Journal of Personalized Medicine

Predicting Psychopathological Onset

Early Signs of Neuropsychiatric Diseases

Edited by
Marco Costanzi

Printed Edition of the Special Issue Published in
Journal of Personalized Medicine

www.mdpi.com/journal/jpm

MDPI

Predicting Psychopathological Onset: Early Signs of Neuropsychiatric Diseases

Predicting Psychopathological Onset: Early Signs of Neuropsychiatric Diseases

Editor

Marco Costanzi

MDPI • Basel • Beijing • Wuhan • Barcelona • Belgrade • Manchester • Tokyo • Cluj • Tianjin

Editor
Marco Costanzi
Human Sciences
LUMSA
Rome
Italy

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Journal of Personalized Medicine* (ISSN 2075-4426) (available at: www.mdpi.com/journal/jpm/special_issues/Psychopathological_Neuropsychiatric).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-4542-4 (Hbk)
ISBN 978-3-0365-4541-7 (PDF)

Contents

Journal of *Personalized Medicine*

MDPI

Editorial

Predicting Psychopathological Onset: Early Signs of Neuropsychiatric Diseases

Marco Costanzi

Department of Human Sciences, LUMSA University, 00193 Rome, Italy; m.costanzi@lumsa.it

Millions of people worldwide are affected by neuropsychiatric disorders, such as anxiety, major depression, bipolar disorder, schizophrenia, obsessive–compulsive disorder, autism spectrum disorders, eating disorders, addiction, and dementia. The identification of the early signs of these pathologies is an important goal to be reached in order to improve treatment effectiveness and to prevent poor outcomes.

The aim of this Special Issue is to collect valuable contributions from scientists worldwide working on the role that biological, behavioral, and cognitive markers can have in predicting the onset of neuropsychiatric disorders. We were able to collect 13 original articles and 2 reviews on this topic. The results published in this Special Issue could provide significant support in pre-clinical phases for the identification of vulnerability factors, to better understand the course of the illness, and to predict its outcome, as well as aiding clinicians in the therapeutic decision-making process.

The observation that patients suffering from a rare metabolic syndrome, trimethylaminuria, also show excessive fear, anxiety, social phobia, a sense of marginalization, suicidal ideation, a sense of persecution, and mood alterations seems to provide an interesting biological scenario linking the mind–body system to mental illness. Notably, gut microbiota alterations, which are responsible for the onset of metabolic syndrome, result in dysfunctions of neurotransmitter release and vagus nerve activation, which might determine the widest spectrum of the psychiatric disorders shown by the affected patients. Therefore, the microbiota–gut–brain axis may become a potential new target for improving the treatment of neuropsychiatric disorders [1]. Cattaneo and collaborators, by reviewing the literature in the field, suggest that the heart–brain relation is important in understanding the etiopathogenetic mechanisms of several psychopathologies and in pursuing mental health. In their work, the authors suggest an interesting relationship between the stress level of an organism and persistent alterations in the neurovegetative system, including the vasovagal system, which, in turn, results in a low heart rate variability. Such a low heart rate variability correlates with emotional dysregulation and frontal lobe dysfunctions, which are considered hallmarks of psychopathological dimensions [2].

Prefrontal cortex circuits are mainly involved in executive functions, such as the inhibitory control mechanisms that control active forgetting processes. Active forgetting plays a pivotal role in suppressing stressful intrusive memories. The suppression of these unwanted memories appears to be critical in preserving mental health, whereas deficits in the inhibitory control of these memories correlate with several psychopathological disorders, such as depression, schizophrenia, post-traumatic stress disorder, and obsessive–compulsive disorder [3].

The results discussed in the abovementioned papers provide an interesting scenario in which the interplay between the biological, psychophysiological, cognitive, and affective domains should be carefully taken into account when considering the possibility of predicting the onset of neuropsychiatric disorders.

The association between genetic variants and several neuropsychiatric disorders has been extensively demonstrated. However, several factors (e.g., the lack of reproducibility of the genetic association data published to date, the weakness of statistical associations,

Citation: Costanzi, M. Predicting Psychopathological Onset: Early Signs of Neuropsychiatric Diseases. *J. Pers. Med.* **2022**, *12*, 778. https://doi.org/10.3390/jpm12050778

Received: 29 April 2022
Accepted: 9 May 2022
Published: 11 May 2022

the heterogeneity of the phenotype, and the massive influence of the environment on human behavior) have to be adequately considered when the role of single polymorphic variants are related to the onset of specific neuropsychiatric disorders. By selecting 24 polymorphisms in genes related to human behavior previously associated with criminal behavior, Zampatti and collaborators found that these genetic variants are not clearly associated with antisocial behavior, suggesting that environmental factors could better explain the onset of violent and criminal behaviors [4].

Therefore, it is worth considering the simultaneous presence of several factors, as well as the relationship between them, when predicting the development of psychopathological trajectories.

Emotional dysregulation (i.e., the inability to monitor and evaluate emotional experiences, as well as the inability to modulate emotional reactions to meet situational demands) and temperamental features (i.e., biological and constitutional characteristics of behavioral tendencies) appear as independent factors in predicting suicidal ideation in young adults with bipolar and depressive disorders [5]. A specific form of emotional dysregulation (namely, the alexithymia), together with body image concerns, positively correlate with exercise addiction. In this relationship, self-esteem emerges as a moderating factor, playing an important role as a protective factor [6]. Another form of addiction that particularly affects adolescents is pathological gambling. Terrone and collaborators found that a chain of multiple risk factors can predict gambling onset. Such a chain begins with an insecure attachment, which negatively influences the developmental perspective and affects the theory of mind towards one's best friend [7]. Although restricted to specific neuropsychiatric disorders, these findings on addiction seem to provide insight into the need to consider possible relationships among several risk factors. This approach may have important clinical implications by orienting preventive activities (e.g., the formation of a positive peer relationship and performing regular exercise), as well as addressing tailored treatments for addicted individuals [6,7].

As concerns the role played by alterations in the cognitive domain in neuropsychiatric disorders, Bechi Gabrielli and collaborators revealed how deficits in executive functions may be considered a potential hallmark for bipolar disorder [8]. Euthymic bipolar patients, who are in the remission phase, show deficits in the trade-off between attentional boost and attentional competition (i.e., a lack of the attentional boost effect), suggesting that temporal selective attention processes are defective in these patients [8]. Kutz and collaborators extend the involvement of the cognitive domain in degenerative disorders, pointing out the role of motor functions. By examining the association between finger tapping and cognitive function in patients affected, or supposed to be affected, by mild cognitive impairments, they suggest that results on the diadochokinetic nature of finger tapping have to be carefully taken into account when simple finger movements are considered a hallmark of age-related neurodegeneration. The assessment of the degeneration of the relevant motor systems (e.g., the cerebellum) must be considered to establish tapping as a good classifier for predicting the onset of neurodegenerative disorders [9].

The need to consider several risk factors acting on different domains has prompted researchers to develop new tools to effectively predict the onset of neuropsychiatric disorders.

Byeon developed a nomogram that could help medical professionals in the primary care setting identify people at high risk of depression. The results of his cross-sectional study, in which elderly people underwent a comprehensive evaluation that included a health survey, blood pressure measurements, physical measurements, blood tests, and a standardized depression screening test, point out the importance of continuously monitoring complex risk factors (such as household income, skipping breakfast, moderate-intensity physical activity, subjective stress, and subjective health status) to prevent depression in older adults [10]. Conti and collaborators considered the importance of introducing neuroanatomical criteria in improving the effectiveness of early differential diagnosis and in tailoring specific early intervention in neuropsychiatric disorders that share common clinical signs. By investigating the brain morphology of children with autism spectrum

disorder or childhood apraxia of speech, as well as children with typical development, they successfully applied a machine learning method able to reach an optimal predictive power to differentiate between the two pathological conditions and from typical development [11]. Gori and collaborators developed a new measurement method for the assessment of mentalizing: the Multidimensional Mentalizing Questionnaire (MMQ). In their research, the authors underlined the centrality of the mentalizing construct in different forms of neuropsychiatric disorders and proposed mentalizing as a broad and multifaceted concept that encompasses and combines multiple constructs involved in treating others and ourselves as social agents. In this framework, the MMQ can be usefully adopted in both research and clinical practice, being a valuable self-reporting tool for repeated measurements of a patient's status over the course of therapy, favoring tailored interventions and supporting clinical research [12]. Mangialavori and collaborators, by investigating perinatal affective disorder, highlighted the importance of screening fathers in perinatal health services, which are still predominantly mother-centered, and pointed out the importance of appropriate gender-sensitive screening for detecting fathers' affective symptoms, given the impact of men's psychological distress on the well-being of the whole family [13].

Finally, the negative impact that the COVID-19 pandemic and the associated restrictive measures have had on mental health [14] urgently requires the development of efficient psychological interventions to prevent and tackle mental disorders in addition to adequate socio-sanitary policies aimed at limiting the pandemic [15].

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Donato, L.; Alibrandi, S.; Scimone, C.; Castagnetti, A.; Rao, G.; Sidoti, A.; D'Angelo, R. Gut-Brain Axis Cross-Talk and Limbic Disorders as Biological Basis of Secondary TMAU. *J. Pers. Med.* **2021**, *11*, 87. [CrossRef] [PubMed]
2. Cattaneo, L.A.; Franquillo, A.C.; Grecucci, A.; Beccia, L.; Caretti, V.; Dadomo, H. Is Low Heart Rate Variability Associated with Emotional Dysregulation, Psychopathological Dimensions, and Prefrontal Dysfunctions? An Integrative View. *J. Pers. Med.* **2021**, *11*, 872. [CrossRef] [PubMed]
3. Costanzi, M.; Cianfanelli, B.; Santirocchi, A.; Lasaponara, S.; Spataro, P.; Rossi-Arnaud, C.; Cestari, V. Forgetting Unwanted Memories: Active Forgetting and Implications for the Development of Psychological Disorders. *J. Pers. Med.* **2021**, *11*, 241. [CrossRef] [PubMed]
4. Zampatti, S.; Ragazzo, M.; Fabrizio, C.; Termine, A.; Campoli, G.; Caputo, V.; Strafella, C.; Cascella, R.; Caltagirone, C.; Giardina, E. Genetic Variants Allegedly Linked to Antisocial Behaviour Are Equally Distributed Across Different Populations. *J. Pers. Med.* **2021**, *11*, 213. [CrossRef] [PubMed]
5. Janiri, D.; Moccia, L.; Conte, E.; Palumbo, L.; Chieffo, D.P.R.; Fredda, G.; Menichincheri, R.M.; Balbi, A.; Kotzalidis, G.D.; Sani, G.; et al. Emotional Dysregulation, Temperament and Lifetime Suicidal Ideation among Youths with Mood Disorders. *J. Pers. Med.* **2021**, *11*, 865. [CrossRef] [PubMed]
6. Gori, A.; Topino, E.; Pucci, C.; Griffiths, M.D. The Relationship between Alexithymia, Dysmorphic Concern, and Exercise Addiction: The Moderating Effect of Self-Esteem. *J. Pers. Med.* **2021**, *11*, 1111. [CrossRef] [PubMed]
7. Terrone, G.; Gori, A.; Topino, E.; Musetti, A.; Scarinci, A.; Guccione, C.; Caretti, V. The Link between Attachment and Gambling in Adolescence: A Multiple Mediation Analysis with Developmental Perspective, Theory of Mind (Friend) and Adaptive Response. *J. Pers. Med.* **2021**, *11*, 228. [CrossRef] [PubMed]
8. Bechi Gabrielli, G.; Rossi-Arnaud, C.; Spataro, P.; Doricchi, F.; Costanzi, M.; Santirocchi, A.; Angeletti, G.; Sani, G.; Cestari, V. The Attentional Boost Effect in Young and Adult Euthymic Bipolar Patients and Healthy Controls. *J. Pers. Med.* **2021**, *11*, 185. [CrossRef] [PubMed]
9. Kutz, D.F.; Fröhlich, S.; Rudisch, J.; Müller, K.; Voelcker-Rehage, C. Finger Tapping as a Biomarker to Classify Cognitive Status in 80+-Year-Olds. *J. Pers. Med.* **2022**, *12*, 286. [CrossRef] [PubMed]
10. Byeon, H. Development of a Nomogram for Predicting Depression in the Elderly Using Patient Health Questionnaire-9 among a Nationwide Sample of Korean Elderly. *J. Pers. Med.* **2021**, *11*, 645. [CrossRef] [PubMed]
11. Conti, E.; Retico, A.; Palumbo, L.; Spera, G.; Bosco, P.; Biagi, L.; Fiori, S.; Tosetti, M.; Cipriani, P.; Cioni, G.; et al. Autism Spectrum Disorder and Childhood Apraxia of Speech: Early Language-Related Hallmarks across Structural MRI Study. *J. Pers. Med.* **2020**, *10*, 275. [CrossRef] [PubMed]
12. Gori, A.; Arcioni, A.; Topino, E.; Craparo, G.; Lauro Grotto, R. Development of a New Measure for Assessing Mentalizing: The Multidimensional Mentalizing Questionnaire (MMQ). *J. Pers. Med.* **2021**, *11*, 305. [CrossRef] [PubMed]

13. Mangialavori, S.; Giannotti, M.; Cacioppo, M.; Spelzini, F.; Baldoni, F. Screening for Early Signs of Paternal Perinatal Affective Disorder in Expectant Fathers: A Cluster Analysis Approach. *J. Pers. Med.* **2021**, *11*, 10. [CrossRef] [PubMed]
14. Guerrini Usubini, A.; Cattivelli, R.; Varallo, G.; Castelnuovo, G.; Molinari, E.; Giusti, E.M.; Pietrabissa, G.; Manari, T.; Filosa, M.; Franceschini, C.; et al. The Relationship between Psychological Distress during the Second Wave Lockdown of COVID-19 and Emotional Eating in Italian Young Adults: The Mediating Role of Emotional Dysregulation. *J. Pers. Med.* **2021**, *11*, 569. [CrossRef] [PubMed]
15. Santirocchi, A.; Spataro, P.; Costanzi, M.; Doricchi, F.; Rossi-Arnaud, C.; Cestari, V. Predictors of the Intention to Be Vaccinated against COVID-19 in a Sample of Italian Respondents at the Start of the Immunization Campaign. *J. Pers. Med.* **2022**, *12*, 111. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Article

Gut-Brain Axis Cross-Talk and Limbic Disorders as Biological Basis of Secondary TMAU

Luigi Donato [1,2], Simona Alibrandi [1,3], Concetta Scimone [1,2,*], Andrea Castagnetti [4], Giacomo Rao [5], Antonina Sidoti [1] and Rosalia D'Angelo [1]

[1] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Division of Medical Biotechnologies and Preventive Medicine, University of Messina, 98125 Messina, Italy; ldonato@unime.it (L.D.); salibrandi@unime.it (S.A.); asidoti@unime.it (A.S.); rdangelo@unime.it (R.D.)
[2] Department of Biomolecular Strategies, Genetics and Avant-Garde Therapies, I.E.ME.S.T., 90139 Palermo, Italy
[3] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98125 Messina, Italy
[4] Wellmicro Start Up, Innovative Spin-Off Alma Mater Studiorum Università di Bologna, 40129 Bologna, Italy; andrea.castagnetti@wellmicro.com
[5] Central Health Superintendence, Prevention and Research Division, INAIL, 00144 Rome, Italy; giacomorao@tiscali.it
* Correspondence: cscimone@unime.it; Tel.: +39-0902213136

Abstract: Background: Trimethylaminuria (TMAU) is a rare metabolic syndrome characterized by the accumulation and the excretion of trimethylamine (TMA), a volatile diet compound produced by gut microbiota. Gut microbiota alterations are mainly involved in the secondary TMAU, whose patients show also different psychiatric conditions. We hypothesized that the biological activity of several molecules acting as intermediate in TMA metabolic reaction might be at the basis of TMAU psychiatric comorbidities. **Methods**: To corroborate this hypothesis, we performed the analysis of microbiota of both psychiatric suffering secondary TMAU patients and TMAU "mentally ill" controls, comparing the alteration of metabolites produced by their gut bacteria possibly involved in neurotransmission and, in the same time, belonging to biochemical pathways leading to TMA accumulation. **Results**: Microbiota analyses showed that *Clostridiaceae*, *Lachnospiraceae* and *Coriobacteriaceae* alterations represented the bacterial families with highest variations. This results in an excessive release of serotonin and an hyperactivation of the vagus nerve that might determine the widest spectrum of psychiatric disorders shown by affected patients. These metabolites, as short chain fatty acids, lactate and neurotransmitter precursors, are also related to TMA accumulation. **Conclusions**: Knowledge of microbiota-gut-brain axis may become a potential new strategy for improving metabolic diseases and to treat linked psychiatric disorders.

Keywords: TMAU; psychiatric disorders; microbiota

Citation: Donato, L.; Alibrandi, S.; Scimone, C.; Castagnetti, A.; Rao, G.; Sidoti, A.; D'Angelo, R. Gut-Brain Axis Cross-Talk and Limbic Disorders as Biological Basis of Secondary TMAU. *J. Pers. Med.* **2021**, *11*, 87. https://doi.org/10.3390/jpm11020087

Academic Editor: Marco Costanzi
Received: 9 January 2021
Accepted: 28 January 2021
Published: 31 January 2021

1. Introduction

Trimethylaminuria (TMAU) is a metabolic syndrome characterized by the accumulation and the body excretion of trimethylamine (TMA), a compound that can be introduced with diet or synthesized by gut microbiota. TMA is excreted through sweat, breath, urine and other body fluids, determining an unpleasant rotten fish odor. The metabolic and clinical manifestations of TMAU are generally considered benign, as there is no associated organ dysfunction. Such evaluation, as well as the evidence that the condition is frequently unrecognized by clinicians, can have important consequences on the delayed or missed diagnosis [1].

The incidence of heterozygous carriers for this pathology ranges from 0.5 to 11 percent depending on the ethnicity examined [2]. Today, at least two different types of TMAU are

differently recognized: The Type 1, caused by a deficit of the Flavin-containing monooxygenase 3 (FMO3) enzyme, and the secondary TMAU, determined by other-than-genetics factors, such as gut microbiota alterations [3].

The *FMO3* gene belongs to the family of FMO genes, and encodes for a transmembrane protein localized to the endoplasmic reticulum of several tissues, particularly in the liver [4]. The FMO3 triggers the NADPH-dependent oxygenation of various sulfur-, nitrogen- and phosphorous-containing xenobiotics such as therapeutic drugs, pesticides, and dietary compounds like TMA and tyramine. In particular, the FMO3 catalyzes the N-oxygenation of TMA, synthesized after the ingestion of choline, lecithin and L-carnitine rich foods, in trimethylamine-N-oxide (TMAO), which is an in-odorous molecule [5]. Consequently, when the pathological condition is suspected or known to occur in a family, the genetic test of the *FMO3* gene can be helpful in identifying members who present the disorder or carry causative variant. Most of TMAU cases are indeed inherited with an autosomal recessive pattern [6].

Although *FMO3* mutations occur in most of TMAU patients, an increasing number of cases are caused by other factors [7]. A fish-like body odor could result from an excessive intake of certain proteins with diet or from increase of specific bacteria families in the digestive system. Among secondary TMAU causes, indeed, the dysbiosis of the gut microbiota is the most frequent. The normal flora present in certain body districts could play a key role in determining the age of onset and, above all, the phenotype, particularly variable from patient to patient. The intestinal microbiota is involved in the conversion of choline, carnitine, lecithin - present in some foods - into derivatives of TMA, which are then absorbed by the intestinal mucosa. Several species of commensal microorganisms characterized by a more active metabolism, as well as an overexpressed microbiota, could determine a greater accumulation of TMA, thus causing a more serious phenotype, and/or an early clinical onset [8].

The TMAU pathological condition is uncommon in the society [8], and due to the fish odor, affected people are often marginalized. This social impact is commonly considered the first cause of the psychiatric conditions as depression, anxiety, behavior disorders that affect people with TMAU. The patients feel shame and embarrassment, fail to maintain relationships, avoid contact with people who comment on their condition and are obsessive about masking the odor with hygiene products and even smoking. Moreover, the malodorous aspect can have serious and destructive effects also on schooling, personal life, career and relationships, resulting in social isolation, low self-esteem and suicide. Several evidences suggest that biological and physiopathological cellular alterations could link TMAU with nervous disturbs [9].

From a careful analysis of the structure of TMA, it is possible to observe a strong structural analogy with homocysteine and, therefore, it is likely to hypothesize that, just as in homocystinemia, at the basis of most of the pathological conditions associated with trimethylaminuria there is an excess of TMA derivatives in the blood responsible for excitotoxicity, oxidative stress, inflammatory phenomena and endothelial dysfunction. Oxidative stress and inflammation are both responsible for endothelial dysfunction implying, at the brain level, the alteration of the endothelial junctions and, therefore, an increase of the blood brain barrier (BBB) permeability. Such impairment could determine, in the long run, a relevant excitotoxicity, responsible for neuronal degeneration [10].

The molecular basis of the physiopathological excitotoxic mechanism is a strong structural analogy between homocysteine and glutamate, one of the most important excitatory neurotransmitters in the brain. Thus, the excess of homocysteine is responsible for a prolonged and excessive activation of N-Methyl-d-aspartate (NMDA), post-synaptic glutaminergic receptors. Its activation is accompanied by the influx of Ca^{2+} resulting in molecular damage, loss of mitochondrial membrane potential and increased oxidative stress [11,12], release of metabolites in to the extracellular space. Based on structural homology between homocysteine and TMA, a similar excitotoxic mechanism might be hypothesized to explain psychiatric behavior in TMAU patients. However, given the

poor understanding of the mechanism underlying this rare metabolic disorder, it is still unknown if the psychiatric involvement is a cause, or conversely, a consequence of TMA altered metabolism. Several elements, indeed, let us hypothesize that the biological activity of several molecules acting as intermediate in TMA metabolic reactions might be at the basis of TMAU psychiatric comorbidities. In order to corroborate this hypothesis, we performed the analysis of microbiota of both psychiatric suffering secondary TMAU patients and TMAU "mentally ill" controls, comparing the alteration of their bacterial produced metabolites possibly involved in neurotransmission and, in the same time, belonging to biochemical pathways leading to TMA accumulation.

2. Materials and Methods

2.1. Subjects

Microbiota comparative analysis of 7 secondary TMAU affected patients with behavior disorders (from now formerly indicated as "case") and 5 demographically TMAU matched control subjects without cerebral functional impairments (called "controls"), all between the ages of 20 and 72 years, participated in this work. The secondary TMAU pathological condition was assessed by negativity of genetic test on *FMO3* gene and with urinary TMA dosage. The behavioral alterations were clinical diagnosed, basing on patients' anamneses. Control participants were recruited after clinical assessment of healthy mental state using the Mini-International Neuropsychiatric Interview, excluding from the analysis subjects with past or present diagnosis of a major neuropsychiatric illnesses [13]. We established the nearest matching neighbors evaluating sex, age, race, BMI category (obese vs. not obese), and history of antibiotic use (in the past year) to control for clinical factors and known major drivers of microbiome changes [14] that could act as confounding factors. More details about subjects are available in Table 1. All participants provided written informed consent.

2.2. DNA Extraction and Sequencing

Total genomic DNA was extracted from fecal specimens using the QIAamp PowerFecal DNA kit (Qiagen, Hilden, Germany), following the protocol provided by the manufacturer. Then the DNA was quantified by spectrophotometric reading of the absorbance at 260 nm by the QIAExpert (Qiagen, Hilden, Germany) and the quality was verified by electrophoretic run on the QIAdvanced (Qiagen, Hilden, Germany). The V3 and V4 regions of the 16S rRNA coding gene were amplified with primer SD-Bact-0341-bS-17/SD-Bact-0785-aA-21 [15] in 25 µL of final volume of PCR mix consisting of 2x PCRBIO Taq Mix (PCR biosystem, London, UK) and 2.5 µL of DNA (5 ng/µL). The thermal cycle was set with an initial denaturation at 95 °C for 3 min, 25 denaturation cycles at 95 °C for 30 s, annealing at 55 °C for 30 s, extension to 72 °C for 30 s and a final step extension at 72 °C for 5 min. The 460 bp amplicons were purified using a magnetic bead system (Agencourt AMPure XP; Beckman Coulter, Brea, CA, United States) and the libraries prepared using the Nextera V2 indexes (Illumina, San Diego, CA, United States). The samples were, then, normalized to 4 nM, denatured and diluted to 5 pM before being loaded onto the MiSeq sequencer (Illumina, San Diego, CA, USA).

Raw sequences were processed using a pipeline that combines PANDAseq [16] and QIIME [17]. The high-quality reads were grouped into Operational Taxonomic Units (OTUs) using UCLUST [18] with a 97% similarity threshold. Taxonomy was assigned using the Greengenes database (May 2019).

Table 1. Subject metabolic and behavioral features. TMAU patients with psychiatric symptomatology (1–7) and TMAU control patients without mental disturbs (1c–5c) were selected for our retrospective comparison, mainly in relationship with relevant differences of behavioral phenotypes.

ID	AGE	SEX	TMAU AGE of ONSET	DIET	ANTIBIOTIC MASSIVE USE	PROBIOTIC/FOOD SUPPLEMENTS	BEHAVIOR DISORDER	KIND OF BEHAVIOR DISORDER	OTHER
1	30	M	17	Chocolate, Eggs, Peas	NO	NO	YES	Anxiety, Fear, Suicidal instincts, Mood alteration	/
2	40	F	14	Fish, Vegetables	NO	NO	YES	Excessive emotionality, Anxiety	/
3	54	F	6	Dairy products, Meat, Fish	NO	L-carnitine, bromelain	YES	Migraine, Sleep disorders, Mood alteration, Sense of marginalization, Difficulties in social relations	/
4	45	F	7	Chocolate, Legumes, Eggs, Fish	YES	NO	YES	Chronic and rapid mental fatigue, Frequent headaches, Dizziness, Anxiety, Depression	Low levels of Folate, Plasmatic Vitamin B2 and D, Cu^{2+}, Zn^{2+}; High levels of PTH, homocysteine, Ca^{2+}
5	44	M	34	Coffee, Tea, White Meat, Vegetables, Fish	YES	L. acidophilus, Bifidobacterium lactis, L. rhamnosus, Streptococcus thermophilus and L. Paracasei	YES	Obsessive-compulsive disorder, Sense of marginalization	/
6	36	F	9	Vegetables, Coffee, Eggs	YES	Zinc, selenium, folic acid, iron, inulin, magnesium, L. Helveticus, B. longum spp.longum, Vitamin B6, Vitamin B1 and Vitamin D	YES	Mood alteration, Sense of marginalization, Suicidal instincts	/
7	25	F	4	Fish, Eggs, Chocolate, Legumes	NO	NO	YES	Depression, Obsessive-compulsive disorder, Sense of persecution	/

Table 1. *Cont.*

ID	AGE	SEX	TMAU AGE of ONSET	DIET	ANTIBIOTIC MASSIVE USE	PROBIOTIC/FOOD SUPPLEMENTS	BEHAVIOR DISORDER	KIND OF BEHAVIOR DISORDER	OTHER
1c	47	F	8	Gluten-free foods, Vegetables, Coffee	NO	NO	NO	NO	/
2c	26	M	10	Fish, Chocolate, Red meat, Coffee, Alcohol	NO	Bifidobacterium lactis, L. acidophilus, L. plantarum, L. paracasei; Streptococcus salivarius subsp. thermophilus, Bifidobacterium brevis, Lactobacillus delbrueckii subsp. bulgaricus, Enterococcus faecium.	NO	NO	/
3c	20	M	16	Gluten-free foods, Vegetables, Meat	NO	L. acidophilus, Bifidobacterium lactis, L. rhamnosus, Streptococcus thermophilus and L. Paracasei	NO	NO	/
4c	72	F	2	Gluten-free and Lactose-free foods, Fish	NO	NO	NO	NO	High ROS and Arachidonic Acid
5c	35	M	35	Red meat, Legumes, vegetables, Salmon	NO	NO	NO	NO	Use of alcohol

2.3. Statistical Analysis

The whole statistical analyses were executed using IBM SPSS 26.0 software (https://www.ibm.com/analytics/us/en/technology/spss/). Bonferroni corrected p-values < 0.05 were considered as statistically significant. Significant differences in alpha diversity were elaborated with QIIME by pairwise non-parametric t-test with 999 permutations. Significant differences in beta diversity were computed with QIIME by PERMANOVA, and permDISP permitted us to check for significant differences in dispersion [19,20]. Taxonomic comparisons were performed by Analysis of Composition of Microbiomes (ANCOM), which exploits compositional log-ratios to identify statistically significant taxa [21]. Canonical Correspondence Analysis (CCA) [22] was implemented with the R package "vegan", and its significance (consisting of the variables sex, age and TMAU affected or not) was tested with ANOVA and step-wise analysis, and corrected by Bonferroni post-hoc method.

2.4. Neurotransmission Pathway Analysis of Gut-Brain Axis

Starting by obtaining OUT relative abundance, we hypothesized the possible role of each altered microbial species in relation to neural alterations. Therefore, we deeply explored literature and MetAboliC pAthways DAtabase for Microbial taxonomic groups (MACADAM), a user-friendly database rich of statistics about metabolic pathways at a given microbial taxonomic position [23]. For each prokaryotic complete genome retrieved from RefSeq, MACADAM creates a pathway genome database (PGDB) exploiting Pathway Tools software built on MetaCyc data which includes metabolic pathways, associated metabolites, enzymes and reactions. Too guarantee the highest quality of the genome functional annotation data, MACADAM also includes Functional Annotation of Prokaryotic Taxa (FAPROTAX), a manually curated functional annotation database, MicroCyc, a manually curated collection of PGDBs, and the IJSEM phenotypic database.

3. Results

3.1. Microbiota of Neuro-Disordered TMAU Patients Revealed Huge Differences in Composition and Relative Abundances If Compared with "Brain-Healthy" TMAU Affected Individuals

Microbiota comparative analysis of TMAU cases versus controls highlighted very interesting differences, regarding both bacterial family heterogeneity and concentration (Figure S1). Microbiotas of cases showed a prevalent over-abundance of bacteria (10 families), with *Clostridiaceae* reaching the highest values in 4 cases, and *Enterococcaceae* in 2. The lowest abundance, instead, was highlighted by *Lachnospiraceae* (3 cases) and *Coriobacteriaceae*, reduced in two cases. The most altered family both in cases and controls was the just cited *Lachnospiraceae* which, however, showed an opposite trend, reaching the highest relative abundance in controls (about 72.24%), and the lowest in cases (from 1.86% to 3.78%). The absolute lowest abundances were achieved by *Streptococcaceae* and *Coriobacteriaceae* in cases (0.01%), and by *Enterobacteriaceae* and *Sutterellaceae* in controls (0.01%). Among cases, the n° 6 highlighted the highest number of bacterial family with expression alterations (*Enterococcaceae* = 0.68%; *Erysipelotrichaceae* = 3.9%; *Rikenellaceae* = 6.95%; *Streptococcaceae* = 2.62%; *Lachnospiraceae* = 3.78%; *Coriobacteriaceae* = 6.5%), while the control showing the most differentially expressed bacterial family was the 4c (*Enterobacteriaceae* = 2.8%; *Oxalobacteraceae* = 0.08%; *Erysipelotrichaceae* = 3.8%; *Rikenellaceae* = 6.78%; *Veilloneaceae* = 0.48%; *Roseburia* = 1%). Detailed list of differentially represented bacterial families and genera in case and controls is available in Table 2.

Table 2. Differentially represented bacterial families/genera in TMAU psychiatric cases and controls. Microbiota analysis of TMAU psychiatric cases and controls showed alterations (% relative abundance) for 16 families and 2 genera (Roseburia and Faecalibacterium). Over-representation are highlighted in red, down-representation in light blue. The normal range of % relative abundance is indicated between squared brackets.

ID	1	2	3	4	5	6	7	1c	2c	3c	4c	5c
Enterobacteriaceae [0.1–1.1]	0.85	1.08	0.45	0.1	0.74	0.15	0.15	0.02	0.01	0.1	2.8	0.05
Oxalobacteraceae [0.0–0.0]	0	0.05	0	0	0	0	0	0	0	0	0.08	0
Enterococcaceae [0.0–0.0]	0.02	0	0.02	0	0	0.68	0	0	0	0	0	0
Erysipelotrichaceae [0.1–2.9]	2.8	0.4	0.78	0.1	0.38	3.9	3.3	0.15	0.21	0.1	3.8	2.62
Rikenellaceae [0.2–5.3]	0.48	5.22	1.25	0.2	2.2	6.95	0.2	0.2	0.2	0.2	6.78	0.48
Veilloneaceae [0.8–7.7]	6.35	3.15	1.58	0.8	2.8	5.35	3.35	0.8	0.8	0.8	0.48	1.85
Roseburia [0.0–0.9]	0	0.15	0.25	0.85	0	0.04	1.03	3.09	4.4	0	1	1.53
Streptococcaceae [0.1–1.8]	0.28	0.22	3.48	0.01	0.15	2.62	0.15	0.1	0.1	0.03	0.32	0.08
Clostridiaceae [0.1–1.4]	0.28	1.45	1.25	287.8	134.1	0.28	1.6	0.1	0.1	0..23	0.32	0.18
Lachnospiraceae [12.8–37.26]	20.52	9.98	24.78	1.86	15.8	3.78	23.22	72.24	44.65	0.04	18.58	23.25
Prevotellaceae [0.1–13.66]	0.12	2.3	16.68	0.1	0.7	3.85	40.0	0.02	0.1	0.1	0.13	26.65
Coriobacteriaceae [0.3–5.9]	0.15	1.08	2.12	0.01	0.7	6.5	0.82	0.3	0.3	0.04	0.52	1.7
Bacteroidaceae [3.2–35.36]	55.62	17.5	9.98	3.2	9.2	25.38	1.4	3.2	3.2	3.2	14.58	9.45
Ruminococcaceae [13.7–34.7]	2.42	24.4	23.38	13.7	18.7	24.35	16.23	0.27	1.43	0.13	24.25	19.8
Faecalibacterium [2.5–15.56]	0	3.05	9.35	5.2	5.5	0.58	8.43	6.4	23.97	10.33	8.25	7.2
Porphiromonodaceae [0.2–3.2]	1.25	0.2	0.98	0.22	0.52	1.5	0.55	0.12	0.2	0.2	1.22	0.28
Sutterellaceae [0.1–3.5]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.61	0.1	0.1	0.1
Bifidobacteriaceae [0.1–7.96]	4.38	1.82	0.38	0.39	3.55	3.88	0.1	0.1	0.003	0.11	0.1	1.05

3.2. Altered Bacterial Families of Neuro-Disordered TMAU Patients' Microbiomes Produce Neurotransmitters and/or a Wide Range of Metabolites Involved in Their Biochemical Pathways

All identified microbial families share a very interesting feature, consisting in the common production of a very heterogeneous and rich group of metabolites involved in neurotransmitter biosynthesis and degradation, as well as in their biochemical pathways required to the correct physiology of chemical synapses. Enterobacteriaceae are able to directly synthetize dopamine, norepinephrine and serotonin, while Roseburia, Clostridiaceae and Veilloneaceae could produce the highest number of different metabolites (acetate, lactate, butyrate, propionate, succinate and valeriate). A complete list of all metabolites produced by considered bacteria, involved in nervous physiology, is available in Table 3.

Linking the alterations of microbiota families to each metabolite produced, a possible complex scenario emerged from analysis of biochemical patterns. The short-chain fatty acids (SCFAs) resulted the most altered molecules in both case and controls, even if with different trends, with the propionate more differentially produced in cases. Tryptophan and GABA, instead, showed different levels only in controls, in which resulted down-represented (Table 4).

Table 3. Metabolites produced by altered microbiotas related to neural metabolism. Differentially expressed families and genera of analyzed microbiotas showed a production of metabolites acting as intermediates of neural metabolism.

BACTERIA/ METABOLITES	Lactate	Dopamine	Norepinephrine	Acetate	Serotonin	Succinate	Butyrate	Glycolate	Propionate	Pyruvate	α-ketoglutarate	LPS	Malate	Tryptophan	GABA
Enterobacteriaceae	X	X	X	X	X	X									X
Oxalobacteraceae	X						X	X		X	X		X		
Enterococcaceae	X				X							X			
Erysipelotrichaceae	X			X											
Bifidobacteriaceae	X			X											X
Rikenellaceae				X		X			X						
Sutterellaceae												X		X	
Veilloneaceae	X			X		X			X						
Roseburia	X			X			X		X						
Ruminococcaceae	X			X		X									
Streptococcaceae	X			X	X										
Clostridiaceae	X			X			X		X						
Lachnospiraceae	X			X		X	X								
Prevotellaceae				X		X			X						
Coriobacteriaceae	X			X											
Bacteroidaceae				X		X	X		X						X
Faecalibacteriaceae							X								
Porphiromonodaceae				X		X			X						

Table 4. Correspondence between altered microbiota families/genera and nervous-related metabolite levels. Differential abundances of bacterial families/genera leads to corresponding alterations of related metabolites acting as intermediate in neurophysiology. Considered metabolites only refer to microbiota biosynthesis, and they are retrieved from MACADAM database and literature. "↑" = over-production. "↓" = down-production. "[empty space]" = no expression differences.

ID	1	2	3	4	5	6	7	1c	2c	3c	4c	5c
Acetate	↑			↓	↑	↑	↓	↓		↓	↓	↓
Lactate	↓	↓			↑	↓	↑		↓	↓	↑	
Succinate			↑			↑	↑	↓	↓	↓		
Dopamine									↓	↑	↑	
Norepinephrine				↓					↓	↑	↑	
Serotonin			↑	↓		↑			↓	↓	↑	
α-ketoglutarate		↑									↑	
Malate		↑									↑	
Pyruvate		↑									↑	
LPS	↑	↑								↓	↑	
Propionate	↑	↑	↓	↑	↑		↓	↓				
Butyrate	↓	↓		↓	↑	↓	↑	↑	↑	↑	↑	
Tryptophan								↓		↓		
GABA									↓			

3.3. Pathway Analysis of Differential Abundances of Bacterial Families Suggested a Possible Biochemical Link between Microbiota Produced Metabolites, TMA Biosynthesis and Mood/Behavioral Disorders

Both MACADAM and literature analyses showed a very interesting network involving main metabolites produced by microbiota, TMA precursors and neurophysiological pathway [24]. Differential production levels of SCFAs (acetate, propionate and butyrate, also resulted from mixed acid fermentation, Figure 1), together with lactate and α-ketoglutarate play a fundamental role into biogenesis of glutamate and GABA, whose concentration could interfere with betaine transport, determining a possible accumulation of TMA [25] (Figure 2).

The same biological process could be activated by serotonin, produced from amino acid tryptophan, and whose release is induced by high levels of lactate [26]. Furthermore, the biosynthesis of serotonin is strictly connected to melatonin one, whose involvement in circadian rhythms such as sleep-wake cycle is well known. Interestingly, in condition of elevated oxidative stress and inflammation, the tryptophan could shift from serotonin biosynthesis to quinolinic acid one, a neurotoxic byproduct able to induce depression (Figure 3).

Catecholamine metabolism resulted also involved in TMA accumulation. The concentration of norepinephrine, synthetized by dopamine, could regulate the activity of Phosphatidylethanolamine N-methyltransferase (PEMT) enzyme, which is also able to metabolize the phosphatidylethanolamine into phosphatidylcholine [27], which then could be converted to choline, with final increase of TMA levels (Figure 4).

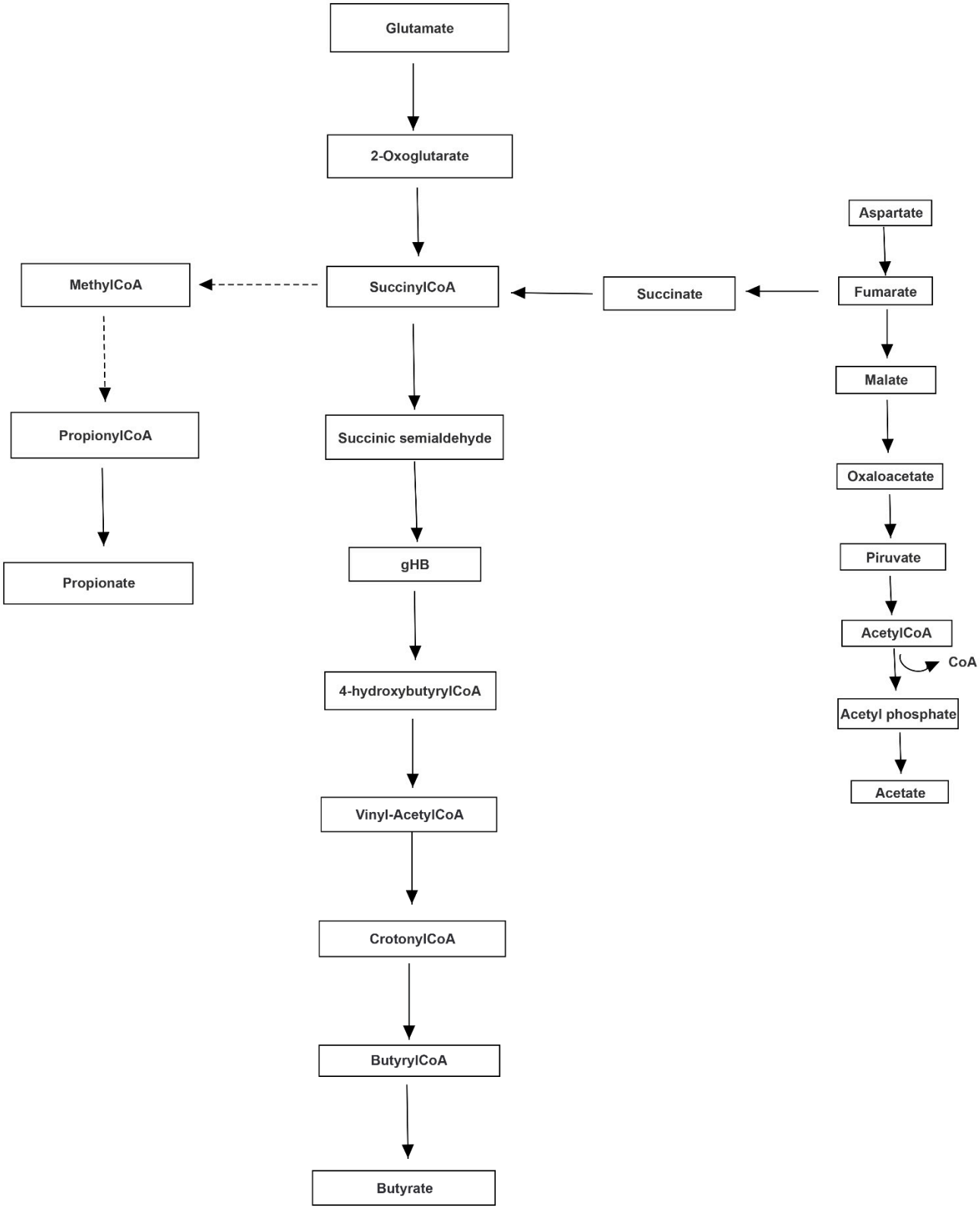

Figure 1. Mixed acid fermentation involving microbiota bacteria. The metabolic way of mixed acid fermentation could produce short chain fatty acids, able to determine an excess of serotonin.

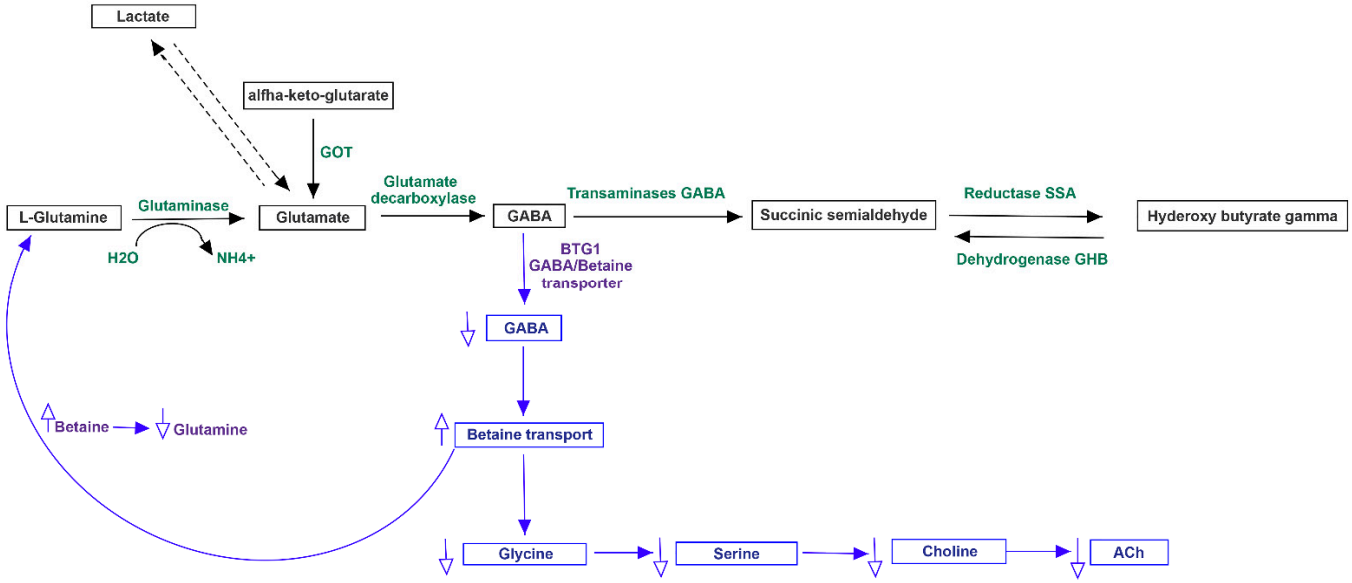

Figure 2. Metabolism of glutamate and GABA linked to ACh. The complex pathway, showing also the involvement of lactate, could play a relevant role in regulation of betaine, a precursor of TMA.

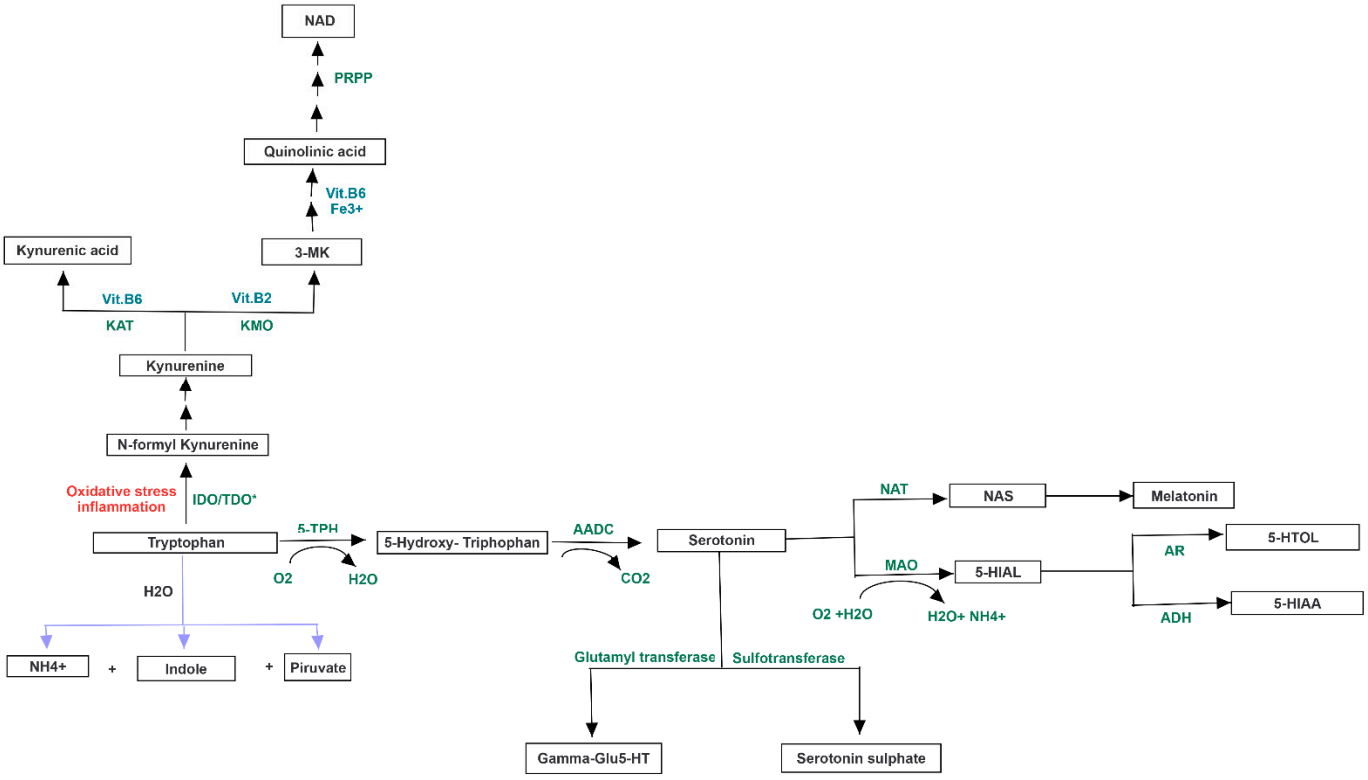

Figure 3. Serotonin metabolism and its "shunt" following oxidative stress and inflammation. Serotonin, produced from tryptophan, could be converted in melatonin. In condition of oxidative stress and inflammation, the amino acid shifts to kynurenine and quinolinic acid pathway, exerting neurotoxic effects.

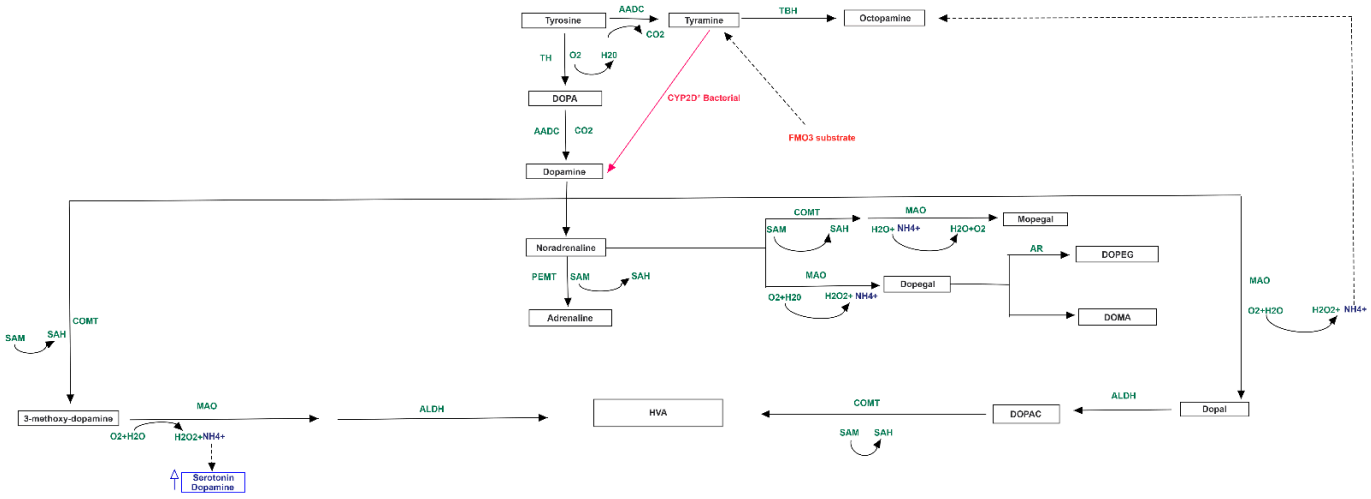

Figure 4. Metabolism of catecholamine and link to serotonin. The scheme also shows that tyramine, produced from tyrosine, is a substrate of FMO3.

The choline quantity could be also raised by acetylcholine, which could also play an important role in carnitine biosynthesis, that could be converted to TMA by bacterial carnitine oxidoreductase (Figure 5).

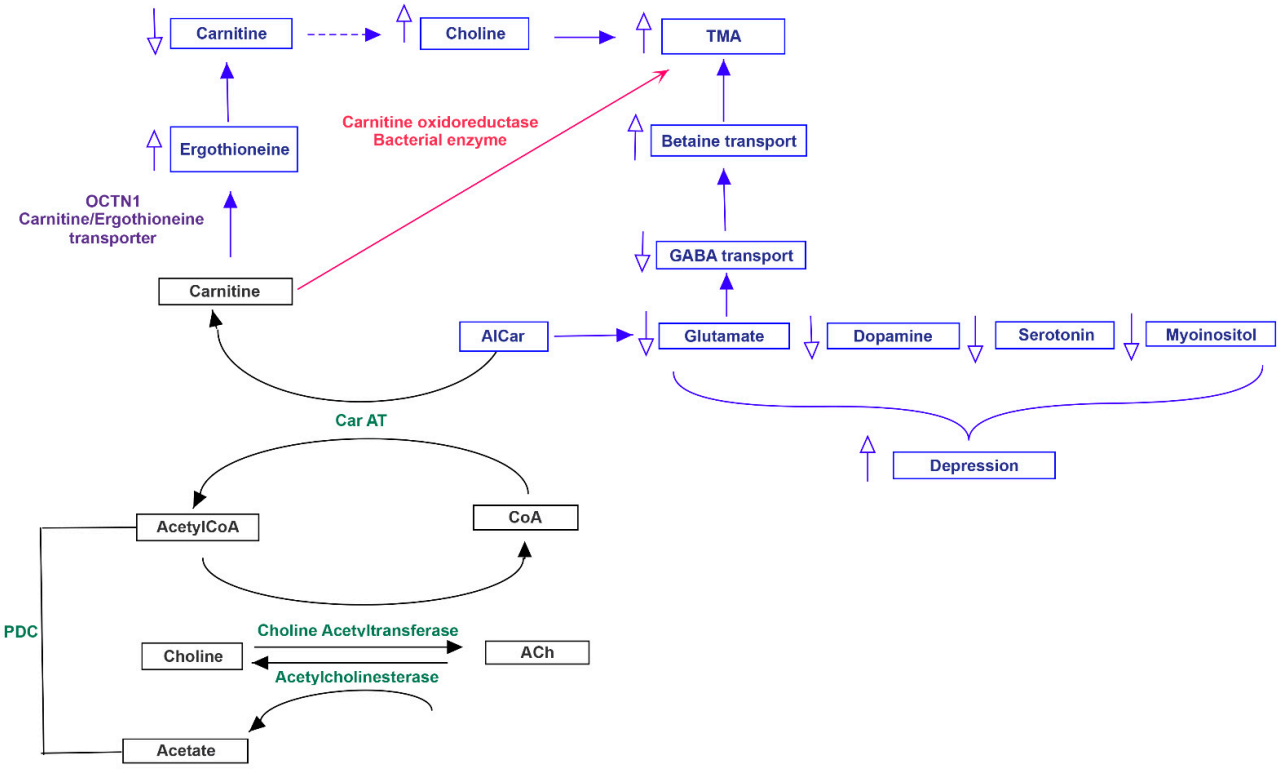

Figure 5. Acetylcholine and carnitine metabolism could influence TMA accumulation and behavioral phenotype. Both carnitine and acetylcholine could alter choline and acetyl-carnitine biosynthesis, determining an accumulation of TMA. In the same time, the acetyl-carnitine could influence the release of main neurotransmitters, determining important behavioral alterations.

Fluctuation of described neurotransmitters could lead to vagus activation/deactivation and limbic deregulation, with behavioral and mood disturbs, like one evidenced by cases in exam. A detailed scheme of all evaluated biochemical pathways linking neurotransmitter and TMA metabolisms is represented in Figure 6.

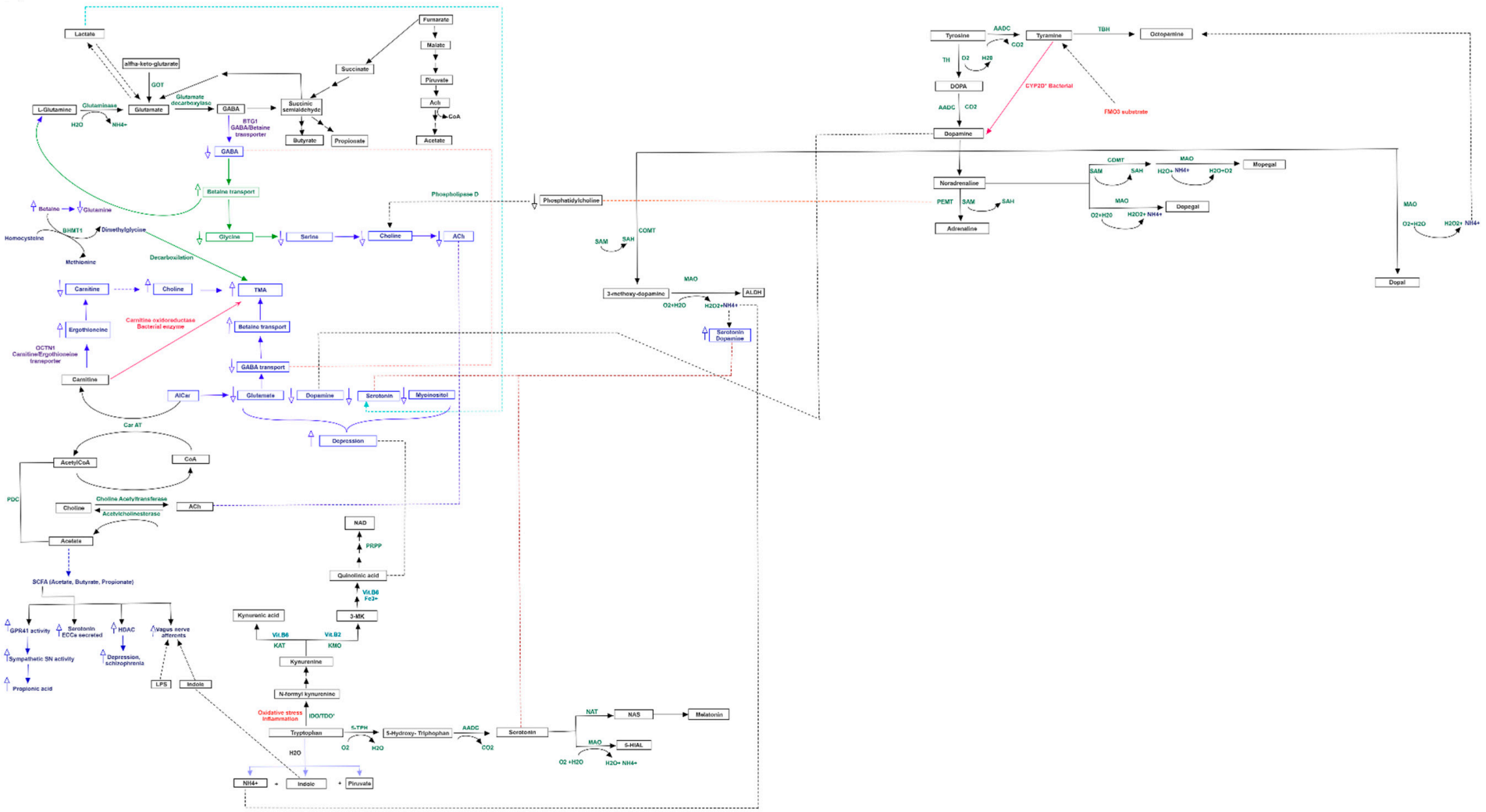

Figure 6. Detailed diagram of biochemical pathways linking neurotransmitter and TMA metabolisms. The figure represents how neurotransmitter and TMA pathways might be correlated. Dashed lines represent indirect and candidate relationships. Empty arrows indicate over- or -down-expression of adjacent metabolite [28].

4. Discussion

Alterations of microbiome is at the basis of an increasing number of metabolic disorders [29]. Recently, it has been highlighted that the gut microbiome is also linked to brain physiopathology [30]. Regarding this, the gut microbiome-brain axis is directly or indirectly associated to neurotransmitters metabolism [31,32]. One of the most challenging scenarios is represented by the possible relationship between metabolic and brain disorders, considered generally unlinked but probably strictly connected [33]. An interesting example is given by TMAU, a metabolic disease characterized by fish odor emission due to the release of high TMA levels, previously accumulated in various body secretions like sweat, urine, blood and vaginal one [34]. While in the primary form of TMAU phenotype is mainly determined by genetic mutations in *FMO3* gene [35], in the secondary one the causes can be different: gut microbiome dysbiosis is one [1]. Patients affected by both primary and secondary forms of TMAU frequently show behavioral disturbs like social exclusion, depression, anxiety, sleep-wake cycle and humoral alterations, until to suicide attempt in extreme cases [36]. These psychological comorbidities, strictly linked to limbic system, represent the most controversial aspects of this pathology, because it is still unknown whether these disturbs are the consequences of social reactions to malodour or could depend on TMA-induced biochemical alterations of nervous system. To deepen this challenging point, we studied 12 patients affected by secondary TMAU, 7 of whom presenting a complex psychological or psychiatric clinical picture (namely called "cases"). All patients were subjected to microbiota analysis, highlighting differences in bacterial abundance and heterogeneity between cases and controls. The bacterial families that showed the most relevant differences in terms of relative abundances were, then, investigated for metabolic pathways. Very interestingly, the highest number of intermediates produced by gut microbiota is transported to central nervous system (CNS), especially to amygdala and hippocampus, through blood stream, even altering the blood brain barrier (BBB) permeability. Furthermore, the same metabolites can directly act on the autonomous nervous system, regulating synapses of vagus nerve in enteric nervous system (ENS) [37]. The most innovative aim of our retrospective comparison was the evaluation of the possible link between TMA and its precursors with metabolism of neurotransmitters involved in limbic system activity. Thus, we proposed a new potential scenario consisting in the explanation of the biochemical patterns involving behavioral disturbs in secondary TMAU affected patients.

Making a brief description of the cases, the patient 5 (Figure 7) potentially produced the lowest number of altered metabolites and showed an over-abundance of *Clostridiaceae* [38], related to high levels of main SCFAs (acetate, propionate and butyrate) and lactate. He manifested serotoninergic syndrome-like phenotype, especially obsessive-compulsive disturbs. This pathological condition is worsened by high lactate levels, which increase butyrate, by the assumption of antibiotics and by supplementation of probiotics consisting of *L. acidophilus*, *Bifidobacterium*, *L. rhamnosus*, *Streptococcus* and *L. paracasei*. Such bacterial families are known to increase the production of lactate, acetate, serotonin, GABA, also determining an accumulation of TMA.

Patients 1 (Figure 8A) and 6 (Figure 8B) showed an analogue serotoninergic syndrome-like symptomatology. The first patient presented an increase of gut *Enterococcaceae* and *Bacteroidaceae*, and a decrease of *Coriobacteriaceae* and *Ruminococcaceae*. The second one, instead, highlighted the highest number of differentially family's composition, consisting of the increase of *Enterococcaceae*, *Erysipelotrichaceae*, *Rikenellaceae*, *Streptococcaceae* and *Coriobacteriaceae*, and the decrease of the only *Lachnospiraceae*. Dysbiosis of such bacteria families in both patients was related to augmented levels of acetate, propionate and LPS, while butyrate and lactate resulted decreased. The over-production of bacterial acetate can be involved into carnitine biosynthesis. The increasing of acetyl-Co, induced by acetate, can activate the carnitine biosynthesis by carnitine acetyl-transferase, thus triggering the accumulation of TMA. The known excitatory effects of lactate on neural metabolism can determinate an increase of both serotonin and glutamate, while provokes

neurotoxicity in neural physiological environment [39]. Thus, low levels of lactate could reduce serotonin and glutamate, whose reduction might decrease GABA biosynthesis in central nervous system, mainly in hippocampus (https://www.proteinatlas.org/ENSG00000145692-BHMT/brain). This portion of limbic system expresses the betaine/GABA transporter BTG-1 [40] which, due to plasma low GABA concentration, might trigger the neuronal internalization of betaine. Betaine can be converted to TMA by betaine-homocysteine-S-methyltransferase (BHMT1) and a following decarboxylation. About serotonin, even if reduced lactate and butyrate levels could reduce it, the increase of acetate and propionate concentration can enhance its biosynthesis. Interestingly, the over-expression of the last two metabolites, together with LPS, could stimulate the afferent component of vagus nerve, inducing what is generally called "gut instincts" or visceral sensations. Such scenario can induce the brain to trigger emotional responses such as fear and anxiety, peculiar of patient 1. In patient 6, the augmented release of serotonin from enterochromaffin cells (ECCs) and the hyperactivation of vagus nerve can be linked to the probiotic supplementation of *L. helveticus* and *B. longum*, well known to increase serotonin and norepinephrine levels production in the hippocampus [41].

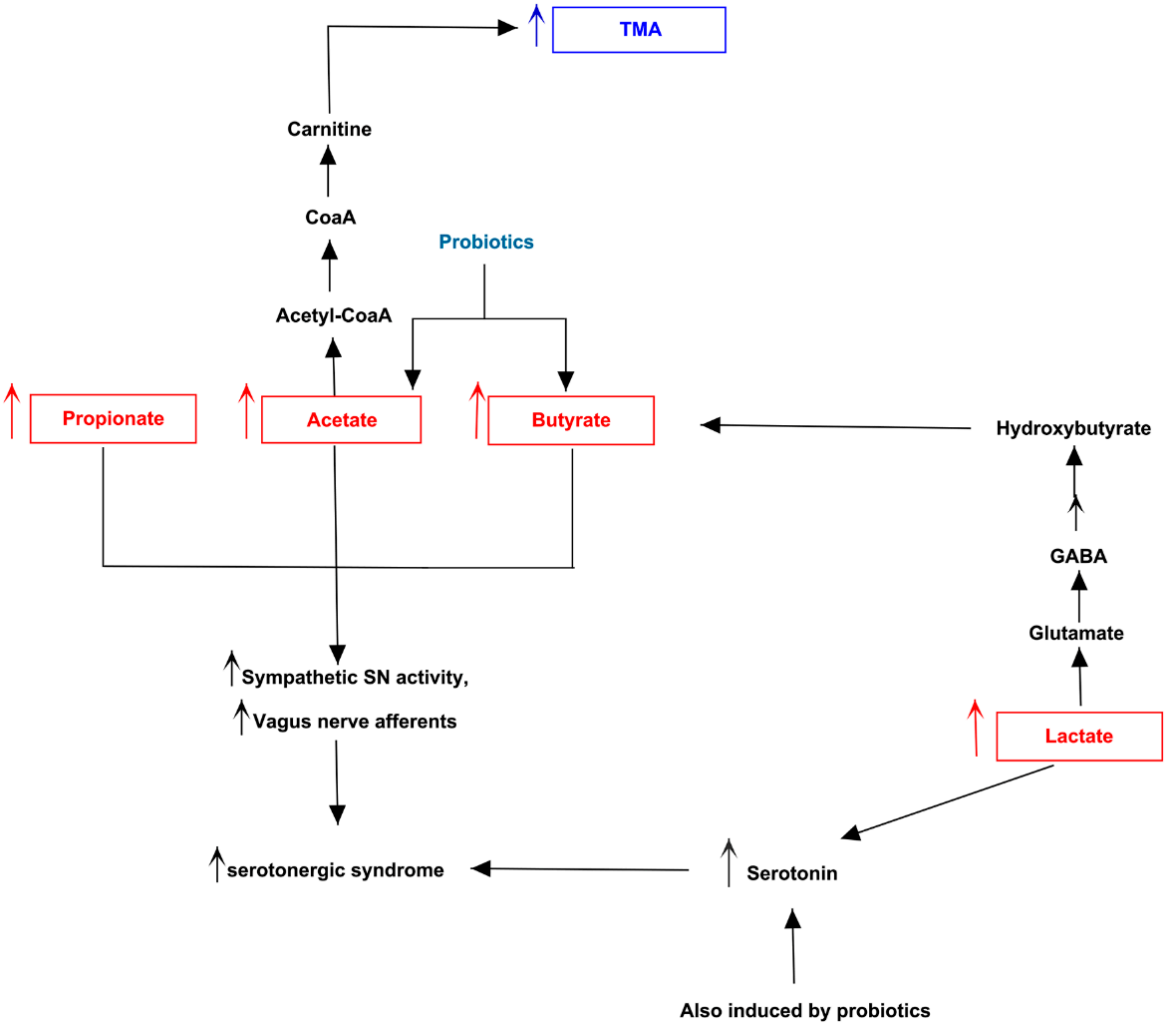

Figure 7. Biochemical pictures of TMAU patient 5. The panel represents how metabolites produced directly or indirectly by patient's microbiota could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

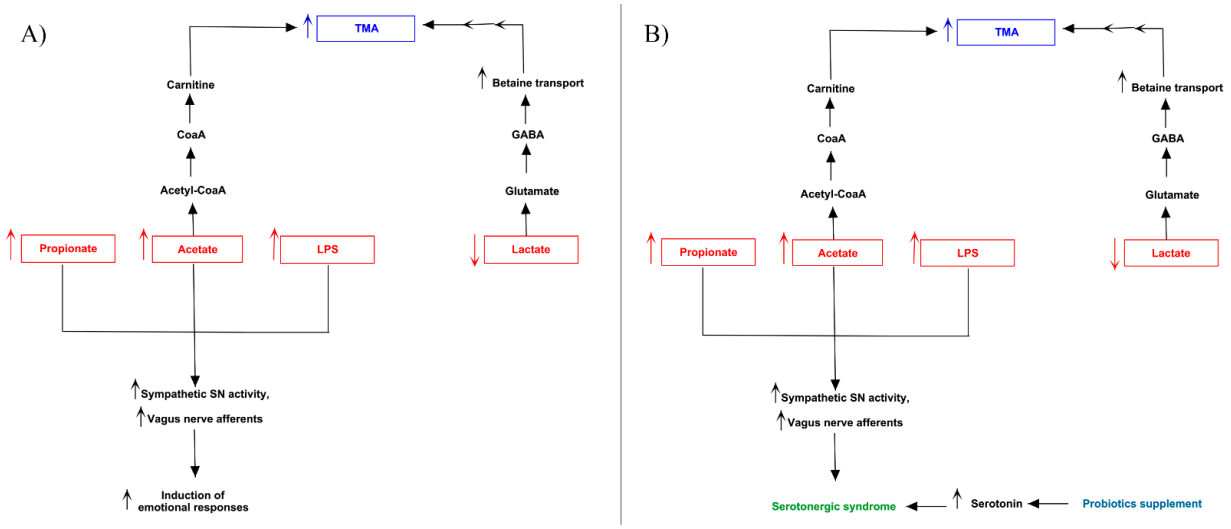

Figure 8. Biochemical pictures of TMAU patients 1 and 6. The panel represents how metabolites produced directly or indirectly by microbiota of patients 1 (**A**) and 6 (**B**) could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

A slightly different situation was evidenced by the patient 3 (Figure 9), who showed increased of *Enterococcaceae*, *Streptococcaceae* and *Prevotellaceae* relative abundance, linked to higher levels of succinate and serotonin and to low levels of propionate. We postulate that over-synthesis of succinate increases the levels of succinyl-CoA, which follows the biochemical pathway starting from succinic semialdehyde and determinates the final production of butyrate. The high levels of succinate and low levels of propionate probably produced by lactic acid mix fermentation, can determine an increase of acetate biosynthesis pathway that, as for patient 1, can imply an accumulation of TMA. Moreover, TMA levels could be increased by the supplement of L-carnitine, converted in TMA by bacterial carnitine oxidoreductase. The probable over-production of butyrate induced by succinate increases the serotonin biosynthesis by ECCs that, together with serotonin secreted by altered gut bacteria, can determine the phenotype typical of the serotoninergic syndrome. This condition reflects the major nervous-related symptoms shown by the patient (migraine, mood alteration, sense of marginalization and social phobia) [42]. Furthermore, the serotonin excess can increase levels of melatonin, explaining alteration of sleep-wake cycle of patient 3.

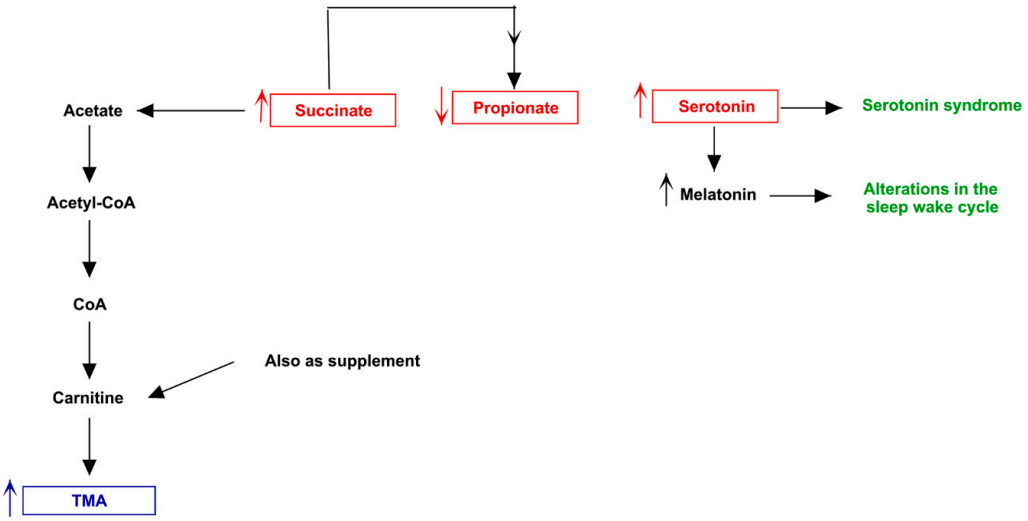

Figure 9. Biochemical pictures of TMAU patient 3. The panel represents how metabolites produced directly or indirectly by patient's microbiota could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

A unique condition was evidenced in patient 4 (Figure 10). He presented a low of acetate, butyrate and Vitamin D levels, and increased concentration of propionate, suggesting a global reduction of vagus nerve activation and serotonin release, already determined by microbiota reduced abundances of *Streptococcaceae*. The low levels of folate characterizing the patient could impair the norepinephrine biosynthesis [43]. This event could shift the catalytic activity of PEMT from epinephrine biosynthesis towards phosphatidylcholine production, which could increase TMA levels via choline pathway. Furthermore, the high concentration of TMA could be also determined by elevated levels of homocysteine shown by the patient, through the reaction that transfer a methyl group from betaine to convert homocysteine to methionine, producing dimethylglycine (DMG) and, in subsequent step, TMA by decarboxylation. The most interesting metabolic pathway related to mood disorders was represented by low levels of plasmatic vitamin B2, which could be accumulated in nervous tissue following increased blood brain barrier (BBB) permeability. This permeability, indeed, is known to be caused by microbiota dysbiosis [44]. Moreover, this inflammatory scenario determined by altered microbiota could trigger the shifting of the tryptophan from serotonin pathway to degradation, producing kynurenine, which cross the BBB and, inside the nervous tissue, is converted into quinolinic acid [45]. This molecule is an antagonist of NMDA receptors and a non-competitive inhibitor of acetylcholine receptors, able to produce oxidative stress and neurotoxic effects, also inducing anxiety and depression, two behavioral alterations of patient 4.

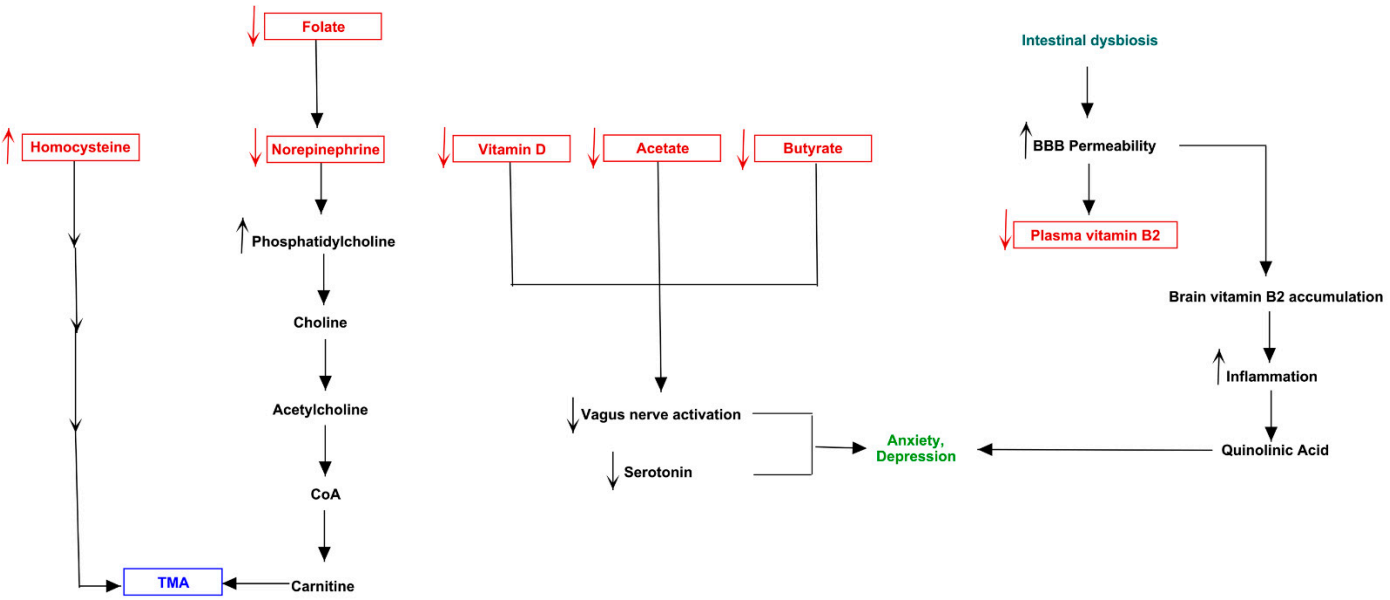

Figure 10. Biochemical pictures of TMAU patient 4. The panel represents how metabolites produced directly or indirectly by patient's microbiota could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

The mixed acid fermentation is the biochemical pathway which produced the highest alteration in neural physiology-related metabolites in patient 2 (Figure 11). The increase of malate, mainly produced by *Oxalobacteraceae* [46], could stimulate the biosynthesis of pyruvate and, soon after, of acetyl-CoA. This metabolite is converted to acetyl phosphate, releasing CoA, with the synthesis of acetate in the final step. The CoA previously produced could enter in carnitine biosynthesis, leading to accumulation of TMA. Additionally, the high levels of alpha-ketoglutarate, together with low levels of lactate, could increase the succinic semi-aldehyde via GABA, determining the production of butyrate as fermentation product. Thus, the overall increase of main SCFAs, together with the elevated levels of propionate produced by altered microbiota, could favorite the ECC endogenous release

of serotonin and the activation of the vagus nerve, along with LPS. Such scenario could explain the excess of anxiety and the uncontrolled emotional status.

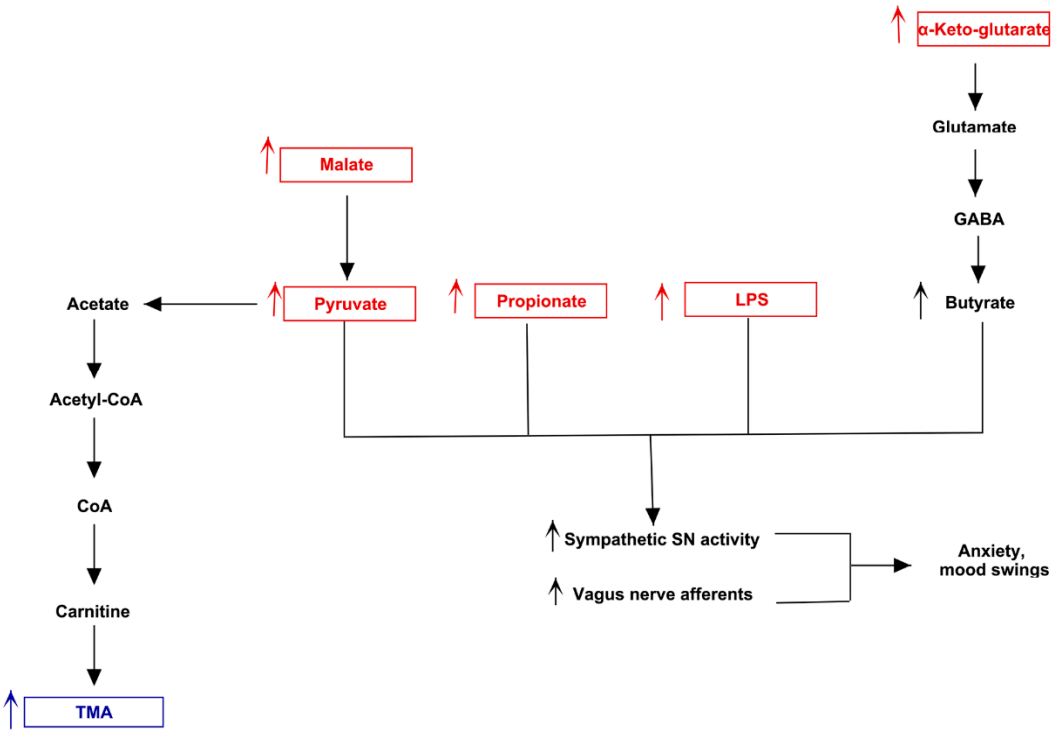

Figure 11. Biochemical pictures of TMAU patient 2. The panel represents how metabolites produced directly or indirectly by patient's microbiota could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

A depressive phenotype was evidenced by patient 7 (Figure 12), who showed an increase of *Erysipelotrichaceae*, *Roseburia*, *Clostridiaceae* and *Prevotellaceae*, with a reduction of *Bacteroidaceae*. The alteration of these families could lead to a down-production of acetate and propionate, determining a global down-regulation of serotonin release and vagus nerve activation, characteristic of depression phenotype. In the meantime, the low levels of acetate could reduce the acetyl-CoA production, arresting the reaction which converts choline to acetylcholine. So, the accumulation of choline could augment TMA levels, leading to TMAU phenotype.

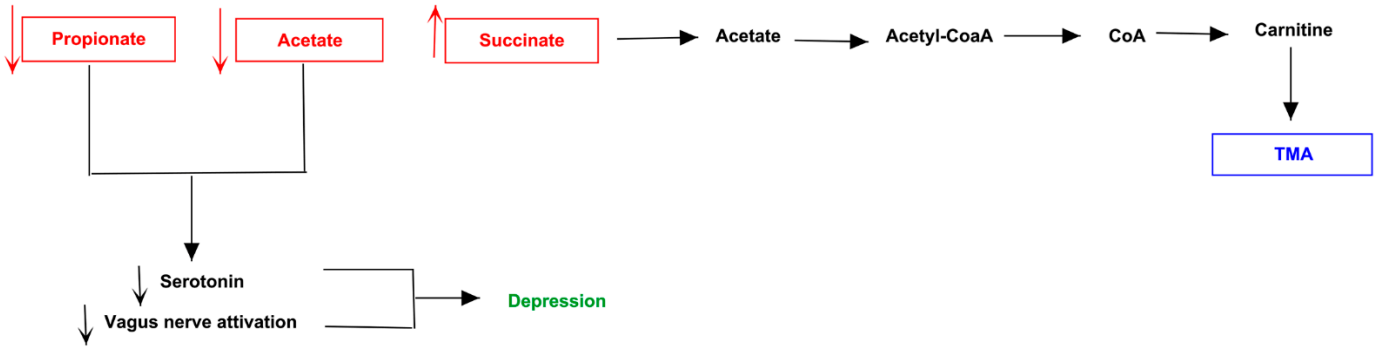

Figure 12. Biochemical pictures of TMAU patient 7. The panel represents how metabolites produced directly or indirectly by patient's microbiota could influence the biosynthesis/release of neurotransmitters (in particular serotonin) and the production/accumulation of TMA.

Based on both microbiota alteration evidences and host biochemical pathways, all analyzed cases showed relevant changes in production of behavioral disorder-related metabolites. In contrast controls here we considered highlighted different alterations in the same pathways. However, the intake of probiotic supplements balanced the pathological phenotype. This latter scenario characterizes controls 2c and 3c, who showed a different spectrum of metabolites. In addition, compensation of probiotics normalized the global concentration of the serotonin, as well as dopamine levels was balanced by *Enterococcus faecium* supplemented in subject 2c [47].

The metabolic picture of control 1c was characterized by a probable down-production of microbiota serotonin, due to decreased levels of several SCFAs and tryptophan. A possible compensation was provided by the human endogenous biosynthesis of serotonin, also enhanced by microbiota butyrate high levels.

An analogue condition was evidenced in control 4c, whose serotonin production induced by SCFAs could be balanced by reduction of vitamin D, which could decrease the neurotransmitter concentration. Moreover, the microbiota synthesis of dopamine might not exert positive effects on neurotransmission, due to the possible conversion of norepinephrine precursor to 6-hydroxydopamine (6-OHDA). Moreover, this could enhance the oxidative stress condition given by the high ROS levels detected in plasma.

Interestingly, the biochemical picture of control 5c highlighted how the increase of only *Prevotellaceae* and *Roseburia* might not be sufficient to determine a psychiatric phenotype. Probably the metabolites produced by both these bacteria are qualitative and quantitative not enough to exert a cytotoxic effect on nervous system. Thus, the integrity of psychic activities might be maintained or very little impaired.

All controls, considering the already discussed biochemical pathways analyzed in relation to cases, showed an accumulation of TMA.

Limitations

Our results suggest that our hypothesis might be truly founded and they highly encourage to confirm them by further experiments. Therefore, we aimed to increase the statistical number of cases and controls, even if this pathology is enough rare to consider reliable our sample size. In order to improve the sample size in a useful way, we are also going to plan a more rigid clinical study, evaluating a stronger methodology. Regarding this, we are also going to improve the psychiatric anamnesis with more details, evaluate the biochemistry and molecular genetics of investigated metabolites, and realize several physiological essays in order to ensure the role of each metabolite in each considered pathway. Such approach could improve the group sampling, trying to avoid several biases caused by the lack of these data.

5. Conclusions

The relationship between gut microbiota and psychiatric disturbs is one of the most challenging topics involving researchers. The vagal nerve is the anatomical structure which permits the communication between the central nervous system (CNS) and enteric nervous system (ENS). Vagal afferent neurons express receptors for gut microbiota metabolites, such as serotonin, that can modulate nutrient metabolism. Furthermore, SCFAs, catecholamines, acetylcholine, the intermediates of mixed acid fermentation and TMAO are able to regulate metabolism through a microbiota-gut-liver axis. However, very little is known about the direct connection between metabolic diseases and mental disorders, involving common pathway in which the considered metabolites play an orchestral role. In our retrospective comparison, we laid the bases for further investigation about biochemical and biological link between secondary trimethylaminuria and psychiatric behaviors. We suppose that the mental disturbs affecting TMAU patients are probably not only related to social consequence of their metabolic disease but also to a physiopathological effect determined by TMA accumulation. The knowledge of this aspects might allow us to personally modulate each gut microbiota. Thus, the related microbiota-gut-brain axis may

become a potential new strategy for improving prognosis of metabolic diseases and treat linked psychiatric disorders.

Supplementary Materials: The following are available online at https://www.mdpi.com/2075-4426/11/2/87/s1, Figure S1: Cladogram of most altered bacterial families in TMAU behavioral disordered cases.

Author Contributions: Conceptualization, L.D.; methodology, L.D. and A.C.; software, L.D. and A.C.; validation, C.S.; formal analysis, L.D.; investigation, L.D. and S.A.; resources, G.R.; data curation, C.S.; writing—original draft preparation, L.D. and S.A.; writing—review and editing, S.A. and A.S.; visualization, C.S.; supervision, R.D. and A.S.; project administration, A.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of "Azienda Policlinico Universitario of Messina" (protocol code 54-20 of 24-06-2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Schmidt, A.C.; Leroux, J.C. Treatments of trimethylaminuria: Where we are and where we might be heading. *Drug Discov. Today* **2020**, *25*, 1710–1717. [CrossRef] [PubMed]
2. Messenger, J.; Clark, S.; Massick, S.; Bechtel, M. A Review of Trimethylaminuria: (Fish Odor Syndrome). *J. Clin. Aesthetic Dermatol.* **2013**, *6*, 45–48.
3. Fraser-Andrews, E.A.; Manning, N.J.; Ashton, G.H.; Eldridge, P.; McGrath, J.; Menage, H.D.P. Fish odour syndrome with features of both primary and secondary trimethylaminuria. *Clin. Exp. Dermatol.* **2003**, *28*, 203–205. [CrossRef] [PubMed]
4. Esposito, T.; Varriale, B.; D'Angelo, R.; Amato, A.; Sidoti, A. Regulation of flavin-containing mono-oxygenase (Fmo3) gene expression by steroids in mice and humans. *Horm. Mol. Biol. Clin. Investig.* **2014**, *20*, 99–109. [CrossRef]
5. Cruciani, G.; Valeri, A.; Goracci, L.; Pellegrino, R.M.; Buonerba, F.; Baroni, M. Flavin Monooxygenase Metabolism: Why Medicinal Chemists Should Matter. *J. Med. Chem.* **2014**, *57*, 6183–6196. [CrossRef]
6. Shephard, E.A.; Treacy, E.P.; Phillips, I.R. Clinical utility gene card for: Trimethylaminuria. *Eur. J. Hum. Genet.* **2012**, *20*, 4–5. [CrossRef]
7. Scimone, C.; Donato, L.; Rinaldi, C.; Sidoti, A.; D'Angelo, R. First case of Currarino syndrome and trimethylaminuria: Two rare diseases for a complex clinical presentation. *J. Dig. Dis.* **2016**, *17*, 628–632. [CrossRef]
8. Vogt, N.M.; Romano, K.A.; Darst, B.F.; Engelman, C.D.; Johnson, S.C.; Carlsson, C.M.; Asthana, S.; Blennow, K.; Zetterberg, H.; Bendlin, B.B.; et al. The gut microbiota-derived metabolite trimethylamine N-oxide is elevated in Alzheimer's disease. *Alzheimer's Res. Ther.* **2018**, *10*, 124. [CrossRef]
9. Mackay, R.J.; McEntyre, C.J.; Henderson, C.; Lever, M.; George, P.M. Trimethylaminuria: Causes and Diagnosis of a Socially Distressing Condition. *Clin. Biochem. Rev.* **2011**, *32*, 33–43.
10. Gu, M.; Mei, X.-L.; Zhao, Y.N. Sepsis and Cerebral Dysfunction: BBB Damage, Neuroinflammation, Oxidative Stress, Apoptosis and Autophagy as Key Mediators and the Potential Therapeutic Approaches. *Neurotox. Res.* **2020**. [CrossRef]
11. Abushik, P.A.; Karelina, T.V.; Sibarov, D.A.; Stepanenko, J.D.; Giniatullin, R.; Antonov, S.M. Homocysteine-induced membrane currents, calcium responses and changes of mitochondrial potential in rat cortical neurons. *Zh. Evol. Biokhim Fiziol.* **2015**, *51*, 258–265. [CrossRef] [PubMed]
12. Machado, F.R.; Ferreira, A.G.; Da Cunha, A.A.; Tagliari, B.; Mussulini, B.H.; Wofchuk, S.; Wyse, A.T. Homocysteine alters glutamate uptake and Na+,K+-ATPase activity and oxidative status in rats hippocampus: Protection by vitamin C. *Metab. Brain Dis.* **2011**, *26*, 61–67. [CrossRef] [PubMed]
13. Reas, D.L.; Pedersen, G.; Karterud, S.; Ro, O. Self-harm and suicidal behavior in borderline personality disorder with and without bulimia nervosa. *J. Consult. Clin. Psychol.* **2015**, *83*, 643–648. [CrossRef] [PubMed]
14. Allaband, C.; McDonald, D.; Vazquez-Baeza, Y.; Minich, J.J.; Tripathi, A.; Brenner, D.A.; Loomba, R.; Smarr, L.; Sandborn, W.J.; Schnabl, B.; et al. Microbiome 101: Studying, Analyzing, and Interpreting Gut Microbiome Data for Clinicians. *Clin. Gastroenterol. Hepatol.* **2019**, *17*, 218–230. [CrossRef]
15. Klindworth, A.; Pruesse, E.; Schweer, T.; Peplies, J.; Quast, C.; Horn, M.; Glockner, F.O. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res.* **2013**, *41*, e1. [CrossRef]
16. Masella, A.P.; Bartram, A.K.; Truszkowski, J.M.; Brown, D.G.; Neufeld, J.D. PANDAseq: Paired-end assembler for illumina sequences. *BMC Bioinform.* **2012**, *13*, 31. [CrossRef]

17. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J.; Bittinger, K.; Bushman, F.D.; Costello, E.K.; Fierer, N.; Pena, A.G.; Goodrich, J.K.; Gordon, J.I.; et al. QIIME Allows Analysis of High-Throughput Community Sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef]
18. Golob, J.L.; Margolis, E.; Hoffman, N.G.; Fredricks, D.N. Evaluating the accuracy of amplicon-based microbiome computational pipelines on simulated human gut microbial communities. *BMC Bioinform.* **2017**, *18*, 283. [CrossRef]
19. Leong, L.E.X.; Taylor, S.L.; Shivasami, A.; Goldwater, P.N.; Rogers, G.B. Intestinal Microbiota Composition in Sudden Infant Death Syndrome and Age-Matched Controls. *J. Pediatr.* **2017**, *191*, 63–68. [CrossRef]
20. Wandro, S.; Osborne, S.; Enriquez, C.; Bixby, C.; Arrieta, A.; Whiteson, K. The Microbiome and Metabolome of Preterm Infant Stool Are Personalized and Not Driven by Health Outcomes, Including Necrotizing Enterocolitis and Late-Onset Sepsis. *mSphere* **2018**, *3*. [CrossRef]
21. Niu, J.; Xu, L.; Qian, Y.; Sun, Z.; Yu, D.; Huang, J.; Zhou, X.; Wang, Y.; Zhang, T.; Ren, R.; et al. Evolution of the Gut Microbiome in Early Childhood: A Cross-Sectional Study of Chinese Children. *Front. Microbiol.* **2020**, *11*, 439. [CrossRef] [PubMed]
22. McNeill, T.W.; Sinkora, G.; Leavitt, F. Psychologic Classification of Low-Back Pain Patients: A Prognostic Tool. *Spine* **1986**, *11*, 955–959. [CrossRef] [PubMed]
23. Le Boulch, M.; Dehais, P.; Combes, S.; Pascal, G. The MACADAM database: A MetAboliC pAthways DAtabase for Microbial taxonomic groups for mining potential metabolic capacities of archaeal and bacterial taxonomic groups. *Database* **2019**, *2019*. [CrossRef] [PubMed]
24. Le Chatelier, E.; Nielsen, T.; Qin, J.; Prifti, E.; Hildebrand, F.; Falony, G.; Almeida, M.; Arumugam, M.; Batto, J.M.; Kennedy, S.; et al. Richness of human gut microbiome correlates with metabolic markers. *Nature* **2013**, *500*, 541–546. [CrossRef]
25. Silva, Y.P.; Bernardi, A.; Frozza, R.L. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. *Front. Endocrinol.* **2020**, *11*, 25. [CrossRef]
26. Jenkins, T.A.; Nguyen, J.C.; Polglaze, K.E.; Bertrand, P.P. Influence of Tryptophan and Serotonin on Mood and Cognition with a Possible Role of the Gut-Brain Axis. *Nutrients* **2016**, *8*, 56. [CrossRef]
27. Murphy, M.M.; Gueant, J.L. B vitamins and one carbon metabolism micronutrients in health and disease. *Biochime* **2020**, *173*, 1–2. [CrossRef]
28. Kutmon, M.; Van Iersel, M.P.; Bohler, A.; Kelder, T.; Nunes, N.; Pico, A.R.; Evelo, C.T. PathVisio 3: An Extendable Pathway Analysis Toolbox. *PLoS Comput. Biol.* **2015**, *11*, e1004085. [CrossRef]
29. Vallianou, N.; Stratigou, T.; Christodoulatos, G.S.; Dalamaga, M. Understanding the Role of the Gut Microbiome and Microbial Metabolites in Obesity and Obesity-Associated Metabolic Disorders: Current Evidence and Perspectives. *Curr. Obes. Rep.* **2019**, *8*, 317–332. [CrossRef]
30. Kim, Y.K.; Shin, C. The Microbiota-Gut-Brain Axis in Neuropsychiatric Disorders: Pathophysiological Mechanisms and Novel Treatments. *Curr. Neuropharmacol.* **2018**, *16*, 559–573. [CrossRef]
31. Strandwitz, P. Neurotransmitter modulation by the gut microbiota. *Brain Res.* **2018**, *1693*, 128–133. [CrossRef] [PubMed]
32. Tian, J.S.; Xia, X.T.; Wu, Y.F.; Zhao, L.; Xiang, H.; Du, G.H.; Zhang, X.; Qin, X.M. Discovery, screening and evaluation of a plasma biomarker panel for subjects with psychological suboptimal health state using (1)H-NMR-based metabolomics profiles. *Sci. Rep.* **2016**, *6*, 33820. [CrossRef] [PubMed]
33. Nagalski, A.; Kozinski, K.; Wisniewska, M.B. Metabolic pathways in the periphery and brain: Contribution to mental disorders? *Int. J. Biochem. Cell Biol.* **2016**, *80*, 19–30. [CrossRef] [PubMed]
34. McNiven, V.; Mamane, S.; Zai, G.; So, J. The Nose Knows . . . or Does it? Olfactory Reference Syndrome in Patients Presenting for Assessment of Unusual Body Odor. *J. Nerv. Ment. Dis.* **2019**, *207*, 145–151. [CrossRef] [PubMed]
35. Phillips, I.R.; Shephard, E.A. Flavin-containing monooxygenase 3 (FMO3): Genetic variants and their consequences for drug metabolism and disease. *Xenobiotica* **2020**, *50*, 19–33. [CrossRef]
36. Caspani, G.; Kennedy, S.; Foster, J.A.; Swann, J. Gut microbial metabolites in depression: Understanding the biochemical mechanisms. *Microb. Cell* **2019**, *6*, 454–481. [CrossRef]
37. Carabotti, M.; Scirocco, A.; Maselli, M.A.; Severi, C. The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol.* **2015**, *28*, 203–209.
38. Oliphant, K.; Allen-Vercoe, E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. *Microbiome* **2019**, *7*, 91. [CrossRef]
39. Newington, J.T.; Harris, R.A.; Cumming, R.C. Reevaluating Metabolism in Alzheimer's Disease from the Perspective of the Astrocyte-Neuron Lactate Shuttle Model. *J. Neurodegener. Dis.* **2013**, *2013*, 234572. [CrossRef]
40. Jin, X.T.; Galvan, A.; Wichmann, T.; Smith, Y. Localization and Function of GABA Transporters GAT-1 and GAT-3 in the Basal Ganglia. *Front. Syst. Neurosci.* **2011**, *5*, 63. [CrossRef]
41. Cheng, L.H.; Liu, Y.W.; Wu, C.C.; Wang, S.; Tsai, Y.C. Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. *J. Food Drug Anal.* **2019**, *27*, 632–648. [CrossRef] [PubMed]
42. Frick, A.; Ahs, F.; Engman, J.; Jonasson, M.; Alaie, I.; Bjorkstrand, J.; Frans, O.; Faria, V.; Linnman, C.; Appel, L.; et al. Serotonin Synthesis and Reuptake in Social Anxiety Disorder: A Positron Emission Tomography Study. *JAMA Psychiatry* **2015**, *72*, 794–802. [CrossRef] [PubMed]
43. Fava, M.; Mischoulon, D. Folate in depression: Efficacy, safety, differences in formulations, and clinical issues. *J. Clin. Psychiatry* **2009**, *70* (Suppl. 5), 12–17. [CrossRef] [PubMed]

44. Spector, R.; Johanson, C.E. Vitamin transport and homeostasis in mammalian brain: Focus on Vitamins B and E. *J. Neurochem.* **2007**, *103*, 425–438. [CrossRef] [PubMed]
45. Kennedy, P.J.; Cryan, J.F.; Dinan, T.G.; Clarke, G. Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology* **2017**, *112*, 399–412. [CrossRef]
46. Baldani, J.I.; Rouws, L.; Cruz, L.M.; Olivares, F.L.; Schmid, M.; Hartmann, A. The Family Oxalobacteraceae. In *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 919–974. [CrossRef]
47. Yano, J.M.; Yu, K.; Donaldson, G.P.; Shastri, G.G.; Ann, P.; Ma, L.; Nagler, C.R.; Ismagilov, R.F.; Mazmanian, S.K.; Hsiao, E.Y. Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. *Cell* **2015**, *161*, 264–276. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Review

Is Low Heart Rate Variability Associated with Emotional Dysregulation, Psychopathological Dimensions, and Prefrontal Dysfunctions? An Integrative View

Lorena Angela Cattaneo [1], Anna Chiara Franquillo [2,3,*], Alessandro Grecucci [4,5], Laura Beccia [1], Vincenzo Caretti [2,3] and Harold Dadomo [6]

1 Schema Therapy Center, 21047 Saronno, Italy; dott.ssacattaneo@gmail.com (L.A.C.); stcsaronno@gmail.com (L.B.)
2 Department of Human Sciences, LUMSA University, 00193 Rome, Italy; v.caretti@lumsa.it
3 Consorzio Universitario Humanitas, 00193 Rome, Italy
4 Department of Psychology and Cognitive Science, DiPSCo, University of Trento, Corso Bettini, 38068 Rovereto, Italy; alessandro.grecucci@unitn.it
5 Center for Medical Sciences, CISMed, University of Trento, 38122 Trento, Italy
6 Neuroscience Unit, Department of Medicine and Surgery, University of Parma, 43125 Parma, Italy; harold.dadomo@gmail.com
* Correspondence: a.franquillo@lumsa.it

Abstract: Several studies have suggested a correlation between heart rate variability (HRV), emotion regulation (ER), psychopathological conditions, and cognitive functions in the past two decades. Specifically, recent data seem to support the hypothesis that low-frequency heart rate variability (LF-HRV), an index of sympathetic cardiac control, correlates with worse executive performances, worse ER, and specific psychopathological dimensions. The present work aims to review the previous findings on these topics and integrate them from two main cornerstones of this perspective: Porges' Polyvagal Theory and Thayer and Lane's Neurovisceral Integration Model, which are necessary to understand these associations better. For this reason, based on these two approaches, we point out that low HRV is associated with emotional dysregulation, worse cognitive performance, and transversal psychopathological conditions. We report studies that underline the importance of considering the heart-brain relation in order to shed light on the necessity to implement psychophysiology into a broader perspective on emotions, mental health, and good cognitive functioning. This integration is beneficial not only as a theoretical ground from which to start for further research studies but as a starting point for new theoretical perspectives useful in clinical practice.

Keywords: heart rate variability; polyvagal theory; neurovisceral integration model; emotional regulation; psychopathology; prefrontal functions

Citation: Cattaneo, L.A.; Franquillo, A.C.; Grecucci, A.; Beccia, L.; Caretti, V.; Dadomo, H. Is Low Heart Rate Variability Associated with Emotional Dysregulation, Psychopathological Dimensions, and Prefrontal Dysfunctions? An Integrative View. *J. Pers. Med.* **2021**, *11*, 872. https://doi.org/10.3390/jpm11090872

Academic Editor: Marco Costanzi

Received: 10 June 2021
Accepted: 29 August 2021
Published: 31 August 2021

1. Introduction

The sympathetic or parasympathetic reactivity of the autonomic nervous system (ANS) has often been cited as one of the most critical factors influencing susceptibility to stress due to its crucial role in mobilizing biological resources during acute "fight or flight" responses to threatening environmental events [1,2]. Individuals often show vast differences in autonomic reactivity, which has been associated with a variety of disorders and pathological conditions, from chronic stress-related disorders to psychopathology [3,4]. Although many authors have identified the relationship between the ANS, psychological functioning, and psychopathology, a comprehensive model of how these factors interact is still lacking. Studies that try to connect the heart and the brain networks via the vagus nerve can serve as a support for this understanding. The vagus nerve supports the communication between the heart and the brain, especially during emotional reactions, and its contribution has been known for a hundred years. In the 19th century, Bernard [5], who gave a significant

contribution to modern physiology, stated the concept of milieu intérieur (translated as the "internal environment"). He stated that "The fixity of the milieu supposes a perfection of the organism such that the external variations are at each instant compensated for and equilibrated. All of the vital mechanisms, however varied they may be, always have one goal, to maintain the uniformity of the conditions of life in the internal environment. The stability of the internal environment is the condition for the free and independent life" [5].

Bernard's conclusions were drawn by observing that the heart's affections rebound on the brain activity and vice versa through the vagus nerve. This is along with previous [6] and recent [7] considerations on the existence of specific multilevel control processes from the brain to the different organs, creating a bidirectional connection between the heart and the brain. Porges [8] introduced the Polyvagal Theory, explaining the role of the vagus nerve as a regulator of the internal viscera and as a mediator of the communication between the heart and the brain. This theory stresses the importance of autonomic functions in regulating human behavior in normal and abnormal conditions. The vagus nerve originates into two different nuclei of the brainstem: the Dorsal Motor Nucleus of the vagus (DNMX) and the Nucleus Ambiguus (NA), each of them ending in the sinoatrial node, but only NA having the control of respiratory sinus arrhythmia (RSA) which is an index of cardiac vagal modulation, and it is associated with emotion regulation (ER) [9].

The DNMX and NA act competitively on the sinoatrial node, adjusting the anabolic parasympathetic activity and the catabolic sympathetic one. In this way, these two branches of the vagus's independent action have different effects on RSA and HRV. The sympathetic nervous system (SNS) innervates the cardioaccelerating center of the heart, the lungs (increased ventilatory rhythm and dilatation of the bronchi), and the non-striated muscles (artery contraction), releasing adrenaline and noradrenaline. On the contrary, the parasympathetic nervous system (PNS), which uses the neurotransmitter acetylcholine (ACh), innervates the cardiomoderator center of the heart, the lungs (slower ventilatory rhythm and contraction of the bronchi), and the non-striated muscles (artery dilatation), reducing the experience of stress. These two systems act agonistically on the heart, respectively, through the stellate ganglion (a collection of sympathetic nerves) and the vagus nerve (a parasympathetic nerve). The interaction of these two branches on the sinoatrial node originates the cardiac variability, measured using electrocardiography by monitoring heart rate variability (HRV), which is a beat-to-beat variability [10]. HRV can be considered as an indicator of physiological stress or arousal. The frequency-domain analysis typically includes three measures: very low frequency ($\leq$0.04 Hz), low frequency (LF, 0.04–0.15 Hz), and high frequency (HF, 0.15–0.4 Hz). The HF component measures vagal activity, while the LF component is related to a combination of both vagal and sympathetic activities, and LF/HF ratio reflects the cardiac sympathovagal balance [11].

Porges [8] adopts a phylogenetic perspective and proposes that mammals, but not reptiles, have a brainstem organization characterized by a ventral vagal complex (including NA) that influences attention, emotion, motion, and communication. He also suggests, with his theory, explanations on how heart rate changes with novel environmental stimuli. Indeed, according to the Polyvagal Theory, there are three evolutionary phylogenetic stages behind the development of the vagus nerve. The dorsal vagal system (archaic, unmyelinated) is phylogenetically the oldest one, and it is associated with immobilization (death feigning, vasovagal syncope, and behavioral shutdown). The sympathetic vagal system is associated with the fight or flight response (active avoidance of the threat). Finally, the last step of this evolution recognizes the ventral vagal system (newest and myelinated), which is associated with social communication and behaviors (facial expression, vocalization, listening).

This model must be considered hierarchical. The last system, named the myelinated vagal system, is the first to be engaged in social and complex human experience. When this system fails in its functionality, the sympathetic vagal system is engaged by displaying the fight or flight response behavior. If this structure fails, the most ancient structure is engaged with the immobilization response [8,12]. In this pattern, psychopathology arises

when the latest structures fail in their functionality, implying a significant dysfunction in ER.

Beauchaine and Thayer [13] stress the validity of respiratory sinus arrhythmia (RSA) as a transdiagnostic biomarker of emotional dysregulation and concomitant psychopathology. Basing his assumption on the RDoC (Research Domain Criteria) project for the re-conceptualization of psychopathology [14–17], they underline the importance of psychophysiological measures as useful tools to use in order to address core psychopathological transdiagnostic mechanisms.

The vagus nerve originating in the DNMX is associated with reflexive regulation of visceral functions, while the vagus nerve originating in the NA is associated with active processes like attention, motion, emotion, and communication. According to Porges [18], mammalians reach the homeostatic balance through bidirectional communications between the peripheral organs and the brain. This entire process is supported by neuroception, a term coined by Porges to describe the mechanism by which our brain can detect dangerous environmental stimuli by analyzing the information coming from our senses through body scanning. When our brain detects (consciously or unconsciously) a threat, disruption of homeostasis happens. Neuroception determines the connection between environmental aspects and specific physiological states that support either fight-flight or social engagement behaviors. The detection of the possible threat activates our organism, leading it to a stress state, which is to say in a disorganization of the autonomic system's rhythmic structure and, consequently, behavioral one if an invalid neuroception of safety and danger occurs. If there is no coherence between the detection of the risk and the visceral response to risk, a dysfunctional, maladaptive physiological reactivity may happen in the long term" [18].

We can estimate the stress degree-level of an organism by measuring RSA, estimated by heart rate variability (HRV) that registers increased heart rate during inspiration and a decrease during expiration. The Polyvagal Theory [19] also provides a plausible explanation for the correlation between atypical autonomic regulation (e.g., reduced vagal influence on the heart) and psychiatric and behavioral disorders, outlining a complex framework of human thinking and behavior.

In a similar vein, Thayer and Lane [20,21] introduced the neurovisceral integrated model of the heart-brain activity, in which prefrontal and limbic structures control HRV. They describe a model in which HRV is both related to attentional and affect regulation. With this model, they deepened the understanding of the central autonomic network (CAN), already pointed out by Benarroch [22], and concluded that it is an integrated component of a complex regulation system by which the brain controls visceromotor, neuroendocrine, and behavioral responses, which are essential for goal-directed behavior and human adaptability too. The CAN includes several regions of the central nervous system (CNS) such as the anterior cingulated, insular, orbitofrontal, and ventromedial prefrontal cortices together with the central nucleus of the amygdala (CeA), the paraventricular and related nuclei of the hypothalamus, the periaqueductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract (NTS), the nucleus ambiguus (NA), the ventrolateral and ventromedial medulla, and the medullary tegmented field. All of these regions are reciprocally interconnected so that information can flow bidirectionally between lower and higher brain levels.

It is evident that all these regions intervene in modulating human behavior by connecting executive functions (prefrontal cortices), physiological reactions, and the autonomic response through the NA and the vagus nerve activities that regulate the sinoatrial node [8]. Thayer et al. [23] pointed out a link between stress, HRV, and cognitive deficits, hypothesizing that HRV indexes important aspects of prefrontal neural function. These assumptions come upon neuroimaging evidence that asserts that the primary output of the CAN is mediated through preganglionic sympathetic and parasympathetic neurons, which innervate the heart via the stellate ganglia and the vagus nerve, respectively. After all, there is increasing evidence in the literature that high HRV correlates with better neuropsychological performances (especially working memory, attentional set-shifting,

and response inhibition), despite low HRV that correlates with worse neuropsychological performances [23–28] In their review, Thayer and Lane [21] also indicate low vagally mediated HRV as an endophenotype for a range of physical and psychological disorders, including psychopathology.

Our paper reviews the scientific works published on the relation between HRV and emotions, HRV and psychopathology and HRV and neuropsychological functions. We aim to define the state-of-art on this topic to delineate new research projects in the field even if no such comprehensive work exists to the very best of our knowledge.

We hypothesize that HRV is a variable that influences different dimensions such as executive functions, ER, and psychopathology, and we decided to deepen these three variables from this hypothesis because they represent essential elements in influencing psychological health and different psychotherapeutic constructs (See Figure 1).

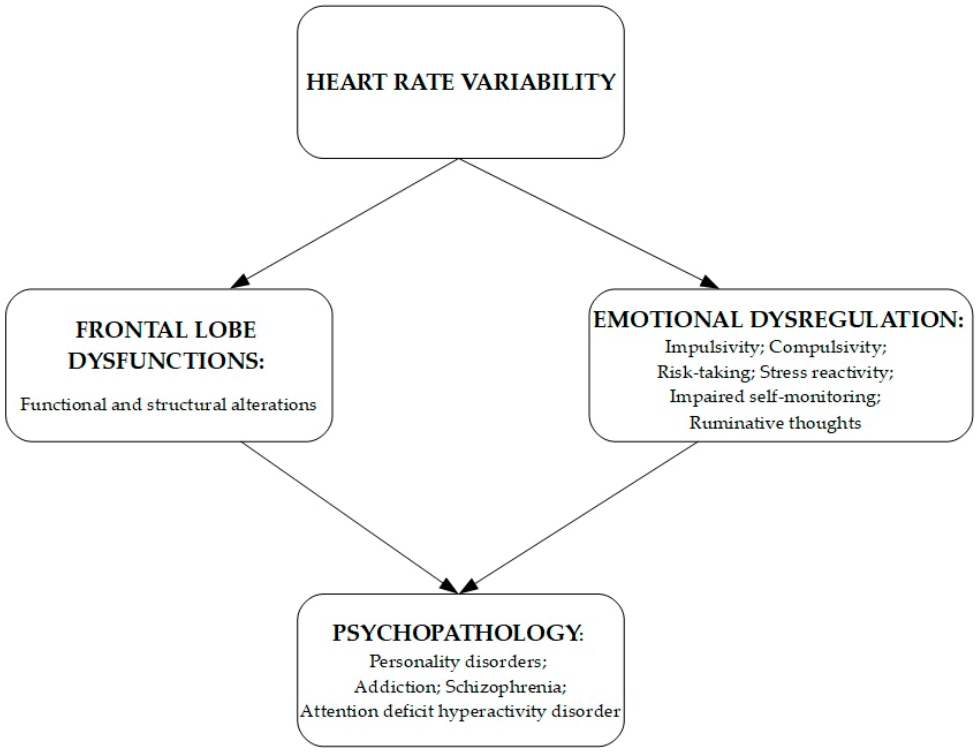

Figure 1. HRV is a variable that in influencing different dimensions such as Frotal Lobe Functions and Emotion Dysregulation contributes to the development of psychopathology.

Therefore, this review intends to evaluate and emphasize the relationship between HRV emotion regulation, executive functions, and psychopathology and how these can be conceptualized according to the Polyvagal theory.

2. Emotional Dysegulation and Heart Rate Variability

Evidence shed light on the link between HRV and emotional responses [8,12,19,29–31]. Participants with higher baseline HRV exhibit appropriate emotional responses during fear-potentiated startle responses and phasic heart rate responses [27,32,33]. By contrast, participants with low baseline HRV are slower in recovering from psychological stressors of cardiovascular and immune responses than controls. This evidence confirms that HRV is an index of self-regulation and consequent subjective well-being [34]. Porges [29] has been one of the first to underline the link between high HRV with adaptive emotional regulation (ER) and coping strategies and between low HRV with emotional dysregulation visible in behaviors characterized by anxiety and rigid attentional threat processing. Emotion regulation ability is associated with greater baseline HRV [21,30] and task-related HRV during successful ER [35,36].

Notably, phasic increases in HRV in response to emotion-inducing situations are associated with better and effective ER [37]. One study showed that the use of reappraisal or suppression strategies during successful ER is associated with increased HRV [38]. From a neural point of view, an increase in HRV during successful emotion regulation is associated with cerebral blood flow changes in areas relevant for ER and inhibitory processes [39].

Still, Thayer and Lane [20] consider cardiac vagal tone indexed by HRV to measure both the integrity and functionality of neural networks involved in emotion-cognition interactions. High HRV portrays the integrity and the healthy functions of these neural networks compared to low HRV, representing a disintegration of their functionality. According to McCraty and Shaffer [40], there is a link between higher levels of resting vagally-mediated HRV and the performance of executive functions such as attentional and emotional processing by the prefrontal cortex. Moreover, the authors report that HRV can be seen as an index of resiliency and flexibility because it indicates the ability to self-regulate and adapt to challenging situations and threatening events.

Appelhans and Luecken [30] claim that HRV is an index of emotionality and considered pain as a homeostatic emotion with an inverse association between LF and pain sensitivity (Appelhans and Luecken) [41]. According to the authors, pain is a homeostatic emotion that is influenced by the affective system in its different parts and global characteristics, supporting, with this perspective, the model of neurovisceral integration [20,27], which sees the CAN as responsible for some aspects of homeostatic regulation. According to Park and Thayer [31], HRV associates with top-down and bottom-up cognitive processes of emotional stimuli, and higher resting HRV is linked with more functional and efficient top-down and bottom-up cognitive processing of emotional stimuli and a consequent more efficient ER. In contrast, lower resting HRV is linked with hypervigilant and maladaptive cognitive responses to emotional stimuli, representing an obstacle to adaptive ER. They also suggest that maladaptive cognitive processes of emotional stimuli (observed in people with low HRV) may contribute to health issues observed in a wide range of people with low HRV.

The Model of Neurovisceral Integration suggests that vagally mediated heart rate variability (vmHRV) represents a psychophysiological index of inhibitory control and is associated with emotion regulation capacity. A study from Visted et al. [42] explored the correlation between ER abilities assessed using the Difficulties in Emotion Regulation Scale (DERS) [43]. They found that difficulties in ER negatively correlated with resting vmHRV, with specific troubles linked to the inability to behave following personal goals. This evidence confirms what was previously found by Williams et al. [44] that using the DERS scale reported a significant negative association between resting vmHRV and difficulties in ER, with problems linked to anxiety and ruminative tendencies.

The link between HRV and cognitive functions does not confine only to top-down inhibitory processes but extends to other cognitive domains.

In an interesting work, Xiu et al. [45] connected working memory, HRV, and ER, suggesting that working memory training could improve ER abilities. Specifically, they found that high frequency-heart rate variability (HF-HRV) increased after 20 days of working memory training in the ER condition, meaning that working memory training can influence ER. Even though there is a need for more studies to confirm these results, they represent a relevant indication of the correlation between cognition-emotion and evidence that higher resting HRV is mainly associated with flexible and adaptive top-down and bottom-up cognitive processing. These adaptive cognitive skills contribute to effective ER. In contrast, lower resting HRV seems to associate with hypervigilant and maladaptive bottom-up and top-down cognitive responses to emotional stimuli, making this cognitive deficiency deleterious for ER and confirming what was previously said. For this reason, we can claim that maladaptive cognitive processing of emotional stimuli observed in people with lower HRV may be disadvantageous for emotional and physical health, and this could explain why low HRV occurs in people within a wide range of psychopathologies.

Beauchaine and Thayer [13] state that emotional dysregulation is related to poor executive control over behavior because of the structural and functional connections between the prefrontal cortex and the parasympathetic nervous system via the vagus nerve (complex interactions between cortical and subcortical pathways, including amygdala circuits). In the regulation and modulation of negative emotional stimuli, such as anxiety which create many cognitive, somatic, and behavioral responses, among the limbic structures, the amygdala appears to have an essential role in regulating emotions. Anxiety-related responses are modulated by GABAergic modulation, and neurosteroids seem to modify, interacting with GABA, neuronal excitability. This evidence makes neurosteroids representing a core for developing new anxiolytic drugs [46] Furthermore, anxiolytic drugs seem to have good effects on amygdala functionality that link with anxiety disorders when dysregulated [47]. Less self-reported anxiety seems to be associated with diminished activity in areas connected with negative emotions and increased activity in regions linked to regulatory processes after administering allopregnanolone, a progesterone-derived neurosteroid known for its anxiolytic properties [48].

Given the importance of HRV on a whole series of parameters and variables, it has been shown how antiarrhythmic agents alter HRV with a direct effect on the ANS and myocardial contractility. Different comparative analyses showed that Amiodarone, despite interacting with the ANS centrally [49] and peripherally [50,51] did not affect HRV. On the other hand, Flecainide and Propafenone have vagolytic [52] and beta-blocking [53] properties, which could further modulate sympathetic and parasympathetic activity in the heart [54,55] decreasing all the parameters of HRV in the time domain and the frequency domain including the markers of vagal activity [56]. On the contrary, Oymatrine, compared to Propafenone, increases HRV [57]. Although this may prove to be an essential field of study, caution in using these drugs to manage HRV is imperative. It is well known how much some potent antiarrhythmic drugs may increase the incidence of sudden death, as observed by different studies [58].

3. The Importance of Emotional Regulation and Heart Rate Variability in General Psychopathology

As seen above, high HRV is associated with a successful adaptation [12,19,23,31]. Adapting to different environmental stressors determines good individual functionality, but subjects with varying degrees of psychopathology seem to lack this capacity. As said before, since ER is an essential skill for psychological health and it represents one's ongoing adjustment to continuous environmental stimuli and changes [59], an adequate emotional ability is crucial for general health since it facilitates the selection of optimal responses by inhibiting and rejecting dysfunctional options [21]. By contrast, links between low HRV and psychopathology are emerging. A meta-analysis by Zahn et al. [60] supports the notion of a relationship between low HRV and worse self-control in inhibiting or diverting dominant impulses related to dysfunctional thoughts, behaviors, and emotions [61].

Regarding anxiety disorders, low HRV can be considered an endophenotype of panic disorder [62,63]. A study from Zhang et al. [64] explored LF/HF in patients with panic disorders, knowing that notably, LF/HF ratio seems associated with sympathetic modulation. Patients with PD exhibited an impairment in sympathovagal modulation compared to healthy controls, corroborating the idea that an autonomic imbalance in patients with PD is the consequence of mental stress, which causes this autonomic imbalance. HRV is heritable [65–67] and is state-independent. Namely, it also occurs in the absence of panic symptoms [68,69], co-aggregates within family members [62,63], and is lower in children of patients with panic disorder than in children of healthy controls [70]. All of these observations confirm the fact that low HRV is an endophenotype for panic disorder.

An interesting association also exists between reduced HRV and epilepsy [71]. The autonomic imbalance in patients with epilepsy can also represent a risk for cardiovascular disease, and this means that HRV can be used as a guide to prevent and assess patients with risk for cardiovascular disease. Some recent evidence shows correlations between low HRV and schizophrenia [72,73]. A literature review from Guccione et al. [74] highlights

that several studies had demonstrated a sympathovagal imbalance in individuals diagnosed with schizophrenia. A study from Castro and colleagues [75] demonstrated that schizophrenic patients showed difficulties in recovering HRV.

Meyer et al. [76] showed that individuals diagnosed with borderline personality disorder (BPD) and with post traumatic stress disorder (PTSD) exhibit lower root mean square of the successive differences (RMSSD) compared to healthy individuals. RMSSD is another index used to measure HRV. Impairment in RMSSD may have a connection with early maladaptive experiences and traumatic events. Interestingly, Dixon-Gordon et al. [77] proved that individuals with BPD after using acceptance strategy exhibited high HRV, indicating that such populations can benefit from ER training.

The inability to regulate emotions and behaviors is also typical of attention deficit hyperactivity disorder (ADHD). Rukmani et al. [78] screened out 270 gender AHDH children (7–12 years) for their investigation and selected 10 children without psychiatric and neurological comorbidities. They found that ADHD children presented a sympathovagal imbalance, characterized by a reduction in overall HRV with a sympathetic predominance.

Neuroticism, a personality trait characterized by negative affect, higher anxiety, and major reactivity to external stress, seems to predispose individuals for psychopathology such as schizophrenia [79] and to link with more difficulties in regulating negative emotions. In this regard, according to Di Simplicio et al. [80], during a negative emotion challenge, individuals with high neuroticism traits reported reduced high-frequency HRV (HF-HRV). Since this index represents the parasympathetic part of the systems showing the flexibility of the vagal tone, these results show that people with high negative affectivity (anxiety, depression) and high reactivity have difficulties modulating their physiological response.

A recent meta-analysis from Koch et al. [81] showed that individuals diagnosed with major depression (MD) exhibited a significant reduction in HF-HRV, LF-HRV, LF-HF ration, RMSSD, and, in general, in all HRV measures compared to controls. Although it is widely recognized that a high value in HRV is related to psychophysical well-being, some authors have also found an elevation of HRV in anorexic patients. In this regard, it may be important to evaluate the hypothesis that there may be an ideal HRV range. In fact, a recent meta-analysis evaluated this hypothesis and discovered a distinct U-shaped pattern, with healthy controls clustered towards the center, individuals with anorexia nervosa experienced increased HRV, and all other disorders were associated with lower HRV parameters. This metanalysis has the advantage of opening a crucial and original question, so it would be helpful to verify the assumption experimentally despite some limitations. [82]. In general, all these results strengthen the idea that HRV can be considered a transdiagnostic index for stress, consequent cardiovascular diseases, and generally worse health outcomes.

4. Heart Rate Variability and Neuropsychological Functions

Thayer et al. [23] pointed out the existence of a relationship between HRV and prefrontal neural functions [39,83–85]. Some findings suggest that cortical activity tonically inhibits brainstem cardioacceleratory circuits and corroborates an association between HRV and the medial prefrontal cortex activity. Lane et al. [39] studied the correlation between a spectrally derived index of vagally mediated HRV, the high frequency-HRV (HF-HRV), and cerebral blood flow data obtained by PET. In this study, HF-HRV correlates with blood flow in the right superior prefrontal cortex (BA 8, 9), with the left rostral anterior cingulate cortex (BA 24, 32), with the right dorsolateral prefrontal cortex (BA 46), and the right parietal cortex (BA40). At the same time, emotional arousal shows an association with a decrease in HRV and a concomitant decrease in the activity in the same regions, indicating that high HRV correlates with prefrontal activation both during emotional and neutral situations.

In contrast, low HRV correlates with lower cerebral activity in the same region only during emotional arousal. These outcomes confirm that HF-HRV links to better cognitive performances in threat and non-threat conditions, while LF-HRV correlates to improved cognitive performances only in threat conditions. In line with these results, we can deduce

that low HRV seems linked only with emotional arousal and worse prefrontal performances than high HRV. These findings agree with assumptions from Ter Horst [86] about a general inhibitory role of the prefrontal cortex on heart activity via the vagus nerve. All of these results give strong evidence of the critical role of the prefrontal cortex in the modulation of subcortical cardioacceleratory circuits via an inhibitory pathway associated with vagal function. These findings make consistent the assumption that HRV can be an index of neuropsychological functions, such as attentional, set-shifting, and planning abilities. Besides, further studies suggest that the right prefrontal cortex is preferentially related to inhibitory processes across the different cognitive, motor, and affective tasks [87–90]. Therefore, we can deduce that the right hemisphere may be preferably involved in inhibitory processes, useful for cognitive, affective, and physiological regulation [23,27]. Such alterations in the prefrontal cortex have several implications for psychopathology. Dysfunction in the prefrontal cortex (also named prefrontal dysfunction, or prefrontal executive dysfunction) is characterized by functional (blood perfusion) or structural (grey and white matter) alterations. It has been clinically proven to result in impulsivity, compulsivity, risk taking, impaired self-monitoring, difficulty in disengaging from ruminative thoughts, enhanced stress reactivity and lack of top-down regulation of emotional responses [91]. According to a recent perspective, prefrontal dysfunction characterizes addictions, depression, schizophrenia, and personality disorders [92].

Moreover, inhibitory processes are the core dimension of several neuropsychological functions involving working memory, such as active short-term storage, online processing, and manipulation of information [93]. This working memory is indicated, by broad literature, as the nucleus of prefrontal functioning, namely attentional processes [94–96]. The hypothesis that HRV has reliability in indexing prefrontal activity [23] comes from the study of Hansen et al. [24], where they measured HF-HRV and LF-HRV of the military personnel while performing attentional and memory tasks and non-executive tasks (simple reaction time and response latencies to specific stimuli). The study's outcome showed better cognitive performances in the HF-HRV group than the LF-HRV group (faster reaction times and fewer false-positive responses). More in detail, the HF-HRV group performed better both in executive and non-executive tasks, while the LF-HRV group performed worse only in the executive tasks but not in the non-executive ones. Hence, it seems that HRV is connected only with executive tasks and does not differentiate non-executive performances. A second scientific work from the same author replicated these results [26], and in this study, they looked at the correlation between HRV and cognitive functions in threat and non-threat conditions, analyzing 65 male sailors (mean age 23.1) from the Royal Norwegian Naval Academy. While recording participants' HRV, they displayed them a computerized version of two cognitive tests: the Continuous Performance Test (CPT) [97] in its California Computerized Assessment Package abbreviated version (CalCAP) used for assessing sustained attention, and four sub-tests such as Simple Reaction time Task (SRT), Choice Reaction Time Task (SRT), Serial Pattern Matching 1 (SPM 1), and a Serial Pattern Matching 2 (SPM 2); and a modified version of the Working Memory Task (WMT) from Hugdahl et al. [98] with a task which was an n-back task (2-back task). The sample was divided into threat and non-threat subsamples, and it was administered an electrical shock (unpleasant but not painful) by a pulsating (18Hz) adjustable DC shock generator in the second group but not in the first one.

The results found out that there are individual differences in autonomic, cognitive, and behavioral aspects of emotional regulation both in ordinary and challenging contexts. In fact, in scenarios requiring vigilance, high HRV subjects demonstrated a better capacity to hold prolonged focused attention than low HRV subjects. Moreover, subjects with low HRV appeared more sensitive to environmental changes than high HRV ones. Furthermore, high HRV subjects showed superior cognitive performance in threat and non-threat conditions, while low HRV subjects showed bad performances during non-threat conditions and improved performance in threat conditions. These outcomes seem to delineate that poor neuropsychological performances can be found in subjects with low HRV at rest. These

data confirm previous hypotheses from Frankenhaeuser et al. [99] and Broadbent [100] about individual differences in cognitive performances due to specific physiological patterns and specific environmental stimuli. Frankenhauser et al. [99] prior observed that subjects with high HR and low HR performed better respectively in a contest of understimulation and overstimulation. Stenfors et al. [101] measured executive functions and cardiovascular parameters, analyzing HRV indices in 119 healthy working adults (79% female) and focusing on Standard Deviation of NN (SDNN), Root of the Mean Squares of Successive Differences (RMSSD), High Frequency (HF) power band from spectral analysis, and QT Variability Index (QTVI). They also included specific adjustments for demographic variables such as age. The outcomes show that age seems responsible for the confusion in the correlation between HRV and executive functions and explains the association between executive measures with SDNN and RMSSD parameters. However, there is an index called QTVI that the age variable does not invalidate. This last parameter proves a clear correlation with prefrontal performances: indeed, while low QTVI registers better prefrontal performances, high QTVI correlates with worse prefrontal performances, specifically for inhibition, shifting, updating, and speed capacities.

In contrast, no correlations were observed between any cardiovascular parameter and working memory task performances. These data increase our understanding of how external variables (e.g., age, education degree, level of physical activity) can affect the correlation between HRV parameters and performances in executive functions, suggesting that, because of its independence from age, QTVI is an index better should be used. Because of that, future studies are needed to deepen the clarification of external variables' potential interference in the correlations between heart indices and brain performances.

Moving on, Gathright et al. [102] investigated executive functions' hypothetical role in mediating depressive symptoms, measured by BDI-II, the Beck's Depression Inventory [103], and resting HRV in heart failure patients. Analyzing 109 patients with HF (Heart Failure), the authors found an association between higher BDI-II scores and lower resting HF-HRV among participants with poorer executive functions. This evidence can suggest two interpretative hypotheses: in HF-HRV patients, there are similar structural brain changes responsible for lower executive functions, increased depression, and poorer autonomic functioning, whereas individuals with good executive functions keep a healthy lifestyle that does not allow depression to impact negatively on the autonomic function. Evidence of the link between HRV and prefrontal functions comes from the study of executive functions in different disorders. Recent studies point toward the direction of structural [104] and functional [105] abnormalities in psychopathy when performing executive tasks [106] Morgan and Lilienfeld [104], in their meta-analytic review of thirty-nine studies about the relationship between psychopathy and executive functions, found that antisocial groups performed 0.62 standard deviations worse on executive function tests in comparison to control groups. Gorenstein [105] has found several neuropsychological tests measuring prefrontal functions (namely Wisconsin Card Sorting Task, Sequential Matching Memory Task, and Necker Cube Task), psychopaths exhibit the same patterns of frontal lesion patients. Based on such assumption, he concluded that psychopathy relies on deficits associated with the frontal lobe dysfunction, but in contrast, Hare argued that Gorestein's conclusions are undermined by inhomogeneous samples regarding age, education, IQ, and substance use [90]. He replicated Gorestein's experiment with forty-six convicts and carried out a series of variance and covariance analyses using exact age, education, IQ, and substance use as covariates, not finding any group differences in task performance.

Still, Hansen et al. [107] attempted to study the link among HRV, psychopathy dimensions, and neuropsychological function, using Hare's four-facet model, the continuous performance test (CPT), and a working memory test to study the relationship between all these variables.

Before describing the evidence determined from this study, it is needed a description of Hare's four-facet model, which provides a representation of psychopathy in four facets: interpersonal style (the tendency to manipulate other subjects, to act pathological lying,

and to expose a grandiose sense of self-worth), affective style (characterized by lack of empathy and remorse or guilt), impulsive lifestyle (typified by sensation-seeking and irresponsibility), antisocial behavior (defined by the use of violence). They examined 33 male prisoners and found that the interpersonal style facet showed a positive relationship with HRV during baseline. The interpersonal style facet showed the most substantial influence on HRV during the test conditions and exhibited better performance than those with low scores on cognitive tasks involving executive function. This evidence suggests that psychopathy might have different underlying physiological and cognitive mechanisms and that HRV seems associated with specific psychopathy facets and specific cognitive mechanisms. Moreover, they seem to give evidence of an association among low HRV, worse cognitive prefrontal performances, and several psychopathological dimensions but primarily for the inhibitory one. The autonomic imbalance, considered an index of disinhibition of sympathoexcitatory neural circuits usually under tonic inhibitory control via the prefrontal cortex, could be the final common pathway linking psychosomatics psychopathology [27].

In conclusion, at this stage of the debate, it is clear that there is a correlation between heart activity and prefrontal brain activity. Actual scientific data suggest that the prefrontal cerebral regions may play a part in influencing heart activity, especially for their inhibitory role mediated by the vagus nerve activity on the sinoatrial node. According to the above evidence, research needs further studies are required to clearly understand which heart activity parameters are strictly connected with specific prefrontal cerebral tasks.

5. Conclusions

HRV has traditionally been treated as a simple, one-way, dependent variable to be observed to assess the influence of heart rate on global sympathetic and parasympathetic regulatory systems [18]. However, the perspective that emerges from this research shifts our attention to a complex system that incorporates and influences complex neurophysiological mechanisms, adaptive functions, and above all, it is a bidirectional system between central elements and peripheral/autonomic elements.

In line with this, there is evidence in the literature of a strict relation between HRV, executive functions, and emotional dysregulation. In particular, there is evidence of a solid correlation between high resting HRV and better cognitive functions, especially executive functions [23,39,83–85]. Subjects with high rest HRV can dispose of a better skill in adaptation to environmental stressors and better cognitive responses to emotional stimuli. This means being able to process and react in a functional way to emotional stimuli and distress.

Conversely, people with low HRV show worse activation in the prefrontal cortex, the rostral cingulate cortex, and the parietal cortex [39], and worse ability to dominate mental and behavioral impulses [48]. Furthermore, there is evidence of a correlation between low HRV and dysfunctional ER [29,39,42,108].

Being that emotional dysregulation the basis of many psychopathological dimensions, we can state that HRV is linked to psychopathology (See Figure 2).

Evidence of correlation between low HRV and anxiety [63], panic disorder [64], epilepsy [71], schizophrenia [72,73], personality disorders [76], and ADHD [78] are given.

There are also suggestions that low HRV could be an endophenotype of specific psychopathologies (e.g., panic disorder), providing data from neurophysiological-cardiovascular level to interpret and test hypotheses relating to psychological processes such as ER and cognitive performances.

In fact, as conceptualized by the Polyvagal Theory, physiological states may influence a wide range of social behaviors emitted, such as the ability to regulate emotional expressions and neural regulation of the social engagement system. This framework explains how HRV may be a marker of specific central pathways activation coming from cortical and subcortical areas (involving the temporal cortex, the central nucleus of the amygdala, and

the periaqueductal gray) involving the regulation of both the vagal component and the somatomotor component of the social engagement system.

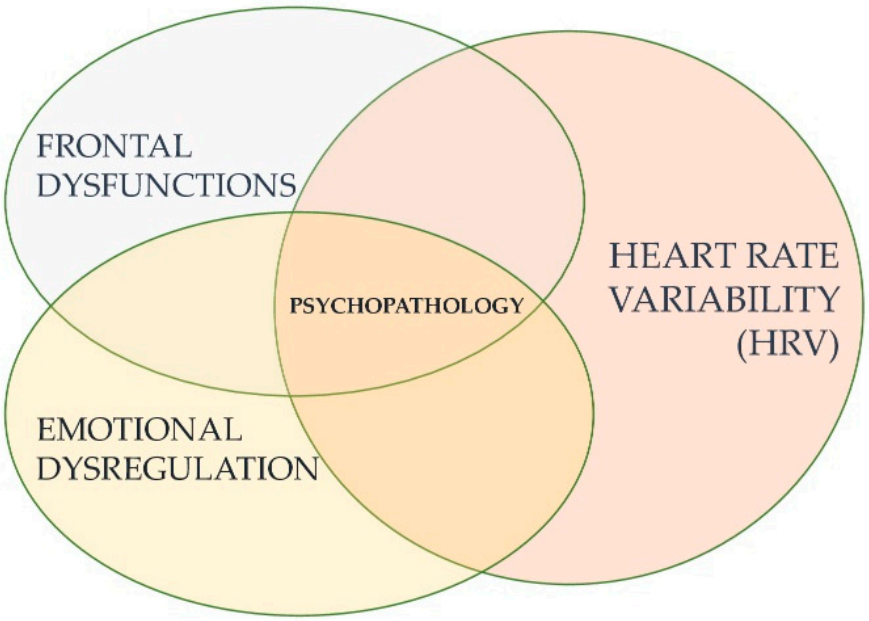

Figure 2. The relation between low HRV, executive functions, and emotional dysregulation is linked to psychopathology.

This perspective may drive research towards new specific hypotheses or neural mechanisms and mediators, opening fundamental questions about the adaptive characteristics of specific psychophysiological responses. More studies are needed to confirm this hypothesis and determine if low HRV can be considered a predictor of psychopathology mediated through cognitive dysfunctions. It is explicit that a relation between heart and brain exists and good heart functionality with mental, emotional, and physical health. Future research is needed to deepen which HRV parameters are linked with specific neuropsychological functions that may undergo bad cognitive performances, ER, and consequent psychopathological dimensions.

This evidence would provide the possibility to integrate the assessment of psychophysiology into the comprehension of psychopathological features and cognitive issues, providing future directions for improvement in research and on the assessment in clinical practice. In fact, given the role of the ANS flexibility and adaptability, future research may account for autonomic problems, even in psychopathological conditions considered at-risk in order to improve the evaluation of situations acknowledged prodromic for worse outcomes.

Author Contributions: Conceptualization, L.A.C., L.B. and H.D.; methodology, L.A.C., A.C.F. and H.D.; writing original draft preparation, L.A.C., A.C.F., A.G. and H.D., writing—review and editing, A.C.F., A.G., V.C. and H.D.; visualization, A.C.F. and H.D.; supervision, L.B. and V.C.; project administration, H.D. All authors have read and agreed to the published version of the manuscript.

Funding: This manuscript did not benefit from any specific funds.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: This study did not report any data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Richter, M.; Wright, R.A. Autonomic Nervous System (ANS). In *Encyclopedia of Behavioral Medicine*; Gellman, M.D., Turner, J.R., Eds.; Springer: New York, NY, USA, 2013. [CrossRef]
2. Gibbons, C.H. Basics of autonomic nervous system function. *Handb. Clin. Neurol.* **2019**, *160*, 407–418. [PubMed]
3. Brook, R.D.; Julius, S. Autonomic imbalance, hypertension, and cardiovascular risk. *Am. J. Hypertens.* **2000**, *13*, 112S–122S. [CrossRef]
4. Thayer, J.F.; Friedman, B.H. *A Neurovisceral Integration Model of Health Disparities in Aging*; National Academies Press: Washington, DC, USA, 2004; pp. 567–603.
5. Bernard, C. *Lectures on the Phenomena of Life Common to Animals and Plants*; Hoff, H.E.; Guillemin, R.; Guillemin, L., Translators; Charles C Thomas: Springfield, IL, USA, 1974.
6. Darwin, C. *The Expression of Emotions in Man and Animals*; Harper Collins: London, UK, 1999; pp. 71–72.
7. Noble, D. Claude Bernard, the first systems biologist, and the future of physiology. *Exp. Physiol.* **2008**, *93*, 16–26. [CrossRef] [PubMed]
8. Porges, S.W. Orienting in a defensive world: Mammalian modifications of our evolutionary heritage. A polyvagal theory. *Psychophysiology* **1995**, *32*, 301–318. [CrossRef]
9. Tonhaizerova, I.; Mestanik, M.; Mestanikova, A.; Jurko, A. Respiratory sinus arrhythmia as a non-invasive index of 'brain-heart' interaction in stress. *Indian J. Med. Res.* **2016**, *144*, 815–822. [CrossRef]
10. Servant, D.; Logier, R.; Mouster, Y.; Goudemand, M. La variabilité de la fréquence cardiaque. Intérêts en psychiatrie [Heart rate variability. Applications in psychiatry]. *L'Encéph. Rev. Psychiatr. Clin. Biol. Thér.* **2009**, *35*, 423–428. [CrossRef]
11. Shaffer, F.; Ginsberg, J.P. An overview of heart rate variability metrics and norms. *Front. Public Health* **2017**, *5*, 258. [CrossRef]
12. Porges, S.W.; Furman, S.A. The early development of the autonomic nervous system provides a neural platform for social behaviour: A polyvagal perspective. *Infant Child Dev.* **2011**, *20*, 106–118. [CrossRef]
13. Beauchaine, T.P.; Thayer, J.F. Heart rate variability as a transdiagnostic biomarker of psychopathology. *Int. J. Psychophysiol.* **2015**, *98*, 338–350. [CrossRef]
14. Insel, T.; Cuthbert, B.; Garvey, M.; Heinssen, R.; Pine, D.S.; Quinn, K.; Sanislow, C.; Wang, P. Research domain criteria (RDoC): Toward a new classification framework for research on mental disorders. *Am. J. Psychiatry* **2010**, *167*, 748–751. [CrossRef]
15. Sanislow, C.A.; Pine, D.S.; Quinn, K.J.; Kozak, M.J.; Garvey, M.A.; Heinssen, R.K.; Wang, P.S.-E.; Cuthbert, B.N. Developing constructs for psychopathology research: Research domain criteria. *J. Abnorm. Psychol.* **2010**, *119*, 631–639. [CrossRef] [PubMed]
16. Cuthbert, B.N.; Kozak, M.J. Constructing constructs for psychopathology: The NIMH research domain criteria. *J. Abnorm. Psychol.* **2013**, *122*, 928–937. [CrossRef]
17. Shankman, S.A.; Gorka, S.M. Psychopathology research in the RDoC era: Unanswered questions and the importance of the psychophysiological unit of analysis. *Int. J. Psychophysiol.* **2015**, *98*, 330–337. [CrossRef]
18. Porges, S.W. The polyvagal perspective. *Biol. Psychol.* **2007**, *74*, 116–143. [CrossRef]
19. Porges, S.W. The polyvagal theory: New insights into adaptive reactions of the autonomic nervous system. *Clevel. Clin. J. Med.* **2009**, *76* (Suppl. 2), S86–S90. [CrossRef]
20. Thayer, J.F.; Lane, R.D. A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* **2000**, *61*, 201–216. [CrossRef]
21. Thayer, J.F.; Lane, R.D. Claude Bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* **2009**, *33*, 81–88. [CrossRef]
22. Benarroch, E.E. The central autonomic network: Functional organization, dysfunction, and perspective. In *Mayo Clinic Proceedings*; Elsevier: Amsterdam, The Netherlands, 1993; Volume 68, pp. 988–1001. [CrossRef]
23. Thayer, J.F.; Hansen, A.L.; Saus-Rose, E.; Johnsen, B.H. Heart rate variability, prefrontal neural function, and cognitive performance: The neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann. Behav. Med.* **2009**, *37*, 141–153. [CrossRef]
24. Hansen, A.L.; Johnsen, B.H.; Thayer, J.F. Vagal influence on working memory and attention. *Int. J. Psychophysiol.* **2003**, *48*, 263–274. [CrossRef]
25. Luft, C.D.B.; Takase, E.; Darby, D. Heart rate variability and cognitive function: Effects of physical effort. *Biol. Psychol.* **2009**, *82*, 186–191. [CrossRef]
26. Hansen, A.L.; Johnsen, B.H.; Thayer, J.F. Relationship between heart rate variability and cognitive function during threat of shock. *Anxiety Stress Coping* **2009**, *22*, 77–89. [CrossRef] [PubMed]
27. Thayer, J.F.; Brosschot, J.F. Psychosomatics and psychopathology: Looking up and down from the brain. *Psychoneuroendocrinology* **2005**, *30*, 1050–1058. [CrossRef] [PubMed]
28. Luque-Casado, A.; Perales, J.C.; Cárdenas, D.; Sanabria, D. Heart rate variability and cognitive processing: The autonomic response to task demands. *Biol. Psychol.* **2016**, *113*, 83–90. [CrossRef]
29. Porges, S.W. Autonomic regulation and attention. In *Attention and Information Processing in Infants and Adults: Perspectives from Human and Animal Research*; Campbell, B.A., Hayne, H., Richardson, R., Eds.; Lawrence Erlbaum Associates, Inc.: Mahwah, NJ, USA, 1992; pp. 201–223.
30. Appelhans, B.M.; Luecken, L.J. Heart rate variability as an index of regulated emotional responding. *Rev. Gen. Psychol.* **2006**, *10*, 229–240. [CrossRef]

31. Park, G.; Thayer, J.F. From the heart to the mind: Cardiac vagal tone modulates top-down and bottom-up visual perception and attention to emotional stimuli. *Front. Psychol.* **2014**, *5*, 278. [CrossRef]
32. Melzig, C.A.; Weike, A.I.; Hamm, A.O.; Thayer, J.F. Individual differences in fear-potentiated startle as a function of resting heart rate variability: Implications for panic disorder. *Int. J. Psychophysiol.* **2009**, *71*, 109–117. [CrossRef]
33. Ruiz-Padial, E.; Sollers, J.J., III; Vila, J.; Thayer, J.F. The rhythm of the heart in the blink of an eye: Emotion-modulated startle magnitude covaries with heart rate variability. *Psychophysiology* **2003**, *40*, 306–313. [CrossRef]
34. Geisler, F.C.; Vennewald, N.; Kubiak, T.; Weber, H. The impact of heart rate variability on subjective well-being is mediated by emotion regulation. *Personal. Individ. Differ.* **2010**, *49*, 723–728. [CrossRef]
35. Ingjaldsson, J.T.; Laberg, J.C.; Thayer, J.F. Reduced heart rate variability in chronic alcohol abuse: Relationship with negative mood, chronic thought suppression, and compulsive drinking. *Biol. Psychiatry* **2003**, *54*, 1427–1436. [CrossRef]
36. Smith, T.W.; Cribbet, M.R.; Nealey-Moore, J.B.; Uchino, B.N.; Williams, P.G.; MacKenzie, J.; Thayer, J.F. Matters of the variable heart: Respiratory sinus arrhythmia response to marital interaction and associations with marital quality. *J. Personal. Soc. Psychol.* **2011**, *100*, 103–119. [CrossRef]
37. Thayer, J.F.; Yamamoto, S.S.; Brosschot, J.F. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int. J. Cardiol.* **2010**, *141*, 122–131. [CrossRef] [PubMed]
38. Butler, E.A.; Gross, J.J.; Barnard, K. Testing the effects of suppression and reappraisal on emotional concordance using a multivariate multilevel model. *Biol. Psychol.* **2014**, *98*, 6–18. [CrossRef]
39. Lane, R.D.; McRae, K.; Reiman, E.M.; Chen, K.; Ahern, G.L.; Thayer, J.F. Neural correlates of heart rate variability during emotion. *Neuroimage* **2009**, *44*, 213–222. [CrossRef]
40. McCraty, R.; Shaffer, F. Heart rate variability: New perspectives on physiological mechanisms, assessment of self-regulatory capacity, and health risk. *Glob. Adv. Health Med.* **2015**, *4*, 46–61. [CrossRef]
41. Appelhans, B.M.; Luecken, L.J. Heart rate variability and pain: Associations of two interrelated homeostatic processes. *Biol. Psychol.* **2008**, *77*, 174–182. [CrossRef]
42. Visted, E.; Sørensen, L.; Osnes, B.; Svendsen, J.L.; Binder, P.E.; Schanche, E. The association between self-reported difficulties in emotion regulation and heart rate variability: The salient role of not accepting negative emotions. *Front. Psychol.* **2017**, *8*, 328. [CrossRef]
43. Gratz, K.L.; Roemer, L. Multidimensional Assessment of Emotion Regulation and Dysregulation: Development, Factor Structure, and Initial Validation of the Difficulties in Emotion Regulation Scale. *J. Psychopathol. Behav. Assess.* **2004**, *26*, 41–54. [CrossRef]
44. Williams, D.P.; Cash, C.; Rankin, C.; Bernardi, A.; Koenig, J.; Thayer, J.F. Resting heart rate variability predicts self-reported difficulties in emotion regulation: A focus on different facets of emotion regulation. *Front. Psychol.* **2015**, *6*, 261. [CrossRef]
45. Xiu, L.; Zhou, R.; Jiang, Y. Working memory training improves emotion regulation ability: Evidence from HRV. *Physiol. Behav.* **2016**, *155*, 25–29. [CrossRef] [PubMed]
46. Nuss, P. Anxiety disorders and GABA neurotransmission: A disturbance of modulation. *Neuropsychiatr. Dis. Treat.* **2015**, *11*, 165. [CrossRef]
47. Salomons, T.V.; Nusslock, R.; Detloff, A.; Johnstone, T.; Davidson, R.J. Neural emotion regulation circuitry underlying anxiolytic effects of perceived control over pain. *J. Cogn. Neurosci.* **2014**, *27*, 222–233. [CrossRef]
48. Sripada, R.K.; Marx, C.E.; King, A.P.; Rampton, J.C.; Ho, S.S.; Liberzon, I. Allopregnanolone elevations following pregnenolone administration are associated with enhanced activation of emotion regulation neurocircuits. *Biol. Psychiatry* **2013**, *73*, 1045–1053. [CrossRef] [PubMed]
49. Cohen-Armon, M.; Schreiber, G.; Sokolovsky, M. Interaction of the antiarrhythmic drug amiodarone with the muscarinic receptor in rat heart and brain. *J. Cardiovasc. Pharmacol.* **1984**, *6*, 1148–1155. [CrossRef] [PubMed]
50. Kotoda, M.; Ino, H.; Kumakura, Y.; Iijima, T.; Ishiyama, T.; Matsukawa, T. Analgesic effects of amiodarone in mouse models of pain. *J. Pain Res.* **2019**, *12*, 1825. [CrossRef]
51. Jayasuriya, G.M.; Elmslie, G.; Burstein, E.S.; Ellis, J. Dronedarone Modulates M1 and M3 Muscarinic Receptors with Subtype Selectivity, Functional Selectivity, and Probe Dependence. *Pharmacology* **2017**, *99*, 128–138. [CrossRef]
52. Lavalle, C.; Magnocavallo, M.; Straito, M.; Santini, L.; Forleo, G.B.; Grimaldi, M.; Badagliacca, R.; Lanata, L.; Ricci, R.P. Flecainide How and When: A Practical Guide in Supraventricular Arrhythmias. *J. Clin. Med.* **2021**, *10*, 1456. [CrossRef]
53. Mo, W.; Michel, M.C.; Lee, X.W.; Kaumann, A.J.; Molenaar, P. The β3-adrenoceptor agonist mirabegron increases human atrial force through β1-adrenoceptors: An indirect mechanism? *Br. J. Pharmacol.* **2017**, *174*, 2706–2715. [CrossRef]
54. Massie, B.M.; Fisher, S.G.; Deedwania, P.C.; Singh, B.N.; Fletcher, R.D.; Singh, S.N. Effect of amiodarone on clinical status and left ventricular function in patients with congestive heart failure. *Circulation* **1996**, *93*, 2128–2134. [CrossRef]
55. Michel, M.C.; Bond, R.A.; Summers, R.J. Adrenoceptors-New roles for old players. *Br. J. Pharmacol.* **2019**, *176*, 2339–2342. [CrossRef]
56. Fauchier, L.; Babuty, D.; Autret, M.L.; Poret, P.; Cosnay, P.; Fauchier, J.P. Effect of flecainide on heart rate variability in subjects without coronary artery disease or congestive heart failure. *Cardiovasc. Drugs Ther.* **1998**, *12*, 483–486. [CrossRef]
57. Guo, Z.B.; Fu, J.G.; Zhao, Y. Therapeutic efficacy of oxymatrine on arrhythmia and heart rate variability in patients with coronary heart disease. *Zhongguo Zhongxiyi jiehe zazhi Chin. J. Integr. Tradit. West. Med.* **2006**, *26*, 311–315.

58. Hallstrom, A.L.; Pratt, C.M.; Leon Greene, H.; Huther, M.; Gottlieb, S.; DeMaria, A.; Young, J.B.; Cardiac Arrhythmia Suppression Trial Investigators. Relations between heart failure, ejection fraction, arrhythmia suppression and mortality: Analysis of the Cardiac Arrhythmia Suppression Trial. *J. Am. Coll. Cardiol.* **1995**, *25*, 1250–1257. [CrossRef]
59. Frijda, N.H. The laws of emotion. *Am. Psychol.* **1988**, *43*, 349–358. [CrossRef]
60. Zahn, D.; Adams, J.; Krohn, J.; Wenzel, M.; Mann, C.G.; Gomille, L.K.; Jacobi-Scherbening, V.; Kubiak, T. Heart rate variability and self-control—A meta-analysis. *Biol. Psychol.* **2016**, *115*, 9–26. [CrossRef] [PubMed]
61. De Ridder, D.T.; Lensvelt-Mulders, G.; Finkenauer, C.; Stok, F.M.; Baumeister, R.F. Taking stock of self-control: A meta-analysis of how trait self-control relates to a wide range of behaviors. *Personal. Soc. Psychol. Rev.* **2012**, *16*, 76–99. [CrossRef] [PubMed]
62. Friedman, B.H.; Thayer, J.F. Autonomic balance revisited: Panic anxiety and heart rate variability. *J. Psychosom. Res.* **1998**, *44*, 133–151. [CrossRef]
63. Friedman, B.H. An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone. *Biol. Psychol.* **2007**, *74*, 185–199. [CrossRef]
64. Zhang, Y.; Zhou, B.; Qiu, J.; Zhang, L.; Zou, Z. Heart rate variability changes in patients with panic disorder. *J. Affect. Disord.* **2020**, *267*, 297–306. [CrossRef] [PubMed]
65. Wang, X.; Thayer, J.F.; Treiber, F.; Snieder, H. Ethnic differences and heritability of heart rate variability in African-and European American youth. *Am. J. Cardiol.* **2005**, *96*, 1166–1172. [CrossRef]
66. Snieder, H.; Van Doornen, L.J.; Boomsma, D.I.; Thayer, J.F. Sex differences and heritability of two indices of heart rate dynamics: A twin study. *Twin Res. Hum. Genet.* **2007**, *10*, 364–372. [CrossRef] [PubMed]
67. Thayer, J.F.; Merritt, M.M.; Sollers, J.J., III; Zonderman, A.B.; Evans, M.K.; Yie, S.; Abernethy, D.R. Effect of angiotensin-converting enzyme insertion/deletion polymorphism DD genotype on high-frequency heart rate variability in African Americans. *Am. J. Cardiol.* **2003**, *92*, 1487–1490. [CrossRef] [PubMed]
68. Friedman, B.H.; Thayer, J.F. Anxiety and autonomic flexibility: A cardiovascular approach. *Biol. Psychol.* **1998**, *47*, 243–263. [CrossRef]
69. Friedman, B.H.; Thayer, J.F.; Borkovec, T.D.; Tyrell, R.A.; Johnson, B.H.; Columbo, R. Autonomic characteristics of nonclinical panic and blood phobia. *Biol. Psychiatry* **1993**, *34*, 298–310. [CrossRef]
70. Srinivasan, K.; Ashok, M.V.; Vaz, M.; Yeragani, V.K. Decreased chaos of heart rate time series in children of patients with panic disorder. *Depress. Anxiety* **2002**, *15*, 159–167. [CrossRef] [PubMed]
71. Lotufo, P.A.; Valiengo, L.; Benseñor, I.M.; Brunoni, A.R. A systematic review and meta-analysis of heart rate variability in epilepsy and antiepileptic drugs. *Epilepsia* **2012**, *53*, 272–282. [CrossRef]
72. Clamor, A.; Koenig, J.; Thayer, J.F.; Lincoln, T.M. A randomized-controlled trial of heart rate variability biofeedback for psychotic symptoms. *Behav. Res. Ther.* **2016**, *87*, 207–215. [CrossRef] [PubMed]
73. Liu, Y.W.; Tzeng, N.S.; Yeh, C.B.; Kuo, T.B.; Huang, S.Y.; Chang, C.C.; Chang, H.A. Reduced cardiac autonomic response to deep breathing: A heritable vulnerability trait in patients with schizophrenia and their healthy first-degree relatives. *Psychiatry Res.* **2016**, *243*, 335–341. [CrossRef]
74. Guccione, C.; di Scalea, G.L.; Ambrosecchia, M.; Terrone, G.; Di Cesare, G.; Ducci, G.; Schimmenti, A.; Caretti, V. Early signs of schizophrenia and autonomic nervous system dysregulation: A literature review. *Clin. Neuropsychiatry J. Treat. Eval.* **2019**, *16*, 86–97.
75. Castro, M.N.; Vigo, D.E.; Weidema, H.; Fahrer, R.D.; Chu, E.M.; De Achaval, D.; Nogués, M.; Leiguarda, R.C.; Cardinali, D.P.; Guinjoan, S.M. Heart rate variability response to mental arithmetic stress in patients with schizophrenia: Autonomic response to stress in schizophrenia. *Schizophr. Res.* **2008**, *99*, 294–303. [CrossRef]
76. Meyer, P.W.; Müller, L.E.; Zastrow, A.; Schmidinger, I.; Bohus, M.; Herpertz, S.C.; Bertsch, K. Heart rate variability in patients with post-traumatic stress disorder or borderline personality disorder: Relationship to early life maltreatment. *J. Neural Transm.* **2016**, *123*, 1107–1118. [CrossRef]
77. Dixon-Gordon, K.L.; Turner, B.J.; Rosenthal, M.Z.; Chapman, A.L. Emotion regulation in borderline personality disorder: An experimental investigation of the effects of instructed acceptance and suppression. *Behav. Ther.* **2017**, *48*, 750–764. [CrossRef] [PubMed]
78. Rukmani, M.R.; Seshadri, S.P.; Thennarasu, K.; Raju, T.R.; Sathyaprabha, T.N. Heart rate variability in children with attention-deficit/hyperactivity disorder: A pilot study. *Ann. Neurosci.* **2016**, *23*, 81–88. [CrossRef] [PubMed]
79. Franquillo, A.C.; Guccione, C.; Angelini, G.; Carpentieri, R.; Ducci, G.; Caretti, V. The role of personality in schizophrenia and psychosis: A systematic review. *Clin. Neuropsychiatry* **2021**, *18*, 28–40. [CrossRef]
80. Di Simplicio, M.; Costoloni, G.; Western, D.; Hanson, B.; Taggart, P.; Harmer, C.J. Decreased heart rate variability during emotion regulation in subjects at risk for psychopathology. *Psychol. Med.* **2012**, *42*, 1775–1783. [CrossRef]
81. Koch, C.; Wilhelm, M.; Salzmann, S.; Rief, W.; Euteneuer, F. A meta-analysis of heart rate variability in major depression. *Psychol. Med.* **2019**, *49*, 1948–1957. [CrossRef] [PubMed]
82. Heiss, S.; Vaschillo, B.; Vaschillo, E.G.; Timko, C.A.; Hormes, J.M. Heart Rate Variability as a Biobehavioral Marker of Diverse Psychopathologies: A Review and Argument for an "Ideal Range". *Neurosci. Biobehav. Rev.* **2021**, *121*, 144–155. [CrossRef]
83. Lane, R.D.; Reiman, E.M.; Ahern, G.L.; Thayer, J.F. Activity in medial prefrontal cortex correlates with vagal component of heart rate variability during emotion. *Brain Cogn.* **2001**, *47*, 97–100.

84. Nugent, A.C.; Bain, E.E.; Thayer, J.F.; Drevets, W.C. Anatomical correlates of autonomic control during a motor task. *Psychosom. Med.* **2007**, *69*, A-74.
85. Nugent, A.C.; Bain, E.E.; Thayer, J.F.; Sollers, J.J.; Drevets, W.C. Alterations in neural correlates of autonomic control in females with major depressive disorder. *Int. J. Psychophysiol.* **2008**, *69*, 195. [CrossRef]
86. Ter Horst, G.J. Central autonomic control of the heart, angina, and pathogenic mechanisms of post-myocardial infarction depression. *Eur. J. Morphol.* **1999**, *37*, 257–266. [CrossRef]
87. Aron, A.R.; Robbins, T.W.; Poldrack, R.A. Inhibition and the right inferior frontal cortex. *Trends Cogn. Sci.* **2004**, *8*, 170–177. [CrossRef]
88. Kalisch, R.; Wiech, K.; Critchley, H.D.; Seymour, B.; O'Doherty, J.P.; Oakley, D.A.; Allen, P.; Dolan, R.J. Anxiety Reduction through Detachment: Subjective, Physiological, and Neural Effects. *J. Cogn. Neurosci.* **2005**, *17*, 874–883. [CrossRef] [PubMed]
89. Garavan, H.; Ross, T.J.; Stein, E.A. Right hemispheric dominance of inhibitory control: An event-related functional MRI study. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 8301–8306. [CrossRef]
90. Konishi, S.; Nakajima, K.; Uchida, I.; Kikyo, H.; Kameyama, M.; Miyashita, Y. Common inhibitory mechanism in human inferior prefrontal cortex revealed by event-related functional MRI. *Brain* **1999**, *122*, 981–991. [CrossRef] [PubMed]
91. Goldstein, R.Z.; Volkow, N.D. Dysfunction of the prefrontal cortex in addiction: Neuroimaging findings and clinical implications. *Nat. Rev. Neurosci.* **2011**, *12*, 652–669. [CrossRef]
92. Gertz, H.J.; Wolf, H.; Arendt, T. Psychiatric disorders of the frontal lobe. *Curr. Opin. Psychiatry* **1999**, *12*, 321–324. [CrossRef]
93. Goldman-Rakic, P.S. The prefrontal landscape: Implications of functional architecture for understanding human mentation and the central executive. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **1996**, *351*, 1445–1453. [CrossRef]
94. Arnsten, A.F.; Goldman-Rakic, P.S. Noise stress impairs prefrontal cortical cognitive function in monkeys: Evidence for a hyperdopaminergic mechanism. *Arch. Gen. Psychiatry* **1998**, *55*, 362–368. [CrossRef]
95. Baddeley, A.D.; Della Sala, S. Working memory and executive control. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* **1996**, *351*, 1397–1404. [CrossRef]
96. Garavan, H.; Ross, T.J.; Li, S.J.; Stein, E.A. A parametric manipulation of central executive functioning. *Cereb. Cortex* **2000**, *10*, 585–592. [CrossRef]
97. Rosvold, H.E.; Mirsky, A.F.; Sarason, I.; Bransome, E.D., Jr.; Beck, L.H. A continuous performance test of brain damage. *J. Consult. Psychol.* **1956**, *20*, 343–350. [CrossRef] [PubMed]
98. Hugdahl, K.; Thomsen, T.; Landrø, N.I.; Ersland, L.; Smievoll, A.I.; Lundervold, A.; Barndon, R.; Sundberg, H.; Iversen, J.K.; Roscher, B. Separating mental arithmetic from working memory: An fMRI-study. *NeuroImage* **2000**, *11*, S384. [CrossRef]
99. Frankenhaeuser, M.; Nordheden, B.; Myrsten, A.L.; Post, B. Psychophysiological reactions to understimulation and overstimulation. *Acta Psychol.* **1971**, *35*, 298–308. [CrossRef]
100. Broadbent, D.E. *Decision and Stress*; Academic Press: London, UK, 1971.
101. Stenfors, C.U.; Hanson, L.M.; Theorell, T.; Osika, W.S. Executive cognitive functioning and cardiovascular autonomic regulation in a population-based sample of working adults. *Front. Psychol.* **2016**, *7*, 1536. [CrossRef] [PubMed]
102. Gathright, E.C.; Walter, F.A.; Hawkins, M.A.; Spitznagel, M.B.; Hughes, J.W.; Gunstad, J. Executive function moderates the relationship between depressive symptoms and resting heart rate variability in heart failure. *J. Behav. Med.* **2016**, *39*, 192–200. [CrossRef]
103. Wang, Y.-P.; Gorenstein, C. Psychometric properties of the Beck Depression Inventory-II: A comprehensive review. *Rev. Bras. Psiquiatr.* **2013**, *35*, 416–431. [CrossRef]
104. Morgan, A.B.; Lilienfeld, S.O. A meta-analytic review of the relation between antisocial behavior and neuropsychological measures of executive function. *Clin. Psychol. Rev.* **2000**, *20*, 113–136. [CrossRef]
105. Gorenstein, E.E. Frontal lobe functions in psychopaths. *J. Abnorm. Psychol.* **1982**, *91*, 368–379. [CrossRef]
106. Hare, R.D. Performance of psychopaths on cognitive tasks related to frontal lobe function. *J. Abnorm. Psychol.* **1984**, *93*, 133–140. [CrossRef]
107. Hansen, A.L.; Johnsen, B.H.; Thornton, D.; Waage, L.; Thayer, J.F. Facets of psychopathy, heart rate variability and cognitive function. *J. Personal. Disord.* **2007**, *21*, 568–582. [CrossRef] [PubMed]
108. Williams, S.R.; Woodruff-Borden, J. Parent emotion socialization practices and child self-regulation as predictors of child anxiety: The mediating role of cardiac variability. *Child Psychiatry Hum. Dev.* **2015**, *46*, 512–522. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Review

Forgetting Unwanted Memories: Active Forgetting and Implications for the Development of Psychological Disorders

Marco Costanzi [1,*], Beatrice Cianfanelli [1], Alessandro Santirocchi [2], Stefano Lasaponara [1,2], Pietro Spataro [3], Clelia Rossi-Arnaud [2] and Vincenzo Cestari [2]

[1] Department of Human Sciences, Lumsa University, 00193 Rome, Italy; b.cianfanelli@lumsa.it (B.C.); s.lasaponara@lumsa.it (S.L.)
[2] Department of Psychology, Sapienza University, 00185 Rome, Italy; alessandro.santirocchi@uniroma1.it (A.S.); clelia.rossi-arnaud@uniroma1.it (C.R.-A.); vincenzo.cestari@uniroma1.it (V.C.)
[3] Department of Economy, Universitas Mercatorum, 00100 Rome, Italy; pietro.spataro@unimercatorum.it
* Correspondence: m.costanzi@lumsa.it

Abstract: Intrusive memories are a common feature of many psychopathologies, and suppression-induced forgetting of unwanted memories appears as a critical ability to preserve mental health. In recent years, biological and cognitive studies converged in revealing that forgetting is due to active processes. Recent neurobiological studies provide evidence on the active role of main neurotransmitter systems in forgetting, suggesting that the brain actively works to suppress retrieval of unwanted memories. On the cognitive side, there is evidence that voluntary and involuntary processes (here termed "intentional" and "incidental" forgetting, respectively) contribute to active forgetting. In intentional forgetting, an inhibitory control mechanism suppresses awareness of unwanted memories at encoding or retrieval. In incidental forgetting, retrieval practice of some memories involuntarily suppresses the retrieval of other related memories. In this review we describe recent findings on deficits in active forgetting observed in psychopathologies, like post-traumatic stress disorder, depression, schizophrenia, and obsessive-compulsive disorder. Moreover, we report studies in which the role of neurotransmitter systems, known to be involved in the pathogenesis of mental disorders, has been investigated in active forgetting paradigms. The possibility that biological and cognitive mechanisms of active forgetting could be considered as hallmarks of the early onset of psychopathologies is also discussed.

Keywords: forgetting; neurotransmitter system; psychopathologies

Citation: Costanzi, M.; Cianfanelli, B.; Santirocchi, A.; Lasaponara, S.; Spataro, P.; Rossi-Arnaud, C.; Cestari, V. Forgetting Unwanted Memories: Active Forgetting and Implications for the Development of Psychological Disorders. *J. Pers. Med.* **2021**, *11*, 241. https://doi.org/10.3390/jpm11040241

Academic Editor: Ruslan Dorfman

Received: 22 January 2021
Accepted: 23 March 2021
Published: 26 March 2021

1. Introduction

There is increasing empirical research suggesting that intrusive memories are a common feature of many mental disorders [1–6]. In patients, intrusions are due to the involuntary retrieval of unwanted memories [7–9], which tend to be repetitive, uncontrollable, and distressing [1]. Suppression of unwanted memories appears to be a critical ability to avoid their unintended influence, thus preserving mental health [9,10]. In healthy subjects, the ability to intentionally suppress memory retrieval (a phenomenon known as suppression-induced forgetting) has been associated with a lesser presence of distressing, intrusive memories for a traumatic movie [11]. On the contrary, impaired suppression-induced forgetting has been associated with worse mental health and has been found in individuals suffering from post-traumatic stress disorder [12], rumination [13,14], anxiety [15,16], and depression [17–20].

Forgetting is the inability to recall previously consolidated memories. Natural time-dependent decay of memory traces, change of context between acquisition and retrieval, and interference have all been considered mechanisms responsible for the inability to recall memories. Aside from the aforementioned passive mechanisms, a number of more recent

studies indicate that forgetting is also due to active processes, which actively work to eliminate memories from the brain [21–24].

On the cognitive side, two main mechanisms for active forgetting have been proposed: incidental and intentional forgetting [22,25,26]. Here, we use the terms "incidental" and "intentional" slightly differently from the way they have been typically adopted in memory studies. In the latter, incidental instructions refer to situations in which participants are not explicitly told to memorize to-be-encoded items and are unaware of the impeding memory test. Intentional instructions refer to situations in which participants are explicitly told to remember the presented items in view of a later memory task [27]. That is, in these studies the two terms are used to indicate the degree of intentionality of the encoding processes. In contrast, in the present reviews the same terms are used to reflect the degree of intentionality of the forgetting processes: along this line, incidental forgetting refers to situations in which participants are not explicitly instructed to forget, whereas intentional forgetting refers to situations in which participants are asked to deliberately forget some previously learned information [26].

More specifically, incidental forgetting occurs when retrieval of some memories involuntarily suppresses the retrieval of other, related, memories (retrieval-induced forgetting [21]) or when, according to a number of authors, memories that threaten our positive self-image are involuntarily repressed (e.g., Freudian repression). Although repression is at the heart of a heated debate [28], it has been described as occurring when a thought or a memory is too painful for an individual, so the person unconsciously pushes the information out of consciousness and becomes unaware of its existence [29]. Retrieval-induced forgetting (RIF), on the other hand, occurs when retrieval practice of items belonging to a category causes forgetting of unpracticed items belonging to the same category, in the absence of any instruction to voluntarily forget these items [30–33]. Such RIF studies typically involve three stages: study, retrieval practice, and final test [34]. During the study phase, participants are presented with a series of category–exemplar pairs (e.g., fruits–orange, drinks–vodka), under the instructions of studying them for a subsequent test or simply thinking about the associations. In the retrieval practice phase, participants are asked to retrieve half of the exemplars from half of the categories by completing category-plus-item-specific cues (e.g., fruit: or___). Often, participants undergo several rounds of retrieval practice before beginning the final phase, in which their ability to retrieve the exemplars is tested. Three types of exemplars are examined in the final recall task: Rp+ items refer to practiced exemplars (orange); Rp− items refer to non-practiced items from practiced categories (lemon); and Nrp items refer to exemplars from non-practiced categories (vodka). Two results classically emerge when using this paradigm: first, Rp+ items are recalled better than Rp− and Nrp items; second, Rp− items are recalled less well than Nrp items. It is exactly the latter finding which is usually referred to as RIF (see Figure 1A). Although several mechanisms have been proposed to account for this phenomenon, most of them can be grouped into the broad category of inhibition-based forgetting theories [34]. According to this perspective, attempts to retrieve practiced exemplars from memory cause associated items to become activated. Since this activation creates competition, non-practiced items from the same category are inhibited in order to selectively retrieve the target items. In this way, inhibition has the key function to reduce interference from non-practiced items during retrieval practice. However, in the final memory task, this same inhibition leads to poorer recall of Rp− items [30,31]. So, for example, when orange is retrieved during retrieval practice, the associated item, lemon, may become incidentally activated; to facilitate the retrieval of orange, lemon is inhibited, thus rendering it less accessible on the final test.

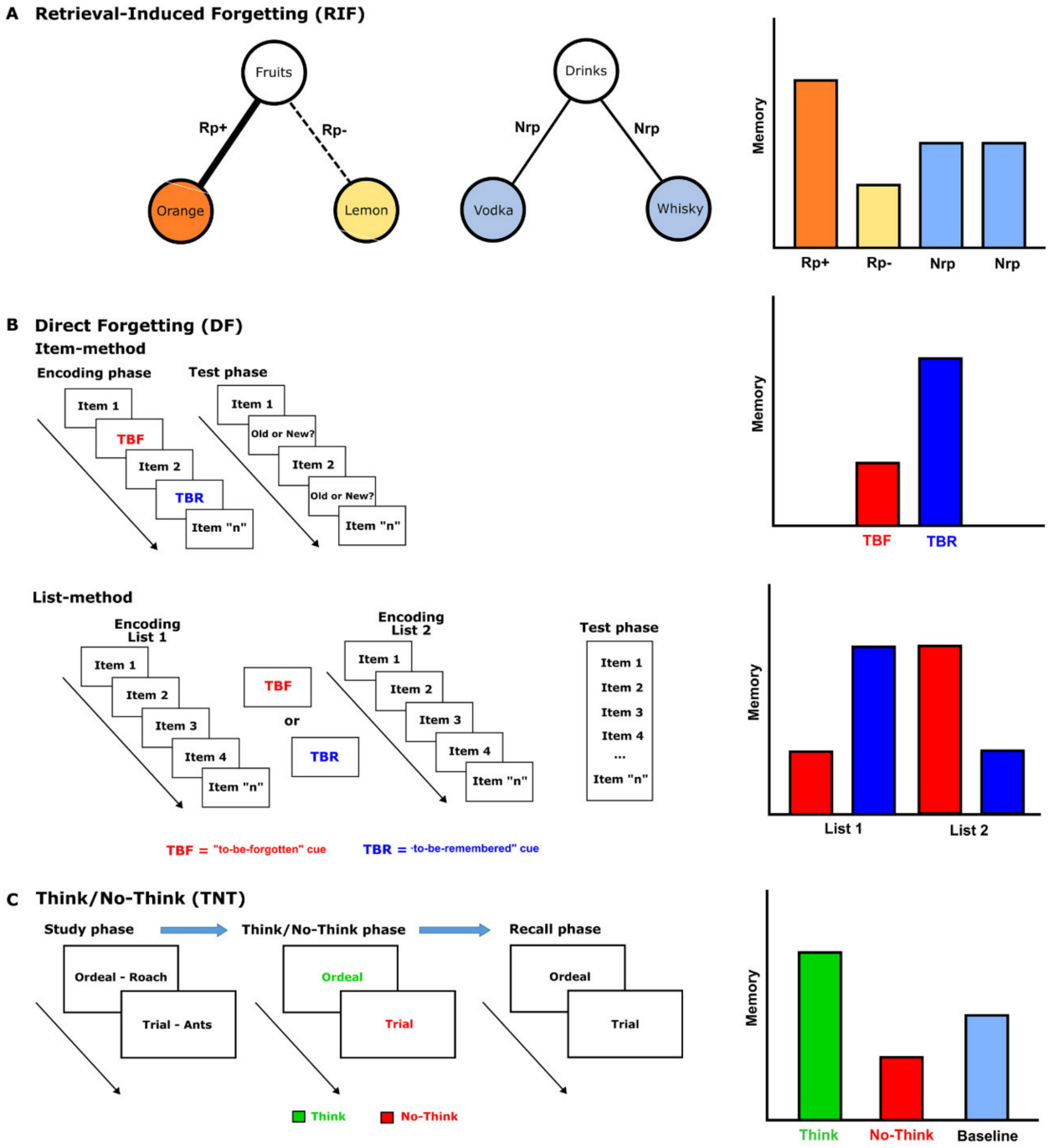

Figure 1. Schematic representations and procedural overview along with the typical pattern of findings of (**A**) the semantic retrieval-induced forgetting (RIF) paradigm, (**B**) item and list-methods for studying directed forgetting (DF), and (**C**) the think/no-think task (TNT).

Intentional forgetting, on the other hand, occurs when cognitive mechanisms are voluntarily engaged to weaken memories. Directed-forgetting (DF) and think/no-think (TNT) are experimental paradigms used to investigate intentional forgetting. In the former, two procedures have been used, depending on whether the instructions to remember or to forget are directed towards single items (item-method) or a list of items (list-method) [35]. In the item-method DF, participants are presented with individual items during the encoding phase, and each item is followed by either a "remember" or a "forget" cue. The common finding is that, in a later recall task, items followed by the instruction "to-be-forgotten" are worse remembered than those followed by the instruction "to-be-remembered". In the

list-method DF, participants are instead presented with two different lists of items during the encoding phase: the first list is followed by a forget or remember cue, whereas the second list is usually followed by a remember cue. In this case, two different effects emerge in the final memory task: the forgetting of list-1 items (i.e., impaired recall for the first list of items when subjects are instructed to forget this list, relative to when they are instructed to remember it) and the enhancement of list-2 items (i.e., improved recall for the second list of words when subjects are instructed to forget the first list, relative to when they are instructed to remember the first list; Figure 1B) [36]. One of the earliest theories that was proposed to account for DF is the selective rehearsal hypothesis [37]. Put simply, it states that presentation of the forget cue leads participants to stop the rehearsal of to-be-forgotten items and devote all their resources to the processing of to-be-remembered items, thus producing the memory enhancement [38]. An alternative hypothesis proposes that the benefit in the recall of list-2 might be explained by differences in the type of strategies used to encode the two lists (i.e., participants instructed to forget list-1 items typically use a deeper strategy to encode list-2 items). There is indeed evidence showing that modulating the encoding strategies in the list-method DF (e.g., using a shallow or a deep strategy to encode both lists) abolishes the benefits in remembering the list-2 but do not alter the inhibition of list-1 [39]. These results suggest that participants treat the "to-be-forgotten" and "to-be-remembered" lists as separate events and do not maintain the context in which they initially encoded list-1 when they are encoding list-2 [39,40]. Lastly, the third hypothesis is provided by the active inhibition account. According to this view, when participants are presented with the forget cue after the first list, they initiate an inhibitory process that suppresses activation of that list, so as to facilitate the learning of the subsequent list. The result is that memory of the first list suffers from inhibition, whereas memory of the second list benefits because the first list can no longer cause proactive interference [35,37]. In this respect, Anderson and Hanslmayr (2014) argued that the list- and item-methods differ in the target of forgetting: the item method leads to the inhibition of individual items, while the list method typically directs people to inhibit a set of items defined by temporal context (i.e., "the previous list"). Hence, in both item- and list-methods the encoding seems to be disrupted by an active inhibitory control mechanism that limits long-term memory formation for to-be-forgotten items or lists, respectively [35]. This interpretation has been recently supported by neurophysiological evidence showing that memory suppression in both item- and list-method DF is mediated by the inhibitory activity of prefrontal cortex on the medial temporal lobe [33]. Specifically, studies using connectivity analyses showed that increased activity in the right dorsolateral prefrontal cortex during forget trials predicted decreased activity in the left hippocampus, especially during successful intentional forgetting [41]. In another study, it was found that stimulating the dorsolateral prefrontal cortex with repetitive transcranial magnetic stimulation during a forget instruction increased the magnitude of the DF effect in the list-method [42].

Lastly, in the TNT paradigm, forgetting is obtained by asking subjects to suppress thoughts about stimuli cued by the instruction "no-think" [35]. In this paradigm, participants study cue–target pairs (i.e., word pairs such as ordeal–roach) and are then repeatedly trained to recall the second terms (the targets: roach) in response to the first terms (the cues: ordeal). Then, in the TNT phase, the cues are re-presented together with think/no-think instructions: when the cues appear with the "think" signal, participants have to recall the targets ("think" items); in contrast, when the cues appear with the "no-think" signal, they must avoid recalling the targets ("no-think" items). To measure the effectiveness of the think/no-think instructions, participants receive a final test in which they are given each cue and are asked to recall the associated target. Here, the typical finding is that "no-think" items are worse remembered than "think" items (Figure 1C). One important difference between the TNT and DF paradigms is that only the former assesses the ability to inhibit information that has been well learned [43]. In the DF procedures, participants study test items on an item-by-item or list-by-list basis, making it difficult to determine whether forgetting is due to inhibition or to simply not encoding the information into

memory. In contrast, in the TNT paradigms participants are first required to learn the studied items until being able to retrieve half or two-thirds of them. This methodology ensures that participants have successfully encoded the items that are then asked to reinforce ("think" trials) or inhibit ("no-think" trials). The difference between the two paradigms implies that inhibitory control acts at different levels of the memory processing: in the DF paradigm memory recall is impaired by inhibition at encoding, while in TNT paradigm memories are suppressed by inhibition at retrieval [35]. More specifically, the participants' task in the TNT paradigm is to prevent encoded information from coming to mind; to this purpose, an active mechanism must stop the retrieved items from reaching consciousness (Murray et al., 2011). According to Anderson and Hanslmayr [35] one such mechanism is inhibition. That is, when participants are instructed to "not think" about a learned word, they must actively inhibit the desire to think about, or recall, that particular word. In agreement with this proposal and similar to DF, retrieval suppression in TNT paradigm appears to be achieved by inhibitory control mechanisms mediated by the prefrontal cortex [35]. In particular, Anderson et al. (2004) found that "no-think" trials were accompanied by increased activation of bilateral dorsolateral and ventrolateral prefrontal cortex, as well as by reduced activity bilaterally in the hippocampus.

Both incidental and intentional forgetting are involved in emotional memory control [10,44,45], and deficits in regulating such memories are known to play a key role in the onset of psychopathological disorders [45,46]. In fact, recent neurobiological studies seem to confirm that the brain has the capacity to actively erase memories through the actions of molecular cascades involved in several neuronal functions [22,47]. In particular, dopamine-related intracellular cascades, receptor trafficking, spine shrinkage involving NMDA-dependent long-term depotentiation, and adult neurogenesis remodeling of hippocampal circuits emerge as biological mechanisms actively involved in memory forgetting [22,47].

The discovery of neurobiological pathways actively involved in forgetting could be relevant in investigating possible overlapping mechanisms between deficits in suppression of unwanted memories and the onset of psychopathological disorders [35,48,49].

Changes in neurotransmission have been shown to alter behaviors and to play a pivotal role in the onset of psychopathological disorders. Among neurotransmitters and hormones, the dopaminergic system seems to be relevant for the onset of anxiety disorders, schizophrenia, and pathological gambling, as well as for mood swings; the noradrenergic system seems to be involved in the occurrence of attentional deficit hyperactive disorder (ADHD), depression and anxiety disorders; the cholinergic system is involved in Alzheimer disease (AD), ADHD, chronic fatigue, and depression; the serotoninergic system is involved in depression, impulse control disorders, obsessive-compulsive disorder (OCD), and suicidal behavior; the glutamatergic system has been implicated in the development of schizophrenia and OCD; the GABAergic system is involved in anxiety disorders; and glucocorticoids have been implicated in stress-induced pathologies, like post-traumatic stress disorder (PTSD) [50–55]. Interestingly, such neurotransmitters and hormones are involved in memory formation and forgetting [56–61].

In the first part of the present article, we review findings on active forgetting in psychological disorders, especially on its possible role as a potential cognitive marker for the early identification of psychopathologies. In the second part, we review literature on the role of the main neurotransmitter and hormone systems in forgetting.

2. Intentional and Incidental Forgetting in Post-Traumatic Stress Disorder

Post-traumatic stress disorder (PTSD) occurs when people are exposed to a horrific traumatic event, which involves threatened death, actual or threatened serious injury, or actual or threatened sexual violation [62]. PTSD patients usually show an impairment in the voluntary retrieval of autobiographical memory linked to the traumatic experience, an increased incidence of involuntary memories (i.e., flashback), as well as overgeneralization and avoidance of contexts resembling trauma. Not all people exposed to similar

traumatic events develop PTSD. Many of them exhibit time-limited distress, and intrusions decline naturally over the first few months after trauma [63]. However, traumatic memories can be unforgettable and interfere with normal life also in the absence of PTSD. Recently, Millon and colleagues (2018) found that women who experienced sexual violence in adolescence, but who did not develop PTSD, reported particularly strong memories for stressful life events in adulthood. In these women, intrusive memories of autobiographical stressful events correlated with the presence of altered cognitions related to traumatic life experiences and with an increased level of anxiety and depression [64].

The diversity of responses to the traumatic event suggests that differences in how people process traumatic experiences in the memory system could play a causal role in the development and maintenance of PTSD [65–67]. Large individual differences in the ability to suppress unwanted memories have been observed in healthy subjects [68]. Furthermore, experimental findings on the inability of PTSD patients to inhibit trauma-related thoughts suggest that difficulties in memory forgetting due to inhibitory control deficits could be involved in the onset of PTSD [15,17,66,68–71]. In the past twenty years, a number of studies investigating intentional forgetting in PTSD patients have been carried out using both the DF and TNT paradigms [11,12,67,72–81]. In comparison with healthy controls and traumatized patients who have not developed a PTSD, PTSD patients tested with the DF paradigms showed memory deficits for "to-be-remembered" neutral and emotional words, but not for trauma-related ones, indicating a reduced ability to memorize information [67,73,75,77]. Despite this general memory impairment, the forgetting rate of PTSD patients is similar to that of healthy controls, especially when trauma-related memories are considered [67,73,75,76,78,81]. These results indicate that PTSD patients have a preserved DF effect, suggesting that the ability to inhibit memory during the encoding is spared in PTSD.

However, although the DF effect seems to be largely preserved in PTSD patients, there is evidence showing a reduced DF effect in specific conditions in which the vulnerability to dissociate as a consequence of traumatic stressors in these patients is taken into account. In PTSD, dissociation is considered as an alteration of memory functioning due to the inability to disengage attention from threatening stimuli.

Zoellner and collaborators (2003) found that PTSD patients submitted to a dissociative-inducing procedure (based on the administration of Velten-like phrases derived from the Peritraumatic Dissociative Experiences Questionnaire) did not show the DF effect [81]. Similarly, healthy subjects at risk for developing a PTSD, who reported high score in the Dissociative Experiences Scale, displayed a reduction in the DF effect for trauma-related words when a concurrent secondary task (dived-attention condition) was performed during the primary DF task [76].

Furthermore, EEG studies in adults with a history of childhood abuse and high scores in the Post-traumatic Stress Diagnostic Scale revealed an increase in the alpha coherence of frontoparietal networks—known to be involved in the allocation of attention during working memory tasks [82]—after word presentation in a DF task. Such an increase in alpha coherence was higher in participants with earlier traumas [83].

On the basis of results obtained in PTSD patients submitted to the DF paradigm, it is possible to envisage that differences in the ability to allocate attentional resources could affect memory inhibition, predicting the onset of PTSD after trauma exposure.

As concerns the suppression of memory retrieval assessed through the TNT paradigm, PTSD patients showed an impairment in the ability to suppress memory retrieval for both emotional and neutral pictures [12,77,79]. The deficit in suppressing memories positively correlated with symptom severity: the higher the score in the Post-traumatic Stress Diagnostic Scale, the higher the recognition of items associated to the no-think instruction [12,79].

Interestingly, a magnetoencephalography analysis in PTSD patients revealed that the deficit in suppression-induced forgetting in the TNT task was associated with an increased gamma power—a neural marker of sensory long-term memory traces—recorded during

the presentation of no-think items, suggesting that PTSD patients experienced a rebound of sensory memory representations when trying to stop unwanted memories [79].

Regarding incidental forgetting, Amir and collaborators (2009) found that traumatized subjects with or without PTSD submitted to a retrieval practice paradigm for neutral, negative, and positive words showed a slight reduction in the typical RIF effect observed in non-traumatized control subjects. Moreover, PTSD patients remembered fewer practiced words than traumatized subjects without PTSD and healthy non-traumatized controls [84].

Taken together, the results on active forgetting in PTSD suggest that a deficit in the ability to intentionally suppress the retrieval of unwanted memories could be a cognitive hallmark for the risk of developing PTSD after a trauma.

3. Intentional and Incidental Forgetting in Depression

The central role of memory intrusion as a core feature for the development and maintenance of depression has been pointed out in a recent meta-analysis. Adults with depression are as likely to experience intrusive memories as adults with PTSD [85].

Although memory intrusion appears as a common feature of both PTSD and depression, differences in the mechanisms through which unwanted memories are inhibited emerge from studies assessing intentional forgetting.

While the DF effect seems to be largely unaffected by PTSD, depressed patients submitted to the DF paradigm recalled a higher number of to-be-forgotten words than healthy controls, especially when words were negative and/or illness-related [86,87]. It is important to note that in the studies by Power and collaborators (2000) and Wingenfeld and collaborators (2013), both showing a deficit of DF effect in depression, all the depressed patients met criteria for a diagnosis of Major Depressive Disorder (DSM-IV; APA, 1994). More recently, Xie and collaborators (2018) carried out an event-related potential analysis in healthy subjects with depressive tendencies, assessed through the Beck Depressive Inventory II (average of BDI-II scores was 19 ± 1, which indicates mild depression according to Beck et al., 1996 [88]), during a DF task with neutral and negative words. The study showed that difficulty in suppressing the encoding of negative material in individuals with depressive tendencies correlated with abnormalities in P2 and LPP elicited by word-valence and in P1 and N2 elicited by to-be-forgotten instructions. These results suggested that individuals with early signs of depression could have either an inefficient ability to suppress negative material or an excessive processing of it during encoding [89]. Only one study reported an intact DF effect in patients with a current diagnosis of a depressive episode (average of BDI-II score was 23 ± 1, which indicates moderate depression according to Beck et al., 1996 [88]), but differences in the selection of emotional words among the studies was reported by the authors to account for discrepant results [90].

No differences in the ability to suppress memory retrieval were reported between depressed patients (average of BDI-II score was 31 ± 2, which indicates severe depression according to Beck et al., 1996 [88]) and healthy subjects submitted to a TNT task [91]. However, differences between depressed and non-depressed subjects emerged in brain activity recorded during a TNT task, suggesting that depressed patients use different strategies to inhibit memories when compared to healthy controls [92].

Interestingly, when healthy subjects affected by a mild depression (average of BDI-II score was 19.1 ± 0.6; Beck et al., 1996 [88]) were submitted to a TNT task with neutral words a deficit in suppressing no-think items appeared in these patients. Furthermore, the inability to suppress memory retrieval correlated with the BDI score [19,93]. A mediation analysis revealed that the relationship between the level of depressive symptoms (BDI score) in these subclinical patients and the forgetting rate was fully mediated by working memory capacity [93].

Although speculative, results obtained in depressed patients submitted to intentional forgetting paradigms suggest that deficits in suppressing the retrieval of unwanted memories in TNT could characterize the first stage of depression (namely, in healthy subjects suffering from mild depression). In cases of severe depression, patients learn to suppress

retrieval of unwanted memories, although with mechanisms that differ from those used by non-depressed individuals.

In the only study investigating incidental forgetting in depression, Groome and Sterkaj (2010) found that depressed patients, who met DSMIV criteria for the major depressive disorder, submitted to a retrieval practice paradigm for neutral words achieved significantly lower RIF scores than healthy subjects, indicating that depression is associated with a reduced RIF effect [91].

4. Intentional and Incidental Forgetting in Schizophrenia

The inability to suppress unwanted information and memory intrusions have been observed in schizophrenic patients, in particular in those with an early onset of the disorder. Recent studies have shown a dysfunctional cognitive control over emotional distraction in patients with schizophrenia [94–97]. Moreover, an increase in false recognition of distractors in a word recognition task has been found to correlate with the hallucination score [98]. Memory intrusions are also observed in nonclinical individuals with high hallucination scores [99,100], suggesting that deficit in the executive control involved in the inhibition of encoding and retrieval of unwanted memories might be a cognitive marker of the vulnerability to schizophrenia.

In a number of studies testing schizophrenic patients in DF paradigms in which neutral and emotional words were used as stimuli, a deficit in the inhibition of to-be-forgotten items has been observed [101–106]. A similar deficit has also been observed in patients affected by Velo-cardio-facial syndrome, a neurogenetic disorder associated with a very high risk for developing schizophrenia [107]. On the contrary, when negative pictures were used as stimuli, schizophrenic patients seemed to display an intact DF effect [108], suggesting that processing of verbal and visual information is differentially affected in schizophrenia.

In studies investigating incidental forgetting through the retrieval practice paradigm for word pairs, an intact RIF effect in the following cued recall test was observed in schizophrenic patients [104,109–111].

Together, these results suggest that deficit in intentional, but not in incidental forgetting, could be considered as a cognitive hallmark for the development of schizophrenia.

5. Intentional and Incidental Forgetting in Obsessive-Compulsive Disorder

OCD is characterized by recurrent, intrusive, and unwanted thoughts, impulses, and images, often associated with compulsive behaviors that are repetitive, time-consuming, and often ritualized.

Compulsions are generally performed in an attempt to either avoid or neutralize the obsessions and to reduce anxiety. Benzina and collaborators (2016), in reviewing a number of studies on the neuropsychological abnormalities in OCD, revealed an impairment in the decision-making process, behavioral flexibility, verbal and non-verbal episodic memory, inhibitory control, as well as altered attentional processes [112]. Although there is not a broad consensus on the biological and cognitive markers of OCD [112–114], the cognitive profile of the disorder seems to be marked by a deficit in executive functions [115–118]. Chamberlain and collaborators (2005) argued that failures in cognitive and behavioral inhibition could explain clinical symptoms as well as executive deficits observed in memory tasks and in tasks requiring inhibition of prepotent responses [119]. Abnormalities in the cortico-limbic network responsible for the inhibitory control have also been found in OCD patients [120].

Contrasting results emerge when inhibitory control was investigated in OCD patients through the DF paradigm [121–125]. In their pioneering work, Whilelm and collaborators (1996) explored the DF effect in OCD patients in a task in which emotional (both positive and negative) and neutral words had to be forgotten or remembered. Results showed that OCD patients reported significantly more negative to-be-forgotten words than healthy controls in the recall test and in the following recognition task, suggesting that an abnormal encoding of negative information occurred in these patients [121]. Evidence for an

abnormal encoding of to-be-intentionally inhibited information based on the emotional valence in OCD was also observed by Bohne and collaborators [122]. Indeed, OCD patients displayed a specific deficit in inhibiting the retrieval of information with negative valence [122].

Tolin and collaborators (2002) extended the lack of DF effect in OCD patients for disease-relevant words, independently of their valence (positive and negative). Interestingly, this lack of DF effect was observed in OCD patients, but not in anxious subjects who displayed a forgetting rate comparable to that of healthy controls [123].

In a more recent study, Moritz and collaborators (2011), by using a DF paradigm in which washing, checking, neutral, and negative words were presented, failed to find a lack of DF effect in OCD patients. However, they reported an unusual counter effect, according to which to-be-forgotten words referring to washing and checking were better remembered than to-be-remembered words in healthy controls [124], suggesting a bias in stimuli selection. In the same year, Konishi and collaborators (2011) found a normal DF effect in OCD for neutral words. Despite an intact DF effect, OCD patients appeared to recall fewer to-be-remembered words than healthy controls [125].

As concerns incidental forgetting, Jelinik and collaborators (2012) found an intact RIF effect in OCD patients submitted to a retrieval practice paradigm, in which OCD-relevant, neutral, and negative words were used as stimuli to be encoded. However, when the RIF effect was specifically analyzed for salient OCD-relevant words, a slight deficit was observed in patients [126].

A lack of RIF effect was reported in OCD patients suffering from mild depression submitted to a retrieval practice task in which neutral words were used as stimuli [116].

Together the above reported studies seem to suggest that OCD patients are unable to intentionally forget negative disease-relevant stimuli.

6. Neurotransmitters and Active Forgetting

Several neurotransmitter systems are known to be involved in the acquisition, consolidation, and retrieval of memories. In recent years, neurobiologists discovered neural, cellular, and molecular processes actively involved in erasing the substrate of memory or in suppressing its accessibility [22,23]. In this section we review studies concerning the involvement of neurotransmitters in active forgetting by underlining, where possible, their role in incidental and intentional forgetting.

6.1. Glutamate

Glutamate is recognized as the major excitatory neurotransmitter in the mammalian brain and exerts its excitatory function through both ionotropic and metabotropic receptors. NMDA, AMPA, and kainate are inotropic receptors, while mGluRs are a class of metabotropic receptors [127].

Among glutamate receptors, NMDA receptors are largely known to be involved in learning and memory processes, as well as in long-term potentiation. Pre- or post-training administration of either competitive (e.g., AP5) or non-competitive (e.g., MK-801, ketamine) NMDA antagonists prevents acquisition and impairs memory consolidation in different animal species submitted to different tasks [128]. Moreover, there is evidence about the involvement of NMDA receptors in acquisition and retention of extinction [129,130], in long-term depotentiation [131], as well as in spontaneous forgetting [132]. In particular, Quartermain and collaborators (1991) found that the administration of milacemide, a metabolic precursor of glycine, which in turn potentiates NMDA activity, suppressed the time-dependent decay of memory for both active and passive avoidance tasks [132]. In a recent work, Sachser and collaborators (2016) found that pharmacological inhibition of NMDA receptors, through systemic administration of memantine and MK801, prevented the normal forgetting of object-location memory in rats, as well as long-term potentiation (LTP) decay driven by the GluN2B subunit of NMDA receptor. In addition, they found that the time-dependent memory loss required Ca^{2+} influx. Systemic administration of L-type

voltage-dependent Ca^{2+} channel (LVDCC) blocker (nimodipine) was, in fact, effective in maintaining long-term memory for object location. Similarly, the inhibition of the calcium-dependent phosphatase calcineurin (CaN) prevented memory loss [133]. Interestingly, CaN activity through NMDA and LVDCC are involved in AMPA trafficking, it is thus possible to hypothesize that the removal of AMPA receptors from the membrane through the influx of calcium after memory consolidation induced forgetting [134].

Blocking the synaptic removal of the GluA2 subunit of AMPA receptors prevents the natural time-dependent forgetting of long-term memories [135,136].

More recently, a differential involvement of GluN2A and GluN2B subunits of the NMDA receptors on memory retention and forgetting has been observed [137]. The intra-hippocampal administration of the GluN2A antagonist, but not the GluN2B antagonist, after the acquisition of a Morris water maze task suppressed spatial memory decay in rats. Conversely, the GluN2B antagonist, but not the GluN2A antagonist, downregulated memory retention in the following memory test [137].

Together these results suggest the active role of the glutamatergic system in forgetting.

6.2. GABA

The GABAergic system is the main inhibitory system in the mammalian central nervous system [138]. It is known to influence neuronal development and synaptic plasticity, as well as learning and memory processes [139,140]. Two receptor systems bind GABA: $GABA_A$ and $GABA_B$ receptors.

$GABA_A$-ionotropic receptors are particularly abundant in the amygdala [141], where they play a major role in the neural mechanism underlying inhibition of aversive memory, such as fear extinction [142], while $GABA_B$-metabotropic receptors are necessary to control both short- and long-term memory formation [143].

In infant rats (16-, 18-day-old) submitted to a fear conditioning task, and tested 48 h after training, a pre-test administration of a $GABA_A$ inverse agonist prevented the long-term memory forgetting typically observed in developing rats. The latter suggests a role for $GABA_A$ in infantile amnesia [144–146].

In a single case human study, chronic intrathecal administration of the $GABA_B$ agonist baclofen induced a transient amnesic syndrome, associated with accelerated forgetting for autobiographical memories, suggesting a role for $GABA_B$ in modulating time-dependent forgetting [147].

6.3. Acetylcholine

The cholinergic system in the brain encompasses two major pathways: (i) the basal forebrain cholinergic system, including the nucleus basalis of Meynert (NBM), the medial septal nucleus, and the diagonal band of Broca (MSDB). The basal forebrain cholinergic system has extensive projections to neocortical regions, as well as to basolateral amygdala and olfactory bulb, hippocampus, and entorhinal cortex. (ii) The brainstem cholinergic system, including the pedunculopontine nucleus and the laterodorsal pontine tegmental nucleus. This brainstem system projects primarily to thalamic structures and to basal forebrain regions.

Acetylcholine released by presynaptic neurons exerts its effect on post-synaptic neurons by binding ionotropic nicotine receptors and metabotropic muscarine receptors. The activation of both nicotine- and muscarinic-cholinergic receptors is involved in either acquisition or extinction learning, indicating that cholinergic regulation plays a fundamental role in memory formation and consolidation [148,149]. In particular, the basal forebrain projections to prefrontal cortex, hippocampus, amygdala, parietal, and sensory regions are known to be involved in learning and memory processes, as well as in extinction of conditioned memories [150,151]. In the hippocampus, nicotine receptors are particularly localized postsynaptically on GABAergic neurons and play a major role in regulating excitatory neurotransmission. Instead, muscarinic receptors are mainly localized on hippocampal glutamatergic pyramidal neurons and are believed to provide direct excitatory

input from basal forebrain afferents, strengthening synaptic plasticity in hippocampal networks [151]. Several emerging lines of evidence suggest that cholinergic modulation of the cortico-hippocampal-amygdala circuit may regulate specific aspects of learning and forgetting [148].

However, contrasting results emerge when the role of cholinergic system in active and passive forgetting are specifically considered.

Edgiton and Rusted (2003) found that an acute dose of nicotine (obtained by smoking a cigarette from the preferred brand), in smokers who were asked to abstain from smoking 2 h before test, increased RIF effect without affecting memory recall. Conversely, in the same subjects the acute administration of scopolamine (a muscarinic cholinergic receptor antagonist), 1 h before training, impaired memory recall, but did not affect RIF [152]. These results suggest that the cholinergic receptor system is differentially involved in memory formation and forgetting, indicating a pivotal role for nicotine receptors in modulating incidental forgetting through inhibitory control. More recently Rusted and Alvares (2008) found that the administration of a single dose of nicotine (obtained by a nasal spray administration) in non-smoker participants enhanced the RIF effect. Interestingly, this enhancement was not observed in subjects submitted to a non-pharmacological procedure aimed at enhancing, like nicotine, the arousal level. These results lend some support for a specific role of nicotine receptors in the inhibitory control mechanism involved in incidental forgetting [153].

6.4. Dopamine

Dopaminergic neurons in the brain largely originate from the substantia nigra (SN) pars compacta and ventral tegmental area (VTA), forming the nigrostriatal and mesocorticolimbic pathways, respectively. In humans, midbrain dopaminergic neurons from VTA project to the prefrontal cortex (PFC) via the mesocortical pathway and to the nucleus accumbens, hippocampus, and amygdala via the mesolimbic pathway. This mesocorticolimbic system plays a role in reward, motivation, arousal, learning, and memory [154].

Several studies on the dopaminergic system have shown that dopamine release facilitates memory consolidation in different species submitted to both appetitive and aversive learning paradigms [155–158]. Interfering immediately after learning with dopamine activity impaired memory consolidation in drosophila, mice, and humans [22,159–162].

Although memory loss due to an impairment in dopamine signaling indicates its involvement in passive forgetting, recent studies seem to demonstrate that the ongoing dopamine activity after learning contributes to memory erasure, suggesting a dopamine-dependent active forgetting [163–166].

In an elegant study on drosophila, Berry and collaborators (2012) found that two different subsets of dopaminergic neurons are involved in retention and forgetting of both aversive and rewarding memories [159]. Blocking the output of dopamine-forgetting neurons after learning resulted in an enhancement of memory expression, while the stimulation of these neurons accelerated memory decay, indicating that dopamine is involved in active forgetting and that memory consolidation may counter the activity of dopamine-forgetting neurons [159]. Post-training stimulation of dopamine-forgetting neurons in drosophila, through locomotor activity, promotes the forgetting of aversive olfactory memories, while the inhibition of these neurons, through post training administration of the GABA-A agonist Gaboxadol, counteracts forgetting by facilitating memory consolidation [164]. These results suggest that the ongoing activity of dopaminergic neurons could determine the memory outcome: a strong dopaminergic activity immediately after acquisition determines forgetting, while its reduction facilitates memory consolidation.

Consistently, the administration of the monoamine stabilizer (-)-OSU6162 blocks the delay-dependent forgetting of object location memory in mice [167]. Interestingly, the (-)-OSU6162 acts as a dopaminergic stabilizer, by either inhibiting or stimulating the dopaminergic transmission depending on the dopaminergic tone. Hence, it is possible to

hypothesize that counteracting aberrations in dopaminergic signaling, without interfering with its normal functioning, might prevent forgetting.

In humans, chronic and recreational cocaine users showed a reduction in the DF effect, indicating that abnormal dopaminergic transmission interferes with the ability to intentionally suppress unwanted memories [167]. Interestingly, a recent neurogenic study demonstrated that in adulthood the presence of one or more polymorphisms associated with higher DA signaling predicted the forgetting rate in a picture recognition task [168]. Furthermore, humans with the Met/Met polymorphism in the catechol-O-methyltransferase gene (which leads to higher prefrontal dopamine availability) displayed a greater RIF effect, when compared with Val/Met and Val/Val allele carriers [169].

Together, these results support the hypothesis that the cortical dopaminergic system is centrally involved in the inhibitory control of memory, determining intentional as well as incidental forgetting.

6.5. Noradrenaline

Noradrenaline is an important hormone and neurotransmitter majorly secreted from the locus coeruleus located in the pons of brain stem. Noradrenergic projections from the locus coeruleus (LC) are directed to (i) the spinal cord and abdomen, where this neurotransmitter is used in sympathetic ganglia; (ii) the neocortex, including PFC; and (iii) the limbic system. The noradrenergic system is involved in multiple complex behavioral regulations, such as the regulation of arousal levels and vigilance [170].

There is evidence indicating that noradrenaline modulates memory formation for emotionally salient events [171]. Amygdala activation during the encoding of emotionally arousing stimuli has been shown to depend on the noradrenergic system [172–175], and pharmacological manipulations that increase the central release of noradrenaline in response to emotional arousal improve episodic memory formation [176–178]. In contrast, the administration of the adrenergic receptor antagonist propranolol blocks this emotional enhancing effect [174,175].

As concerns the role of the noradrenergic system in determining remembering or forgetting, interesting results emerge when the emotional valence of to-be-learned items is considered. Hurlemann and Collaborators (2005) found that the presence of negative material impaired the subsequent recall of episodic memories (retrograde amnesia), while the presence of positive material determined a better recall (retrograde hypermnesia). The pharmacological modulation of the noradrenergic system—blocking or enhancing noradrenergic transmission by the administration of either beta-receptor antagonist or noradrenalin reuptake inhibitor, respectively—was able to modify the levels of amnesia or hypermnesia, suggesting that memory persistence or forgetting was a function of the emotional arousal due to noradrenergic signaling [179]. In rats, the pre-test administration of either beta or alpha noradrenergic agonists has been observed to alleviate the time-dependent forgetting of an active avoidance memory [180]. Conversely, noradrenaline-depleted mice submitted to a water maze task displayed a more rapid forgetting than control mice [181]. Together these results seem to support the idea that noradrenaline-mediated arousal could play a role in determining which information has to be remembered or forgotten.

6.6. Glucocorticoids

It is well established that stress affects memory consolidation and reconsolidation through glucocorticoid release [182–184]. The hypothalamus–pituitary–adrenal (HPA) axis leads to the release of glucocorticoid hormones from the adrenal cortex under stress conditions. Glucocorticoids enter the blood–brain barrier and bind glucocorticoid (GR) and mineralocorticoid receptors MR. GRs are highly ubiquitous and expressed in most brain regions (including PFC), whereas MRs are predominantly expressed in limbic regions such as the hippocampus and amygdala [185]. Through this binding with GR and

MR, glucocorticoids act on memory formation enhancing consolidation and impairing retrieval [186,187].

Although there is a general consensus on the role of glucocorticoids on memory formation, few studies have systematically investigated their role in memory forgetting.

Koessler and collaborators (2009; 2013) found that oral administration of cortisol did not affect RIF, although stress-induced increases in salivary cortisol levels were able to eliminate the RIF effect [188,189].

Similarly, subjects submitted to a stress-induced procedure, able to increase cortisol levels, showed an impairment in the ability to intentionally inhibit retrieval of memory in a TNT paradigm. This deficit in memory control was related to the stress-dependent modulation of functional connectivity between the hippocampus and prefrontal cortex linked to memory suppression [190].

In a recent study, Kuehl and collaborators (2017) showed that acute cortisol administration before a DF task, in which participants were instructed to remember or to forget emotional words, did not affect memory performance [90].

Overall, these results seem to suggest that cortisol released due to stress induction plays a role in both incidental and intentional forgetting.

6.7. Neurotransmitters, Inhibitory Control, and Active Forgetting: An Overview

Several lines of evidence from brain imaging studies, as well as findings from psychiatric and neurological patients, indicate the main role of prefrontal cortex in orchestrating the inhibitory control of response behavior, emotions, as well as the retrieval of unwanted memories. The neural mechanisms and neurotransmitters involved in these PFC functions are extensively described elsewhere in excellent reviews [191–199].

In this paragraph we tentatively summarize the possible role of the neurotransmitter systems described above in the neural mechanisms involved in the inhibitory control of memories that may lead to active forgetting.

Anderson and colleagues (2016; 2020), by extensively reviewing several works on the neural networks involved in the inhibitory control of memories, reported that the prefrontal cortex (PFC) inhibits the activity of subcortical structures (e.g., the hippocampus) during RIF, TNT, and DF [191,196,200]. In particular, a PFC network—which includes connections between dorso-lateral PFC regions (dlPFC) with medial PFC (including the anterior cingulate cortex, aCC), and with the orbitofrontal cortex (OFC)—influences the activity of hippocampus, parahippocampal cortices, and amygdala (see Figure 2). The inhibitory control exerted by PFC on the hippocampus during memory suppression has been extensively demonstrated in functional activation and connectivity studies. An inhibitory control of PFC on the parahippocampal cortices and amygdala has been further observed when memories for a scene's spatial context and for emotional content had to be suppressed, respectively [196,200].

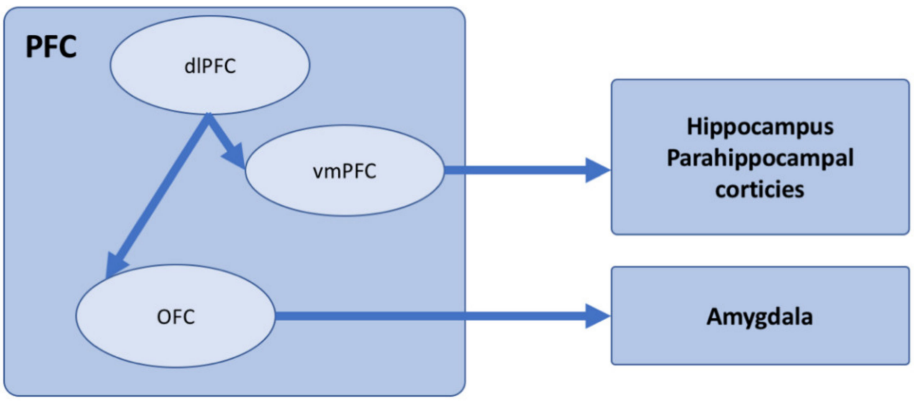

Figure 2. Schematic showing the neural network driving inhibitory control of unwanted memories.

At the cellular level, PFC function is related to the activity of pyramidal glutamatergic neurons and GABA interneurons. Efferent pathways from PFC are mainly due to glutamatergic projections toward cortical and subcortical structures. The activity of glutamatergic neurons is finely tuned by local inhibitory GABAergic interneurons. This cellular network, which consist of pyramidal glutamatergic neurons and GABA interneurons, may be involved in the enhanced processing of relevant stimuli as well as in the inhibition of irrelevant stimuli, thereby facilitating memory formation or forgetting. The inhibitory activity of PFC on cortical and subcortical structures seems to be driven by the firing of excitatory glutamatergic neurons, which directly excite local inhibitory GABAergic neurons located in the subcortical sites (e.g., hippocampus) responsible for memory formation ("direct inhibition") (Figure 3).

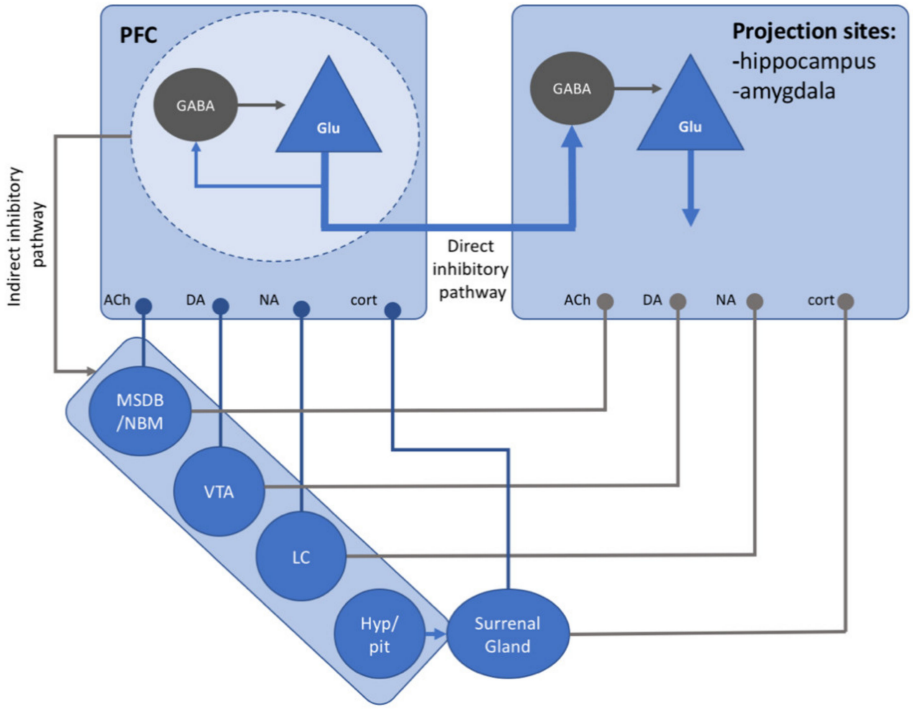

Figure 3. Schematic showing the cellular mechanism of direct and indirect inhibition driven by glutamatergic neurons in the prefrontal cortex (PFC). Afferent pathways from the main neurotransmitter systems, known to modulate PFC activity in inhibitory control, are also depicted.

The PFC control over hippocampus and amygdala can also follow an "indirect" pathway. PFC can indirectly modulate the activity of the hippocampus and amygdala through glutamatergic projections to the ventral tegmental area (VTA), locus coeruleus (LC), and basal forebrain, which in turn send dopamine, noradrenaline, and acetylcholine axons to both the hippocampus and amygdala [201,202]. The "direct inhibition" exerted by PFC glutamatergic projections to GABAergic neurons of subcortical structures like the hippocampus and amygdala appears to be the main pathway through which memories are suppressed [203]. Recently, imaging results obtained in humans submitted to a memory suppression task have revealed that higher concentrations of hippocampal GABA correlated with a greater downregulation during retrieval suppression and greater negative coupling between the PFC and the hippocampus, supporting the idea of a direct inhibitory PFC–hippocampus pathway [204].

Similarly, Depue and collaborators (2007) found that PFC directly inhibits the activity of the hippocampus and amygdala during memory suppression by using an emotional version of the TNT task [205]. Furthermore, direct projections from the PFC to GABAergic inhibitory neurons, located in intercalated cell masses of the amygdala, have been extensively observed in non-human primates and rodents [203,206–208].

Acetylcholine, dopamine, noradrenaline, and glucocorticoid are known to modulate the activity of glutamatergic and GABAergic neurons in the PFC and to shape the dendritic arborization of these neurons [209–213].

Cholinergic neurotransmission in the neocortex is known to be involved in several cognitive functions that include perception, attention, emotion, and memory consolidation [130,213,214]. Recently, a role for cholinergic transmission in the inhibitory control has also been hypothesized. In a recent fMRI study, Kasparbauer and colleagues (2018) found that the dlPFC is activated during the execution of a response inhibition task. Interestingly, nicotine administration increased dlPFC activity when response inhibition was successfully exercised. The magnitude of dlPFC activity due to nicotine administration was related to trait impulsivity: the higher the level of impulsivity, the stronger the reduction in dlPFC activity induced by nicotine when subjects failed to correctly inhibit the response [215]. As previously discussed in Section 6.1, nicotine administration in non-smoker subjects increased the RIF effect. Hence, although speculative, these results seem to indicate that cholinergic transmission, mediated by nicotine receptors in PFC, is essential for modulating the inhibitory control required for active forgetting.

Noradrenergic projections from LC and dopaminergic projections from VTA are classically associated with arousal regulation [216–220]. Release of noradrenaline and dopamine in PFC is low during sleep, moderate during alert situations, and high during uncontrollable stress [204]. Recent findings support the notion that the arousal-related release of both dopamine and noradrenaline into the PFC is involved in gain control mechanisms, which in turn amplify task-relevant signals by reducing neuronal noise. Such a gain control seems to be strongly involved in the inhibitory control process driven by PFC [204,221–224]. Pharmacological and imaging studies revealed that increasing dopaminergic and noradrenergic transmission (by the administration of psychostimulants, the dopamine/norepinephrine transporter blocker methylphenidate, or the noradrenaline inhibitor reuptake atomoxetine) are able to increase response inhibition. Such an increase in response inhibition correlated with the magnitude of PFC activation [196,225–231]. Both noradrenaline and dopamine are potent neuromodulators involved in the regulation of remembering and forgetting. In particular, the level of dopamine during the original encoding experience seems to determine whether memories are forgotten or remembered. Similarly, noradrenaline levels mediated by arousal could play a role in determining which information has to be remembered or forgotten. Hence, by extending results on response inhibition to memory control, it is possible to envisage that the inhibitory control of memories mediated by the PFC is under the influence of dopaminergic and noradrenergic tone. Interestingly, both dopamine and noradrenaline have a U-inverted effect on performance mediated by the PFC function, such that both the lowest and the highest levels of dopamine or noradrenaline impair performance [232].

In the last decades, neuroendocrine research has indicated that glucocorticoids modulate behavioral response and cognitive performance in stress conditions, particularly hippocampus-mediated declarative memory and prefrontal cortex-mediated working memory for emotional material [184–186,233].

Acute stress appears to dampen input onto inhibitory neurons, which results in an increase in glutamate release of PFC neurons. Such a glutamate release depends on glucocorticoid-related mechanisms [199]. Chronic stress also affects PFC function by remodeling the neural architecture of PFC (dendritic shrinking mPFC and dendritic expansion in OFC) [197]. Recent experimental results show that stress-dependent cortisol release impairs the inhibitory control of memories as well as response inhibition in a go/no-go task [174,190,234].

Taken together, results discussed in this section suggest that (i) overlapping PFC networks are involved in behavioral (e.g., stopping a motor response) and cognitive (e.g., memory suppression) inhibition; (ii) active forgetting is due to the inhibitory control exerted by PFC on subcortical structures (i.e., hippocampus, amygdala, and parahippocampal

cortices) responsible for memory formation; and (iii) different neurotransmitter systems modulate the function of PFC networks responsible for inhibitory control.

7. Conclusions

In summary, the results of the studies discussed in this review suggest that deficits in the inhibitory control involved in forgetting of unwanted memories are a common feature of psychopathologies like PTSD, depression, schizophrenia, and OCD. Furthermore, alterations in PFC–subcortical networks responsible for the inhibitory control mechanisms involved in active forgetting have been documented in schizophrenia, depression, OCD, and PTSD [210,231,235,236]. When incidental and intentional forgetting are investigated in patients, different patterns of deficits have been observed among these psychopathologies (see Table 1).

Table 1. Intentional and incidental forgetting affected by psychopathologies.

	Intentional Forgetting		Incidental Forgetting
	DF	**TNT**	**RIF**
Post-traumatic stress disorder	unaffected	affected	slightly affected
Depression *	affected	unaffected	affected
Schizophrenia	affected	n.a.	unaffected
Obsessive compulsive disorder	affected (only for disease-related stimuli)	n.a.	slightly affected (affected in OCD patients suffering of depressive symptoms)

DF: direct-forgetting; TNT: think/no-think; RIF: retrieval-induced forgetting. * Includes patients with a diagnosis of Major depressive disorder and subjects with mild to moderate depression.

Moreover, when pharmaco-behavioral evidence on the role of neurotransmitter systems in the inhibitory control of memories is put in relation to the deficits observed in patients, an interesting pattern of results emerges from the analysis of the literature.

As reported in Section 6.7, glutamatergic pyramidal neurons and GABAergic local interneuron in PFC play a pivotal role in driving the inhibitory control of memories. Alterations in glutamatergic and GABAergic transmissions have been reported in schizophrenia, depression, PTSD, and obsessive-compulsive disorder [237–243]. Moreover, studies on the role of these neurotransmitter systems in mental disorders seem to be promising for better understanding etiopathogenetic mechanisms, as well as for improving the effectiveness of pharmacological therapies [244–250]. Although direct evidence on the role of glutamatergic and GABAergic transmissions in active memory forgetting in psychopathological disorders is still lacking, it is possible to hypothesize that dysfunctions of these neurotransmitter systems are a common basis for deficits in active forgetting observed in the different psychopathologies.

Specific dysfunctions in other neurotransmitter systems, known to modulate the activity of glutamatergic and GABAergic neurons in PFC, might account for the different patterns of deficits that have been observed in active memory forgetting among psychopathologies.

Severe deficits in intentional inhibition of memory retrieval in the TNT paradigm and slight deficits in the incidental inhibition of memory retrieval in the RIF paradigm are observed in PTSD patients. On the other hand, intentional inhibition of memory encoding in DF tasks seems to be largely spared in these patients. On the contrary, a lack of both DF and RIF effects, but not TNT, are observed in depressed patients.

Studies in which intentional and incidental forgetting have been investigated in relation to cortisol release induced by stressful events seem to parallel the deficits in the inhibitory control of memory retrieval observed in PTSD patients. In particular, stress-induced cortisol release impaired RIF and TNT, but not DF effect [184,186,251]. A reduction in the ability to suppress the retrieval of unwanted memories related to an increase in cortisol level has been reported in PTSD patients. It is thus possible to hypothesize that post-traumatic stress might affect the inhibition of traumatic memories through alterations

in cortisol release. Conversely, it is possible to envisage that individual differences in the capability to inhibit the retrieval of unwanted memories might be a risk factor for the development of PTSD after trauma exposure. In healthy subjects, the ability to inhibit unwanted memories negatively correlated with the presence of intrusions by a previously watched traumatic movie [252].

Imaging studies show that emotion dysregulation in PTSD patients is related to a reduced PFC volume and to a weak inhibitory connection between the PFC and amygdala [253]. Furthermore, imaging and lesion studies in animal models of PTSD provide further support to the possible role played by the stress-induced deficits in the inhibitory control of memories due to a breakdown of PFC–amygdala connectivity [252,253].

Alterations in cortisol release, as well as reduced inhibitory control driven by PFC, are also observed in depressed patients [254,255]. PTSD and depression comorbidity due to exposure to traumatic experiences has been reported [256]. Hence, dysregulation in cortisol release due to traumatic stress may account for deficit in active forgetting observed in both PTSD and depressed patients.

The double dissociation observed in intentional forgetting between PTSD and depression might be explained by indirect pieces of evidence. As aforementioned, PTSD patients show an intact DF effect but a reduced TNT effect, while depressed patients display a strong reduction in DF effect, but a normal TNT. At the neurobiological level, a reduction in dopamine release has been strongly associated with depressive symptoms [257], while an increase in noradrenergic transmission following a traumatic event is correlated with PTSD symptoms [258]. Alterations in dopaminergic, but not noradrenergic, transmission seem to be involved in DF. It is thus possible to envisage that differences between PTSD and depression in the level of catecholamine release may account for the behavioral differences observed in patients submitted to the intentional forgetting paradigms.

Furthermore, recent clinical studies have pointed out a role for the cholinergic system in depression. In particular, reduced activity of nicotine receptors has been related to the onset of depression-like behaviors, while nicotine administration improved mood in depressed patients two days after its administration [259]. Pharmacological studies demonstrated a specific role for nicotine acetylcholine receptors in incidental forgetting, investigated through the RIF paradigm [260–262]. It is worth noting that, at the cognitive level, nicotine administration increases the RIF effect, which is impaired in depression, suggesting a close relationship between cholinergic alteration and incidental forgetting deficit in depression.

A specific deficit in DF emerges in studies in which memory forgetting has been investigated in schizophrenic patients. Such patients, differently from depressed patients, showed an intact RIF effect. The fact that a reduction in the RIF effect has been reported in depression, but not in schizophrenia, suggests that assessing the ability to involuntary inhibit unwanted memory could be a useful tool to differentiate diagnosis in these patients, especially at the early onset of these psychopathologies [106,251,263–265].

Although speculative, it is possible to envisage that alterations in dopamine activity (namely, reduced dopamine levels in depression and enhanced in schizophrenia), together with an altered capability to inhibit the encoding of unwanted memories, could be considered as hallmarks of schizophrenia and depression. Interestingly, dopamine levels affect PFC-dependent processes in a U-inverted fashion: the highest and lowest levels of dopamine impair performance [233].

As in depressed and schizophrenic patients, OCD patients showed a lack of DF effect, especially when OCD-related stimuli have to be intentionally suppressed, while RIF is only slightly affected. Interestingly, a severe deficit in RIF effect in OCD patients has been observed in those subjects suffering from depressive symptoms. These pieces of evidence suggest that deficits in the mechanisms driving incidental forgetting are specifically compromised by depressive states.

OCD patients display a profound inability to inhibit intrusive thoughts and compulsive behaviors. Dysfunctions of the PFC network involved the inhibitory control process,

perhaps due to altered dopaminergic transmission, might also help to explain comorbidities between OCD and other psychopathologies such as depression and schizophrenia [112,119,195,266,267].

The above reported results are intriguing for the definition of possible biological and cognitive hallmarks for the development of psychopathologies like PTSD, depression, schizophrenia, and OCD.

There is growing support for the notion that cognitive control abnormalities are a central component of many of the neuropsychological deficits observed in individuals with mental illnesses. Cognitive control refers to a set of mental processes that modulate other cognitive and emotional systems in service of goal-directed adaptive behavior [268]. Among the executive functions related to the cognitive control, the inhibition of unwanted memories through active forgetting emerges as a critical mechanism in order to maintain mental health. Deficits in this function have been observed in several psychopathologies [23,191].

A number of limits in the studies reviewed have to be carefully taken into account before the role of active forgetting can be considered in the assessment of psychopathologies.

First, the mechanisms of forgetting in patients were investigated in studies with small sample sizes; thus, the generalizability of results could be compromised. As reported in a recent review, sample size is one of the critical factors in considering putative bio-behavioral markers of psychopathologies as possible endophenotypes [269]. A second limit regards the ecological validity of stimuli used to investigate suppression of unwanted memories. Most studies used (emotional and/or disorder-related) words. It is possible to hypothesize that different results would be obtained with more ecologically valid stimuli. For example, there is evidence showing that emotional movies are better than words or pictures to investigate emotional memory process in PTSD [270]. Third, all the patients involved in the studies underwent medical treatments that per se could affect the inhibitory mechanisms involved in forgetting. It is thus possible that different patterns of results on forgetting could emerge in unmedicated patients or in real-life conditions. For this reason, studies on the involvement of active forgetting in psychopathology could benefit from future investigations in unmedicated healthy subjects, at risk to develop a mental disorder, or at the early stages of the psychopathological development.

Overall, although preliminary, the results we discussed in this review seem to provide pieces of evidence in favor of the use of active forgetting paradigms to increase pre-clinical tools aimed at the identification of the early onset of psychopathology and to aid clinicians in the therapeutic decision-making process.

Funding: This work was supported by the LUMSA (Fondo di Ateneo per la Ricerca 2019 to MC).

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Brewin, C.R.; Gregory, J.D.; Lipton, M.; Burgess, N. Intrusive images in psychological disorders: Characteristics, neural mechanisms, and treatment implications. *Psychol. Rev.* **2010**, *117*, 210–232. [CrossRef]
2. Brewin, C.R. Episodic memory, perceptual memory, and their interaction: Foundations for a theory of posttraumatic stress disorder. *Psychol. Bull.* **2014**, *140*, 69–97. [CrossRef] [PubMed]
3. Harvey, A.B.; Watkins, E.; Mansell, W.; Shafran, R. *Cognitive Behavioural Processes across Psychological Disorders: A Transdiagnostic Approach to Research and Treatment*; Oxford University Press: Oxford, UK, 2004.
4. Hirsch, C.R.; Holmes, E.A. Mental imagery in anxiety disorders. *Psychiatry* **2007**, *6*, 161–165. [CrossRef]
5. Speckens, A.E.M.; Ehlers, A.; Hackmann, A.; Ruths, F.A.; Clark, D.M. Intrusive memories and rumination in patients with post-traumatic stress disorder: A phenomenological comparison. *Memory* **2007**, *15*, 249–257. [CrossRef] [PubMed]
6. Visser, R.M. Why Do Certain Moments Haunt Us? Conceptualizing Intrusive Memories as Conditioned Responses. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **2020**, *5*, 375–376. [CrossRef] [PubMed]
7. Berntsen, D. *Involuntary Autobiographical Memories*; Cambridge University Press (CUP): Cambridge, UK, 2009.
8. Mace, C. Mindfulness in psychotherapy: An introduction. *Adv. Psychiatr. Treat.* **2007**, *13*, 147–154. [CrossRef]
9. Hu, X.; Bergström, Z.M.; Gagnepain, P.; Anderson, M.C. Suppressing Unwanted Memories Reduces Their Unintended Influences. *Curr. Dir. Psychol. Sci.* **2017**, *26*, 197–206. [CrossRef]

10. Gagnepain, P.; Hulbert, J.; Anderson, M.C. Parallel Regulation of Memory and Emotion Supports the Suppression of Intrusive Memories. *J. Neurosci.* **2017**, *37*, 6423–6441. [CrossRef]
11. Streb, M.; Mecklinger, A.; Anderson, M.C.; Lass-Hennemann, J.; Michael, T. Memory control ability modulates intrusive memories after analogue trauma. *J. Affect. Disord.* **2016**, *192*, 134–142. [CrossRef]
12. Catarino, A.; Küpper, C.S.; Werner-Seidler, A.; Dalgleish, T.; Anderson, M.C. Failing to Forget. *Psychol. Sci.* **2015**, *26*, 604–616. [CrossRef] [PubMed]
13. Fawcett, J.M.; Benoit, R.G.; Gagnepain, P.; Salman, A.; Bartholdy, S.; Bradley, C.; Chan, D.K.-Y.; Roche, A.; Brewin, C.R.; Anderson, M.C. The origins of repetitive thought in rumination: Separating cognitive style from deficits in inhibitory control over memory. *J. Behav. Ther. Exp. Psychiatry* **2015**, *47*, 1–8. [CrossRef]
14. Hertel, P.T.; Maydon, A.; Ogilvie, A.; Mor, N. Ruminators (Unlike Others) Fail to Show Suppression-Induced Forgetting on Indirect Measures of Memory. *Clin. Psychol. Sci.* **2018**, *6*, 872–881. [CrossRef]
15. Marzi, T.; Regina, A.; Righi, S. Emotions shape memory suppression in trait anxiety. *Front. Psychol.* **2014**, *4*, 1001. [CrossRef]
16. Benoit, R.G.; Davies, D.J.; Anderson, M.C. Reducing future fears by suppressing the brain mechanisms underlying episodic simulation. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E8492–E8501. [CrossRef]
17. Hertel, P.T.; Gerstle, M. Depressive Deficits in Forgetting. *Psychol. Sci.* **2003**, *14*, 573–578. [CrossRef]
18. Noreen, S.; Ridout, N. Examining the impact of thought substitution on intentional forgetting in induced and naturally occurring dysphoria. *Psychiatry Res.* **2016**, *241*, 280–288. [CrossRef] [PubMed]
19. Noreen, S.; Ridout, N. Intentional forgetting in dysphoria: Investigating the inhibitory effects of thought substitution using independent cues. *J. Behav. Ther. Exp. Psychiatry* **2016**, *52*, 110–118. [CrossRef]
20. Zhang, D.; Xie, H.; Liu, Y.; Luo, Y. Neural correlates underlying impaired memory facilitation and suppression of negative material in depression. *Sci. Rep.* **2016**, *6*, 37556. [CrossRef] [PubMed]
21. Anderson, M.C.; Green, C. Suppressing unwanted memories by executive control. *Nat. Cell Biol.* **2001**, *410*, 366–369. [CrossRef] [PubMed]
22. Davis, R.L.; Zhong, Y. The Biology of Forgetting—A Perspective. *Neuron* **2017**, *95*, 490–503. [CrossRef]
23. Medina, J.H. Neural, Cellular and Molecular Mechanisms of Active Forgetting. *Front. Syst. Neurosci.* **2018**, *12*, 3. [CrossRef] [PubMed]
24. Hulbert, J.C.; Anderson, M.C. Does retrieving a memory insulate it against memory inhibition? A retroactive interference study. *Memory* **2020**, *28*, 293–308. [CrossRef] [PubMed]
25. Erdelyi, M.H. The unified theory of repression. *Behav. Brain Sci.* **2006**, *29*, 499–511. [CrossRef]
26. Nørby, S. Forgetting and emotion regulation in mental health, anxiety and depression. *Memory* **2018**, *26*, 342–363. [CrossRef]
27. Schacter, D.L.; Bowers, J.; Booker, J. Intention, awareness, and implicit memory: The retrieval intentionality criterion. In *Implicit Memory: Theoretical Issues*; Lewandowsky, S., Dunn, J.C., Kirsner, K., Eds.; Lawrence Erlbaum Associates, Inc.: Mahwah, NJ, USA, 1989; pp. 47–65.
28. Otgaar, H.; Howe, M.L.; Patihis, L.; Merckelbach, H.; Lynn, S.J.; Lilienfeld, S.O.; Loftus, E.F. The Return of the Repressed: The Persistent and Problematic Claims of Long-Forgotten Trauma. *Perspect. Psychol. Sci.* **2019**, *14*, 1072–1095. [CrossRef]
29. Davis, P.J.; Schwartz, G.E. Repression and the inaccessibility of affective memories. *J. Pers. Soc. Psychol.* **1987**, *52*, 155–162. [CrossRef]
30. Anderson, M. Rethinking interference theory: Executive control and the mechanisms of forgetting. *J. Mem. Lang.* **2003**, *49*, 415–445. [CrossRef]
31. Anderson, M.C.; Levy, B.J. Theoretical issues in inhibition: Insights from research on human memory. In *Inhibition in Cognition*; American Psychological Association (APA): Washington, DC, USA, 2008; pp. 81–102.
32. Raaijmakers, J.G.W. Inhibition in Memory. In *Stevens' Handbook of Experimental Psychology and Cognitive Neuroscience*; Wiley: Hoboken, NJ, USA, 2018; pp. 1–34.
33. Raaijmakers, J.G.W. Inhibition in memory. In *Stevens' Handbook of Experimental Psychology and Cognitive Neuroscience, Language and Memory*, 4th ed.; Phelps, E.A., Davachi, L., Wixted, J.T., Eds.; Wiley: Hoboken, NJ, USA, 2018; Volume 1, pp. 251–284. [CrossRef]
34. Murayama, K.; Miyatsu, T.; Buchli, D.; Storm, B.C. Forgetting as a consequence of retrieval: A meta-analytic review of retrieval-induced forgetting. *Psychol. Bull.* **2014**, *140*, 1383–1409. [CrossRef]
35. Anderson, M.C.; Hanslmayr, S. Neural mechanisms of motivated forgetting. *Trends Cogn. Sci.* **2014**, *18*, 279–292. [CrossRef] [PubMed]
36. Geraerts, E.; McNally, J.R. Forgetting unwanted memories: Directed forgetting and thought suppression methods. *Acta Psychol.* **2008**, *127*, 614–622. [CrossRef] [PubMed]
37. Sahakyan, L.; Delaney, P.F.; Nathaniel, L.; Abushanab, B. List-Method Directed Forgetting in Cognitive and Clinical Research: A Theoretical and Methodological Review. *Psychol. Learn. Motiv.* **2013**, *59*, 131–189.
38. Bjork, R.A. Theoretical implication of directed forgetting. In *Coding Processes in Human Memory*; Melton, A.W., Martin, E., Eds.; Winston & Sons, Inc.: Washington, DC, USA, 1972; pp. 217–235.
39. Sahakyan, L.; Delaney, P.F. Can encoding differences explain the benefits of directed forgetting in the list method paradigm? *J. Mem. Lang.* **2003**, *48*, 195–206. [CrossRef]
40. Sahakyan, L.; Kelley, C.M. A contextual change account of the directed forgetting effect. *J. Exp. Psychol. Learn. Mem. Cogn.* **2002**, *28*, 1064–1072. [CrossRef] [PubMed]

41. Rizio, A.A.; Dennis, N.A. The Neural Correlates of Cognitive Control: Successful Remembering and Intentional Forgetting. *J. Cogn. Neurosci.* **2013**, *25*, 297–312. [CrossRef] [PubMed]
42. Hanslmayr, S.; Volberg, G.; Wimber, M.; Oehler, N.; Staudigl, T.; Hartmann, T.; Raabe, M.; Greenlee, M.W.; Bäuml, K.-H.T. Prefrontally Driven Downregulation of Neural Synchrony Mediates Goal-Directed Forgetting. *J. Neurosci.* **2012**, *32*, 14742–14751. [CrossRef]
43. Murray, B.D.; Muscatell, K.A.; Kensinger, E.A. Effects of emotion and age on performance during a think/no-think memory task. *Psychol. Aging* **2011**, *26*, 940–955. [CrossRef] [PubMed]
44. Kim, K.; Yi, D.-J. Out of Mind, Out of Sight. *Psychol. Sci.* **2013**, *24*, 569–574. [CrossRef]
45. Spataro, P.; Saraulli, D.; Cestari, V.; Costanzi, M.; Sciarretta, A.; Rossi-Arnaud, C. Implicit memory in schizophrenia: A meta-analysis. *Compr. Psychiatry* **2016**, *69*, 136–144. [CrossRef]
46. Kang, E.; Wen, Z.; Song, H.; Christian, K.M.; Ming, G.-L. Adult Neurogenesis and Psychiatric Disorders. *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, a019026. [CrossRef]
47. Richards, B.A.; Frankland, P.W. The Persistence and Transience of Memory. *Neuron* **2017**, *94*, 1071–1084. [CrossRef]
48. Liu, Y.; Lin, W.; Liu, C.; Luo, Y.; Wu, J.; Bayley, P.J.; Qin, S. Memory consolidation reconfigures neural pathways involved in the suppression of emotional memories. *Nat. Commun.* **2016**, *7*, 13375. [CrossRef] [PubMed]
49. Nader, K.; Hardt, O.; Lanius, R. Memory as a new therapeutic target. *Dialog Clin. Neurosci.* **2013**, *15*, 475–486.
50. Choudhury, A.; Sahu, T.; Ramanujam, P.L.; Banerjee, A.K.; Chakraborty, I.; Kumar, A.; Arora, N. Neurochemicals, Behaviours and Psychiatric Perspectives of Neurological Diseases. *Neuropsychiatry* **2018**, *8*, 395–424. [CrossRef]
51. Kalueff, A.V.; Nutt, D.J. Role of GABA in anxiety and depression. *Depress. Anxiety* **2006**, *24*, 495–517. [CrossRef] [PubMed]
52. Kaouane, N.; Porte, Y.; Vallée, M.; Brayda-Bruno, L.; Mons, N.; Calandreau, L.; Marighetto, A.; Piazza, P.V.; Desmedt, A. Glucocorticoids Can Induce PTSD-Like Memory Impairments in Mice. *Science* **2012**, *335*, 1510–1513. [CrossRef] [PubMed]
53. Gauthier, I.; Nuss, P. Anxiety disorders and GABA neurotransmission: A disturbance of modulation. *Neuropsychiatr. Dis. Treat.* **2015**, *11*, 165–175. [CrossRef]
54. Poels, E.M.; Kegeles, L.S.; Kantrowitz, J.T.; Javitt, D.C.; Lieberman, J.A.; Abi-Dargham, A.; Girgis, R.R. Glutamatergic abnormalities in schizophrenia: A review of proton MRS findings. *Schizophr. Res.* **2014**, *152*, 325–332. [CrossRef] [PubMed]
55. Zheng, P.; Zeng, B.; Liu, M.; Chen, J.; Pan, J.; Han, Y.; Liu, Y.; Cheng, K.; Zhou, C.; Wang, H.; et al. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci. Adv.* **2019**, *5*, eaau8317. [CrossRef]
56. Castellano, C.; Cabib, S.; Zocchi, A.; Puglisi-Allegra, S. Strain-dependent effects of cocaine on memory storage improvement induced by post-training physostigmine. *Psychopharmacology* **1996**, *123*, 340–345. [CrossRef]
57. Furini, C.R.; Myskiw, J.C.; Benetti, F.; Izquierdo, I. New frontiers in the study of memory mechanisms. *Rev. Bras. Psiquiatr.* **2013**, *35*, 173–177. [CrossRef] [PubMed]
58. Gold, J.J.; Squire, L.R. Quantifying medial temporal lobe damage in memory-impaired patients. *Hippocampus* **2004**, *15*, 79–85. [CrossRef]
59. Meneses, A. Frameworking memory and serotonergic markers. *Rev. Neurosci.* **2017**, *28*, 455–497. [CrossRef] [PubMed]
60. Ridley, R.; Baker, H.; Harder, J.; Pearson, C. Effects of lesions of different parts of the septo-hippocampal system in primates on learning and retention of information acquired before or after surgery. *Brain Res. Bull.* **1996**, *40*, 21–32. [CrossRef]
61. Tellez, R.; Gomez-Viquez, L.; Meneses, A. GABA, glutamate, dopamine and serotonin transporters expression on memory formation and amnesia. *Neurobiol. Learn. Mem.* **2012**, *97*, 189–201. [CrossRef]
62. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; (DSM-5); American Psychiatric Association: Washington, DC, USA, 2013; p. 5.
63. Ehlers, A. Understanding and Treating Unwanted Trauma Memories in Posttraumatic Stress Disorder. *Z. Psychol. J. Psychol.* **2010**, *218*, 141–145. [CrossRef] [PubMed]
64. Millon, E.M.; Chang, H.Y.M.; Shors, T.J. Stressful Life Memories Relate to Ruminative Thoughts in Women with Sexual Violence History, Irrespective of PTSD. *Front. Psychiatry* **2018**, *9*, 311. [CrossRef] [PubMed]
65. Brewin, C.R. The Nature and Significance of Memory Disturbance in Posttraumatic Stress Disorder. *Annu. Rev. Clin. Psychol.* **2011**, *7*, 203–227. [CrossRef]
66. Brewin, C.R. Memory and Forgetting. *Curr. Psychiatry Rep.* **2018**, *20*, 1–8. [CrossRef]
67. McNally, R.J. Experimental approaches to cognitive abnormality in posttraumatic stress disorder. *Clin. Psychol. Rev.* **1998**, *18*, 971–982. [CrossRef]
68. Levy, B.J.; Anderson, M.C. Individual differences in the suppression of unwanted memories: The executive deficit hypothesis. *Acta Psychol.* **2008**, *127*, 623–635. [CrossRef]
69. El Khoury-Malhame, M.; Lanteaume, L.; Beetz, E.M.; Roques, J.; Reynaud, E.; Samuelian, J.-C.; Blin, O.; Garcia, R.; Khalfa, S. Attentional bias in post-traumatic stress disorder diminishes after symptom amelioration. *Behav. Res. Ther.* **2011**, *49*, 796–801. [CrossRef] [PubMed]
70. Fleurkens, P.; Rinck, M.; Van Minnen, A. Specificity and generalization of attentional bias in sexual trauma victims suffering from posttraumatic stress disorder. *J. Anxiety Disord.* **2011**, *25*, 783–787. [CrossRef] [PubMed]
71. Paunovic, N.; Lundh, L.-G.; Öst, L.-G. Attentional and memory bias for emotional information in crime victims with acute posttraumatic stress disorder (PTSD). *J. Anxiety Disord.* **2002**, *16*, 675–692. [CrossRef]

72. Küpper, C.S.; Benoit, R.G.; Dalgleish, T.; Anderson, M.C. Direct suppression as a mechanism for controlling unpleasant memories in daily life. *J. Exp. Psychol. Gen.* **2014**, *143*, 1443–1449. [CrossRef]
73. Baumann, M.; Zwissler, B.; Schalinski, I.; Ruf-Leuschner, M.; Schauer, M.; Kissler, J.M. Directed Forgetting in Post-Traumatic-Stress-Disorder: A Study of Refugee Immigrants in Germany. *Front. Behav. Neurosci.* **2013**, *7*, 94. [CrossRef]
74. Blix, I.; Brennen, T. Intentional Forgetting of Emotional Words after Trauma: A Study with Victims of Sexual Assault. *Front. Psychol.* **2011**, *2*, 235. [CrossRef]
75. Cottencin, O.; Vaiva, G.; Huron, C.; Devos, P.; Ducrocq, F.; Jouvent, R.; Goudemand, M.; Thomas, P. Directed forgetting in PTSD: A comparative study versus normal controls. *J. Psychiatr. Res.* **2006**, *40*, 70–80. [CrossRef] [PubMed]
76. DePrince, A.P.; Freyd, J.J. Forgetting Trauma Stimuli. *Psychol. Sci.* **2004**, *15*, 488–492. [CrossRef] [PubMed]
77. Mary, A.; Dayan, J.; Leone, G.; Postel, C.; Fraisse, F.; Malle, C.; Vallée, T.; Klein-Peschanski, C.; Viader, F.; De La Sayette, V.; et al. Resilience after trauma: The role of memory suppression. *Science* **2020**, *367*, eaay8477. [CrossRef] [PubMed]
78. Tudorache, A.-C.; El-Hage, W.; Tapia, G.; Goutaudier, N.; Kalenzaga, S.; Bouazzaoui, B.; Jaafari, N.; Clarys, D. Inhibitory control of threat remembering in PTSD. *Memory* **2019**, *27*, 1404–1414. [CrossRef]
79. Waldhauser, G.T.; Dahl, M.J.; Ruf-Leuschner, M.; Müller-Bamouh, V.; Schauer, M.; Axmacher, N.; Elbert, T.; Hanslmayr, S. The neural dynamics of deficient memory control in heavily traumatized refugees. *Sci. Rep.* **2018**, *8*, 13132. [CrossRef] [PubMed]
80. Van Schie, K.; Anderson, M.C. Successfully controlling intrusive memories is harder when control must be sustained. *Memory* **2017**, *25*, 1201–1216. [CrossRef] [PubMed]
81. Zoellner, L.A.; Sacks, M.B.; Foa, E.B. Directed forgetting following mood induction in chronic posttraumatic stress disorder patients. *J. Abnorm. Psychol.* **2003**, *112*, 508–514. [CrossRef]
82. Sauseng, P.; Klimesch, W.; Doppelmayr, M.; Pecherstorfer, T.; Freunberger, R.; Hanslmayr, S. EEG alpha synchronization and functional coupling during top-down processing in a working memory task. *Hum. Brain Mapp.* **2005**, *26*, 148–155. [CrossRef] [PubMed]
83. Cook, F.; Ciorciari, J.; Varker, T.; Devilly, G.J. Changes in long term neural connectivity following psychological trauma. *Clin. Neurophysiol.* **2009**, *120*, 309–314. [CrossRef] [PubMed]
84. Amir, N.; Taylor, C.T.; Bomyea, J.A.; Badour, C.L. Temporal allocation of attention toward threat in individuals with posttraumatic stress symptoms. *J. Anxiety Disord.* **2009**, *23*, 1080–1085. [CrossRef]
85. Payne, A.; Kralj, A.; Young, J.; Meiser-Stedman, R. The prevalence of intrusive memories in adult depres- sion: A meta-analysis. *J. Affect Diosord.* **2019**, *53*, 193–202. [CrossRef]
86. Power, M.; Dalgleish, T.; Claudio, V.; Tata, P.; Kentish, J. The directed forgetting task: Application to emotionally valent material. *J. Affect. Disord.* **2000**, *57*, 147–157. [CrossRef]
87. Wingenfeld, K.; Terfehr, K.; Meyer, B.; Löwe, B.; Spitzer, C. Memory Bias for Emotional and Illness-Related Words in Patients with Depression, Anxiety and Somatization Disorders: An Investigation with the Directed Forgetting Task. *Psychopathology* **2013**, *46*, 22–27. [CrossRef]
88. Beck, A.T.; Steer, R.A.; Brown, G.K. *Manual for the Beck Depression Inventory–II*; Psychological Corporation: San Antonio, TX, USA, 1996.
89. Xie, H.; Jiang, D.; Zhang, D. Individuals with depressive tendencies experience difficulty in forgetting negative material: Two mechanisms revealed by ERP data in the directed forgetting paradigm. *Sci. Rep.* **2018**, *8*, 1–14. [CrossRef]
90. Kuehl, L.K.; Wolf, O.T.; Driessen, M.; Schlosser, N.; Fernando, S.C.; Wingenfeld, K. Effects of cortisol on the memory bias for emotional words? A study in patients with depression and healthy participants using the Directed Forgetting task. *J. Psychiatr. Res.* **2017**, *92*, 191–198. [CrossRef]
91. Groome, D.; Sterkaj, F. Retrieval-induced forgetting and clinical depression. *Cogn. Emot.* **2010**, *24*, 63–70. [CrossRef]
92. Sacchet, M.D.; Levy, B.J.; Hamilton, J.P.; Maksimovskiy, A.; Hertel, P.T.; Joormann, J.; Anderson, M.C.; Wagner, A.D.; Gotlib, I.H. Cognitive and neural consequences of memory suppression in major depressive disorder. *Cogn. Affect. Behav. Neurosci.* **2017**, *17*, 77–93. [CrossRef]
93. Noreen, S.; Cooke, R.; Ridout, N. Investigating the mediating effect of working memory on intentional forgetting in dysphoria. *Psychol. Res.* **2020**, *84*, 2273–2286. [CrossRef]
94. Anticevic, A.; Repovs, G.; Corlett, P.R.; Barch, D.M. Negative and Nonemotional Interference with Visual Working Memory in Schizophrenia. *Biol. Psychiatry* **2011**, *70*, 1159–1168. [CrossRef]
95. Diaz, M.T.; He, G.; Gadde, S.; Bellion, C.; Belger, A.; Voyvodic, J.T.; McCarthy, G. The influence of emotional distraction on verbal working memory: An fMRI investigation comparing individuals with schizophrenia and healthy adults. *J. Psychiatr. Res.* **2011**, *45*, 1184–1193. [CrossRef] [PubMed]
96. Horan, W.P.; Hajcak, G.; Wynn, J.K.; Green, M.F. Impaired emotion regulation in schizophrenia: Evidence from event-related potentials. *Psychol. Med.* **2013**, *43*, 2377–2391. [CrossRef] [PubMed]
97. Strauss, G.P.; Kappenman, E.S.; Culbreth, A.J.; Catalano, L.T.; Ossenfort, K.L.; Lee, B.G.; Gold, J.M. Emotion regulation abnormalities in schizophrenia: Directed attention strategies fail to decrease the neurophysiological response to unpleasant stimuli. *J. Abnorm. Psychol.* **2015**, *124*, 288–301. [CrossRef]
98. Brébion, G.; Gorman, J.M.; Malaspina, D.; Amador, X. A model of verbal memory impairments in schizophrenia: Two systems and their associations with underlying cognitive processes and clinical symptoms. *Psychol. Med.* **1999**, *35*, 133–142. [CrossRef] [PubMed]

99. Badcock, J.C.; Mahfouda, S.; Maybery, M.T. Hallucinations and inhibitory functioning in healthy young adults with high and low levels of hypomanic personality traits. *Cogn. Neuropsychiatry* **2015**, *20*, 254–269. [CrossRef] [PubMed]
100. Brébion, G.; Stephan-Otto, C.; Ochoa, S.; Roca, M.; Nieto, L.; Usall, J. Impaired Self-Monitoring of Inner Speech in Schizophrenia Patients with Verbal Hallucinations and in Non-clinical Individuals Prone to Hallucinations. *Front. Psychol.* **2016**, *7*, 1381. [CrossRef]
101. Müller, U.; Ullsperger, M.; Hammerstein, E.; Sachweh, S.; Becker, T. Directed forgetting in schizophrenia. *Eur. Arch. Psychiatry Clin. Neurosci.* **2004**, *255*, 251–257. [CrossRef]
102. Patrick, R.E.; Kiang, M.; Christensen, B.K. Neurophysiological correlates of emotional directed-forgetting in persons with Schizophrenia: An event-related brain potential study. *Int. J. Psychophysiol.* **2015**, *98*, 612–623. [CrossRef] [PubMed]
103. Patrick, R.E.; Christensen, B.K. Reduced directed forgetting for negative words suggests schizophrenia-related disinhibition of emotional cues. *Psychol. Med.* **2013**, *43*, 2289–2299. [CrossRef]
104. Racsmány, M.; Conway, M.A.; Garab, E.A.; Cimmer, C.; Janka, Z.; Kurimay, T.; Pléh, C.; Szendi, I. Disrupted memory inhibition in schizophrenia. *Schizophr. Res.* **2008**, *101*, 218–224. [CrossRef] [PubMed]
105. Sahakyan, L.; Kwapil, T.R.; Jiang, L. Differential impairment of positive and negative schizotypy in list-method and item-method directed forgetting. *J. Exp. Psychol. Gen.* **2020**, *149*, 368–381. [CrossRef]
106. Alderson-Day, B.; Smailes, D.; Moffatt, J.; Mitrenga, K.; Moseley, P.; Fernyhough, C. Intentional inhibition but not source memory is related to hallucination-proneness and intrusive thoughts in a university sample. *Cortex* **2019**, *113*, 267–278. [CrossRef]
107. Debbané, M.; Glaser, B.; Eliez, S. Encoding and retrieval processes in velo-cardio-facial syndrome (VCFS). *Neuropsychology* **2008**, *22*, 226–234. [CrossRef] [PubMed]
108. Okruszek, Ł.; Jarkiewicz, M.; Szrubarz, P.; Wichniak, A.; Michałowski, J.; Marchewka, A.; Łojek, E. Many ways to forget—Neurophysiology of directed forgetting mechanisms in schizophrenia. *Psychiatry Res.* **2019**, *274*, 358–364. [CrossRef] [PubMed]
109. Nestor, P.G.; Piech, R.; Allen, C.; Niznikiewicz, M.; Shenton, M.; McCarley, R.W. Retrieval-induced forgetting in schizophrenia. *Schizophr. Res.* **2005**, *75*, 199–209. [CrossRef] [PubMed]
110. AhnAllen, C.G.; Nestor, P.G.; McCarley, R.W.; Shenton, M.E. The role of retrieval inhibition in the associative memory impairment of schizophrenia. *Psychiatry Res.* **2007**, *150*, 43–50. [CrossRef]
111. Soriano, M.F.; Jimenez, J.F.; Roman, P.; Bajo, M.T. Inhibitory processes in memory are impaired in schizophrenia: Evidence from retrieval induced forgetting. *Br. J. Psychol.* **2009**, *100*, 661–673. [CrossRef]
112. Benzina, N.; Mallet, L.; Burguière, E.; N'Diaye, K.; Pelissolo, A. Cognitive Dysfunction in Obsessive-Compulsive Disorder. *Curr. Psychiatry Rep.* **2016**, *18*, 1–11. [CrossRef]
113. Fullana, M.A.; Abramovitch, A.; Via, E.; López-Sola, C.; Goldberg, X.; Reina, N.; Fortea, L.; Solanes, A.; Buckley, M.J.; Ramella-Cravaro, V.; et al. Diagnostic biomarkers for obsessive-compulsive disorder: A reasonable quest or ignis fatuus? *Neurosci. Biobehav. Rev.* **2020**, *118*, 504–513. [CrossRef] [PubMed]
114. Lipton, M.G.; Brewin, C.R.; Linke, S.; Halperin, J. Distinguishing features of intrusive images in obsessive–compulsive disorder. *J. Anxiety Disord.* **2010**, *24*, 816–822. [CrossRef]
115. Cavedini, P.; Zorzi, C.; Piccinni, M.; Cavallini, M.C.; Bellodi, L. Executive Dysfunctions in Obsessive-Compulsive Patients and Unaffected Relatives: Searching for a New Intermediate Phenotype. *Biol. Psychiatry* **2010**, *67*, 1178–1184. [CrossRef]
116. Demeter, G.; Keresztes, A.; Harsanyi, A.; Csigò, K. Obsessed not to forget: Lack of retrieval-induced sup- pression effect in obsessive-compulsive disorder. *Psychiatry Res.* **2014**. [CrossRef]
117. Olley, A.; Malhi, G.; Sachdev, P. Memory and executive functioning in obsessive–compulsive disorder: A selective review. *J. Affect. Disord.* **2007**, *104*, 15–23. [CrossRef] [PubMed]
118. Rao, N.P.; Reddy, Y.J.; Kumar, K.J.; Kandavel, T.; Chandrashekar, C. Are neuropsychological deficits trait markers in OCD? *Prog. Neuro Psychopharmacol. Biol. Psychiatry* **2008**, *32*, 1574–1579. [CrossRef] [PubMed]
119. Chamberlain, S.; Blackwell, A.; Fineberg, N.; Robbins, T.; Sahakian, B. The neuropsychology of obsessive compulsive disorder: The importance of failures in cognitive and behavioural inhibition as candidate endophenotypic markers. *Neurosci. Biobehav. Rev.* **2005**, *29*, 399–419. [CrossRef]
120. Ewood, J.; Ahmari, S.E. A Framework for Understanding the Emerging Role of Corticolimbic-Ventral Striatal Networks in OCD-Associated Repetitive Behaviors. *Front. Syst. Neurosci.* **2015**, *9*, 171. [CrossRef]
121. Wilhelm, S.; McNally, R.J.; Baer, L.; Florin, I. Directed forgetting in obsessive-compulsive disorder. *Behav. Res. Ther.* **1996**, *34*, 633–641. [CrossRef]
122. Bohne, A.; Savage, C.R.; Deckersbach, T.; Keuthen, N.J.; Jenike, M.A.; Tuschen-Caffier, B.; Wilhelm, S. Visuospatial Abilities, Memory, and Executive Functioning in Trichotillomania and Obsessive-Compulsive Disorder. *J. Clin. Exp. Neuropsychol.* **2005**, *27*, 385–399. [CrossRef]
123. Tolin, D.F.; Hamlin, C.; Foa, E.B. Directed forgetting in obsessive-compulsive disorder: Replication and extension. *Behav. Res. Ther.* **2002**, *40*, 793–803. [CrossRef]
124. Moritz, S.; Hottenrott, B.; Jelinek, L.; Brooks, A.M.; Scheurich, A. Effects of Obsessive-Compulsive Symptoms on Neuropsychological Test Performance: Complicating an Already Complicated Story. *Clin. Neuropsychol.* **2012**, *26*, 31–44. [CrossRef]
125. Konishi, M.; Shishikura, K.; Nakaaki, S.; Komatsu, S.-I.; Mimura, M. Remembering and forgetting: Directed forgetting effect in obsessive-compulsive disorder. *Neuropsychiatr. Dis. Treat.* **2011**, *7*, 365–372. [CrossRef] [PubMed]

126. Jelinek, L.; Rietschel, L.; Kellner, M.; Muhtz, C.; Moritz, S. The effect of practice on the recall of salient information in obsessive–compulsive disorder. *Psychiatry Res.* **2012**, *198*, 89–93. [CrossRef] [PubMed]
127. Niciu, M.J.; Kelmendi, B.; Sanacora, G. Overview of glutamatergic neurotransmission in the nervous system. *Pharmacol. Biochem. Behav.* **2012**, *100*, 656–664. [CrossRef] [PubMed]
128. Castellano, C.; Cestari, V.; Ciamei, A. NMDA receptors and learning and memory processes. *Curr. Drug Targets* **2001**, *2*, 273–283. [CrossRef] [PubMed]
129. Rezvani, A.H.; Levin, E.D.; Buccafusco, J.J. *Involvement of the NMDA System in Learning and Memory*; CRC Press/Taylor & Francis: Abingdon, UK, 2006; Chapter 4.
130. Woodside, B.; Borroni, A.; Hammonds, M.; Teyler, T. NMDA receptors and voltage-dependent calcium channels mediate different aspects of acquisition and retention of a spatial memory task. *Neurobiol. Learn. Mem.* **2004**, *81*, 105–114. [CrossRef] [PubMed]
131. Walker, D.L.; Davis, M. Involvement of NMDA receptors within the amygdala in short- versus long-term memory for fear condiDoning as assessed with fear-potenDated startle. *Behav. Neurosci.* **2000**, *114*, 1019–1033. [CrossRef] [PubMed]
132. Quartermain, D.; Nuygen, T.; Sheu, J.; Herting, R.L. Milacemide enhances memory storage and alleviates spontaneous forgetting in mice. *Pharmacol. Biochem. Behav.* **1991**, *39*, 31–35. [CrossRef]
133. Sachser, R.M.; Santana, F.; Crestani, A.P.; Lunardi, P.; Pedraza, L.K.; Quillfeldt, J.A.; Hardt, O.; Alvares, L.D.O. Forgetting of long-term memory requires activation of NMDA receptors, L-type voltage-dependent Ca^{2+} channels, and calcineurin. *Sci. Rep.* **2016**, *6*, 22771. [CrossRef] [PubMed]
134. Sachser, R.M.; Haubrich, J.; Lunardi, P.S.; Alvares, L.D.O. Forgetting of what was once learned: Exploring the role of postsynaptic ionotropic glutamate receptors on memory formation, maintenance, and decay. *Neuropharmacology* **2017**, *112*, 94–103. [CrossRef] [PubMed]
135. Migues, P.V.; Liu, L.; Archbold, G.E.B.; Einarsson, E.Ö.; Wong, J.; Bonasia, K.; Ko, S.H.; Wang, Y.T.; Hardt, O. Blocking Synaptic Removal of GluA2-Containing AMPA Receptors Prevents the Natural Forgetting of Long-Term Memories. *J. Neurosci.* **2016**, *36*, 3481–3494. [CrossRef]
136. Hardt, O.; Nader, K.; Nadel, L. Decay happens: The role of active forgetting in memory. *Trends Cogn. Sci.* **2013**, *17*, 111–120. [CrossRef] [PubMed]
137. Shinohara, K.; Hata, T. Post-acquisition hippocampal blockade of the NMDA receptor subunit GluN2A but not GluN2B sustains spatial reference memory retention. *Neurobiol. Learn. Mem.* **2018**, *147*, 1–8. [CrossRef]
138. Wong, C.G.T.; Bottiglieri, T.; Snead, O.C. GABA, γ-hydroxybutyric acid, and neurological disease. *Ann. Neurol.* **2003**, *54*, S3–S12. [CrossRef]
139. Haefely, W.; Martin, J.R.; Schoch, P. Novel anxiolytics that act as partial agonists at benzodiazepine receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 452–456. [CrossRef]
140. Olsen, R.W.; Spigelman, I. GABA-a Recaptor Plasticity in Alcohol Withdrawal. In *JASPER'S Basic Mechanisms of the Epilepsies*, 4th ed.; National Center for Biotechnology Information: Bethesda, MD, USA, 2012.
141. Davis, M.; Campeau, S.; Kim, M.; Falls, W.A.; McGaugh, J.L.; Weinberger, N.M.; Lynch, G. Neural Systems of Emotion: The Amygdala's Role in Fear and Anxiety. In *Brain and Memory Modulation and Mediation of Neuroplasticity*; Oxford University Press (OUP): Oxford, UK, 1995; pp. 3–40.
142. Myers, K.M.; Davis, M. Systems-Level Reconsolidation. *Neuron* **2002**, *36*, 340–343. [CrossRef]
143. Davies, C.; Starkey, S.; Pozza, M.; Collingridge, G. GABA-B autoreceptors regulate the induction of LTP. *Nature* **1991**, *349*, 609–611. [CrossRef] [PubMed]
144. Kim, J.H.; McNally, G.P.; Richardson, R. Recovery of fear memories in rats: Role of gamma-amino butyric acid (GABA) in infantile amnesia. *Behav. Neurosci.* **2006**, *120*, 40–48. [CrossRef] [PubMed]
145. Kim, J.H.; Richardson, R. A developmental dissociation of context and GABA effects on extinguished fear in rats. *Behav. Neurosci.* **2007**, *121*, 131–139. [CrossRef]
146. Tang, H.H.Y.; McNally, G.P.; Richardson, R. The effects of FG7142 on two types of forgetting in 18-day-old rats. *Behav. Neurosci.* **2007**, *121*, 1421–1425. [CrossRef] [PubMed]
147. Zeman, A.; Hoefeijzers, S.; Milton, F.; Dewar, M.; Carr, M.; Streatfield, C. The GABAB receptor agonist, baclofen, contributes to three distinct varieties of amnesia in the human brain—A detailed case report. *Cortex* **2016**, *74*, 9–19. [CrossRef] [PubMed]
148. Bergstrom, H.C.; Pinard, C.R. Corticolimbic circuits in learning, memory, and disease. *J. Neurosci. Res.* **2017**, *95*, 795–796. [CrossRef]
149. Blake, M.G.; Boccia, M.M. Basal Forebrain Cholinergic System and Memory. *Curr. Top. Behav. Neurosci.* **2016**, 253–273. [CrossRef]
150. Bentley, P.; Driver, J.; Dolan, R.J. Cholinergic modulation of cognition: Insights from human pharmacological functional neuroimaging. *Prog. Neurobiol.* **2011**, *94*, 360–388. [CrossRef]
151. Bertrand, D.; Wallace, T.L. A Review of the Cholinergic System and Therapeutic Approaches to Treat Brain Disorders. *Curr. Top. Behav. Neurosci.* **2020**, 1–28. [CrossRef]
152. Edginton, T.; Rusted, J.M. Separate and combined effects of scopolamine and nicotine on retrieval-induced forgetting. *Psychopharmacology* **2003**, *170*, 351–357. [CrossRef] [PubMed]
153. Rusted, J.M.; Alvares, T. Nicotine effects on retrieval-induced forgetting are not attributable to changes in arousal. *Psychopharmacology* **2008**, *196*, 83–92. [CrossRef] [PubMed]

154. Klein, M.O.; Battagello, D.S.; Cardoso, A.R.; Hauser, D.N.; Bittencourt, J.C.; Correa, R.G. Dopamine: Functions, signaling, and association with neurological diseases. *Cell. Mol. Nurobiol.* **2019**, *39*, 31–59. [CrossRef] [PubMed]
155. Grogan, J.P.; Bogacz, R.; Tsivos, D.; Whone, A.L.; Coulthard, E. Dopamine and Consolidation of Episodic Memory: Timing Is Everything. *J. Cogn. Neurosci.* **2015**, *27*, 2035–2050. [CrossRef] [PubMed]
156. Karunakaran, S.; Chowdhury, A.; Donato, F.; Quairiaux, C.; Michel, C.M.; Caroni, P. PV plasticity sustained through D1/5 dopamine signaling required for long-term memory consolidation. *Nat. Neurosci.* **2016**, *19*, 454–464. [CrossRef] [PubMed]
157. Péczely, L.; Ollmann, T.; László, K.; Kovács, A.; Gálosi, R.; Kertes, E.; Zagorácz, O.; Kállai, V.; Karádi, Z.; Lénárd, L. Role of ventral pallidal D2 dopamine receptors in the consolidation of spatial memory. *Behav. Brain Res.* **2016**, *313*, 1–9. [CrossRef]
158. Yamasaki, M.; Takeuchi, T. Locus Coeruleus and Dopamine-Dependent Memory Consolidation. *Neural Plast.* **2017**, *2017*, 1–15. [CrossRef]
159. Berry, J.A.; Cervantes-Sandoval, I.; Nicholas, E.P.; Davis, R.L. Dopamine Is Required for Learning and Forgetting in Drosophila. *Neuron* **2012**, *74*, 530–542. [CrossRef]
160. Berry, J.A.; Cervantes-Sandoval, I.; Chakraborty, M.; Davis, R.L. Sleep Facilitates Memory by Blocking Dopamine Neuron-Mediated Forgetting. *Cell* **2015**, *161*, 1656–1667. [CrossRef]
161. Reichenbach, N.; Herrmann, U.; Kähne, T.; Schicknick, H.; Pielot, R.; Naumann, M.; Dieterich, D.C.; Gundelfinger, E.D.; Smalla, K.-H.; Tischmeyer, W. Differential effects of dopamine signalling on long-term memory formation and consolidation in rodent brain. *Proteome Sci.* **2015**, *13*, 13. [CrossRef]
162. Rossignoli, M.T.; Lopes-Aguiar, C.; Ruggiero, R.N.; Da Silva, R.A.D.V.; Bueno-Junior, L.S.; Kandratavicius, L.; Peixoto-Santos, J.E.; Crippa, J.A.; Hallak, J.E.C.; Zuardi, A.W.; et al. Selective post-training time window for memory consolidation interference of cannabidiol into the prefrontal cortex: Reduced dopaminergic modulation and immediate gene expression in limbic circuits. *Neuroscience* **2017**, *350*, 85–93. [CrossRef] [PubMed]
163. Cervantes-Sandoval, I.; Chakraborty, M.; MacMullen, C.; Davis, R.L. Scribble Scaffolds a Signalosome for Active Forgetting. *Neuron* **2016**, *90*, 1230–1242. [CrossRef]
164. Kaun, K.R.; Rothenfluh, A. Dopaminergic rules of engagement for memory in Drosophila. *Curr. Opin. Neurobiol.* **2017**, *43*, 56–62. [CrossRef]
165. Himmelreich, S.; Masuho, I.; Berry, J.A.; MacMullen, C.; Skamangas, N.K.; Martemyanov, K.A.; Davis, R.L. Dopamine Receptor DAMB Signals via Gq to Mediate Forgetting in Drosophila. *Cell Rep.* **2017**, *21*, 2074–2081. [CrossRef]
166. Shuai, Y.; Hirokawa, A.; Ai, Y.; Zhang, M.; Li, W.; Zhong, Y. Dissecting neural pathways for forgetting in Drosophila olfactory aversive memory. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E6663–E6672. [CrossRef] [PubMed]
167. Nilsson, M.K.; Carlsson, M.L. The monoaminergic stabilizer (−)-OSU6162 reverses delay-dependent natural forgetting and improves memory impairment induced by scopolamine in mice. *Neuropharmacology* **2013**, *75*, 399–406. [CrossRef] [PubMed]
168. Ruiz, M.J.; Paolieri, D.; Colzato, L.S.; Bajo, M.T. Directed forgetting of memories in cocaine users. *Exp. Clin. Psychopharmacol.* **2016**, *24*, 423–435. [CrossRef] [PubMed]
169. Papenberg, G.; Bäckman, L.; Nagel, I.E.; Nietfeld, W.; Schröder, J.; Bertram, L.; Heekeren, H.R.; Lindenberger, U.; Li, S.-C. Dopaminergic Gene Polymorphisms Affect Long-term Forgetting in Old Age: Further Support for the Magnification Hypothesis. *J. Cogn. Neurosci.* **2013**, *25*, 571–579. [CrossRef] [PubMed]
170. Wimber, M.; Schott, B.H.; Wendler, F.; Seidenbecher, C.I.; Behnisch, G.; Macharadze, T.; Bäuml, K.H.; Richardson-Klavehn, A. Prefrontal dopamine and the dynamic control of human long-term memory. Trans-cult. *Psychiatry* **2011**, *1*, 15.
171. Dolan, R.J. Emotion, Cognition, and Behavior. *Science* **2002**, *298*, 1191–1194. [CrossRef]
172. Phelps, E.A.; Delgado, M.R.; Nearing, K.I.; LeDoux, J.E. Extinction Learning in Humans. *Neuron* **2004**, *43*, 897–905. [CrossRef]
173. McGaugh, J.L.; Izquierdo, I. The contribution of pharmacology to research on the mechanisms of memory formation. *Trends Pharmacol. Sci.* **2000**, *21*, 208–210. [CrossRef]
174. Strange, B.A.; Hurlemann, R.; Dolan, R.J. An emotion-induced retrograde amnesia in humans is amygdala- and -adrenergic-dependent. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13626–13631. [CrossRef]
175. Strange, B.A.; Dolan, R.J. Adrenergic modulation of emotional memory-evoked human amygdala and hippocampal responses. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 11454–11458. [CrossRef]
176. Van Stegeren, A.H.; Goekoop, R.; Everaerd, W.; Scheltens, P.; Barkhof, F.; Kuijer, J.P.; Rombouts, S.A. Noradrenaline mediates amygdala activation in men and women during encoding of emotional material. *NeuroImage* **2005**, *24*, 898–909. [CrossRef] [PubMed]
177. Harmer, C.J.; Hill, S.A.; Taylor, M.J.; Cowen, P.J.; Goodwin, G.M. Toward a Neuropsychological Theory of Antidepressant Drug Action: Increase in Positive Emotional Bias After Potentiation of Norepinephrine Activity. *Am. J. Psychiatry* **2003**, *160*, 990–992. [CrossRef]
178. Harmer, C.J.; Shelley, N.C.; Cowen, P.J.; Goodwin, G.M. Increased Positive Versus Negative Affective Perception and Memory in Healthy Volunteers Following Selective Serotonin and Norepinephrine Reuptake Inhibition. *Am. J. Psychiatry* **2004**, *161*, 1256–1263. [CrossRef] [PubMed]
179. Hurlemann, R.; Hawellek, B.; Matusch, A.; Kolsch, H.; Wollersen, H.; Madea, B.; Vogeley, K.; Maier, W.; Dolan, R.J. Noradrenergic Modulation of Emotion-Induced Forgetting and Remembering. *J. Neurosci.* **2005**, *25*, 6343–6349. [CrossRef] [PubMed]
180. Quartermain, D.; Leo, P. Strength of scopolamine-induced amnesia as a function of time between training and testing. *Behav. Neural Biol.* **1988**, *50*, 300–310. [CrossRef]

181. Decker, M.W.; McGaugh, J.L. Effects of concurrent manipulations of cholinergic and noradrenergic function on learning and retention in mice. *Brain Res.* **1989**, *477*, 29–37. [CrossRef]
182. Finsterwald, C.; Alberini, C.M. Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: From adaptive responses to psychopathologies. *Neurobiol. Learn. Mem.* **2014**, *112*, 17–29. [CrossRef]
183. Meir Drexler, S.; Wolf, O.T. The role of glucocorticoids in emotional memory reconsolidation. *Neurobiol. Learn. Mem.* **2016**. [CrossRef]
184. Wolf, O.T. Stress and memory in humans: Twelve years of progress? *Brain Res.* **2009**, *1293*, 142–154. [CrossRef]
185. De Quervain, D.; Schwabe, L.; Roozendaal, B. Stress, glucocorticoids and memory:implications for treating fear-related disorders. *Nat. Rev. Neurosci.* **2017**, *18*, 7–19. [CrossRef]
186. Het, S.; Ramlow, G.; Wolf, O. A meta-analytic review of the effects of acute cortisol administration on human memory. *Psychoneuroendocrinology* **2005**, *30*, 771–784. [CrossRef]
187. Wolf, O.T.; Atsak, P.; De Quervain, D.J.; Roozendaal, B.; Wingenfeld, K. Stress and Memory: A Selective Review on Recent Developments in the Understanding of Stress Hormone Effects on Memory and Their Clinical Relevance. *J. Neuroendocr.* **2016**, *28*. [CrossRef] [PubMed]
188. Koessler, S.; Steidle, L.; Engler, H.; Kissler, J. Stress eliminates retrieval-induced forgetting—Does the oral application of cortisol? *Psychoneuroendocrinology* **2013**, *38*, 94–106. [CrossRef] [PubMed]
189. Koessler, S.; Engler, H.; Riether, C.; Kissler, J. No Retrieval-Induced Forgetting Under Stress. *Psychol. Sci.* **2009**, *20*, 1356–1363. [CrossRef] [PubMed]
190. Quaedflieg, C.; Schneider, T.; Daume, J.; Engel, A.; Schwabe, L. Stress impairs intentional memory control through altered theta oscillations in lateral parietal cortex. *J. Neurosci.* **2020**, *40*, 7739–7748. [CrossRef]
191. Engen, H.G.; Anderson, M.C. Memory Control: A Fundamental Mechanism of Emotion Regulation. *Trends Cogn. Sci.* **2018**, *22*, 982–995. [CrossRef]
192. Barkley, R.A. Behavioral inhibition, sustained attention, and executive functions: Constructing a unifying theory of ADHD. *Psychol. Bull.* **1997**, *121*, 65–94. [CrossRef]
193. Conway, M.A.; Fthenaki, A. Disruption of Inhibitory Control of Memory Following Lesions to the Frontal and Temporal Lobes. *Cortex* **2003**, *39*, 667–686. [CrossRef]
194. Bari, A.; Robbins, T.W. Inhibition and impulsivity: Behavioral and neural basis of response control. *Prog. Neurobiol.* **2013**, *108*, 44–79. [CrossRef]
195. Aron, A.R.; Robbins, T.W.; Poldrack, R.A. Inhibition and the right inferior frontal cortex: One decade on. *Trends Cogn. Sci.* **2014**, *18*, 177–185. [CrossRef] [PubMed]
196. Anderson, M.C.; Bunce, J.G.; Barbas, H. Prefrontal–hippocampal pathways underlying inhibitory control over memory. *Neurobiol. Learn. Mem.* **2016**, *134*, 145–161. [CrossRef] [PubMed]
197. McEwen, B.S.; Nasca, C.; Gray, J.D. Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology* **2016**, *41*, 3–23. [CrossRef]
198. Bartholomew, M.E.; Heller, W.; Miller, G.A. Inhibitory control of emotional processing: Theoretical and empirical considerations. *Int. J. Psychophysiol.* **2019**. [CrossRef] [PubMed]
199. McKlveen, J.M.; Moloney, R.D.; Scheimann, J.R.; Myers, B.; Herman, J.P. "Braking" the Prefrontal Cortex: The Role of Glucocorticoids and Interneurons in Stress Adaptation and Pathology. *Biol. Psychiatry* **2019**, *86*, 669–681. [CrossRef]
200. Anderson, M.C.; Hulbert, J.C. Active Forgetting: Adaptation of Memory by Prefrontal Control. *Annu. Rev. Psychol.* **2021**, *72*, 1–36. [CrossRef]
201. Del Arco, A.; Mora, F. Glutamate-dopamine in vivo interaction in the prefrontalcortex modulates the release of dopamine and acetylcholinein the nucleus accumbens of the awake rat. *J. Neural Transm.* **2005**, *112*, 97–109. [CrossRef] [PubMed]
202. Robbins, T.W.; Arnsten, A.F.T. The Neuropsychopharmacology of Fronto-Executive Function: Monoaminergic Modulation. *Annu. Rev. Neurosci.* **2009**, *32*, 267–287. [CrossRef] [PubMed]
203. Duvarci, S.; Pare, D. Amygdala Microcircuits Controlling Learned Fear. *Neuron* **2014**, *82*, 966–980. [CrossRef]
204. Schmitz, T.W.; Correia, M.M.; Ferreira, C.S.; Prescot, A.P.; Anderson, M.C. Hippocampal GABA enables inhibitory control over unwanted thoughts. *Nat. Commun.* **2017**, *8*, 1–12. [CrossRef]
205. Depue, B.E.; Curran, T.; Banich, M.T. Prefrontal Regions Orchestrate Suppression of Emotional Memories via a Two-Phase Process. *Science* **2007**, *317*, 215–219. [CrossRef]
206. Ghashghaei, H.; Barbas, H. Pathways for emotion: Interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience* **2002**, *115*, 1261–1279. [CrossRef]
207. Sotres-Bayon, F.; Quirk, G.J. Prefrontal control of fear: More than just extinction. *Curr. Opin. Neurobiol.* **2010**, *20*, 231–235. [CrossRef] [PubMed]
208. Giustino, T.F.; Emaren, S. The Role of the Medial Prefrontal Cortex in the Conditioning and Extinction of Fear. *Front. Behav. Neurosci.* **2015**, *9*, 298. [CrossRef] [PubMed]
209. Del Arco, A.; Mora, F. Neurotransmitters and prefrontal cortex–limbic system interactions: Implications for plasticity and psychiatric disorders. *J. Neural. Transm.* **2009**, *116*, 941–952. [CrossRef] [PubMed]
210. Wellman, C.L. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *J. Neurobiol.* **2001**, *49*, 245–253. [CrossRef]

211. Likhtik, E.; Johansen, J.P. Neuromodulation in circuits of aversive emotional learning. *Nat. Neurosci.* **2019**, *22*, 1586–1597. [CrossRef]
212. Bast, T.; Pezze, M.; McGarrity, S. Cognitive deficits caused by prefrontal cortical and hippocampal neural disinhibition. *Br. J. Pharmacol.* **2017**, *174*, 3211–3225. [CrossRef]
213. Bloem, B.; Poorthuis, R.B.; Mansvelder, H.D. Cholinergic modulation of the medial prefrontal cortex: The role of nicotinic receptors in attention and regulation of neuronal activity. *Front. Neural Circuits* **2014**, *8*. [CrossRef]
214. Parikh, V.; Bangasser, D.A. Cholinergic Signaling Dynamics and Cognitive Control of Attention. *Behav. Neurobiol. Chronic Pain* **2020**, 71–87. [CrossRef]
215. Kasparbauer, A.-M.; Petrovsky, N.; Schmidt, P.-M.; Trautner, P.; Weber, B.; Sträter, B.; Ettinger, U. Effects of nicotine and atomoxetine on brain function during response inhibition. *Eur. Neuropsychopharmacol.* **2019**, *29*, 235–246. [CrossRef] [PubMed]
216. Berridge, C.W.; Waterhouse, B.D. The locus coeruleus–noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. *Brain Res. Rev.* **2003**, *42*, 33–84. [CrossRef]
217. Jouvet, M. Biogenic Amines and the States of Sleep. *Science* **1969**, *163*, 32–41. [CrossRef] [PubMed]
218. Wise, R.A.; Rompre, P.P. Brain Dopamine and Reward. *Annu. Rev. Psychol.* **1989**, *40*, 191–225. [CrossRef]
219. Aston-Jones, G.; Cohen, J.D. An Integrative Theory of Locus Coeruleus-Norepinephrine Function: Adaptive Gain and Optimal Performance. *Annu. Rev. Neurosci.* **2005**, *28*, 403–450. [CrossRef] [PubMed]
220. Robinson, T.E. The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res. Rev.* **1993**, *18*, 247–291. [CrossRef]
221. Mather, M.; Clewett, D.; Sakaki, M.; Harley, C.W. Norepinephrine ignites local hotspots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behav. Brain Sci.* **2016**, *39*, e200. [CrossRef]
222. Chmielewski, W.X.; Mückschel, M.; Ziemssen, T.; Beste, C. The norepinephrine system affects specific neurophysiological subprocesses in the modulation of inhibitory control by working memory demands. *Hum. Brain Mapp.* **2016**, *38*, 68–81. [CrossRef]
223. Mückschel, M.; Dippel, G.; Beste, C. Distinguishing stimulus and response codes in theta oscillations in prefrontal areas during inhibitory control of automated responses. *Hum. Brain Mapp.* **2017**, *38*, 5681–5690. [CrossRef]
224. Nieuwenhuis, S.; Aston-Jones, G.; Cohen, J.D. Decision making, the P3, and the locus coeruleus–norepinephrine system. *Psychol. Bull.* **2005**, *131*, 510–532. [CrossRef] [PubMed]
225. Solanto, M.V. Neuropsychopharmacological mechanisms of stimulant drug action in attention-deficit hyperactivity disorder: A review and integration. *Behav. Brain Res.* **1998**, *94*, 127–152. [CrossRef]
226. Dalley, J.W.; Cardinal, R.N.; Robbins, T.W. Prefrontal executive and cognitive functions in rodents: Neural and neurochemical substrates. *Neurosci. Biobehav. Rev.* **2004**, *28*, 771–784. [CrossRef]
227. Volkow, N.D.; Wang, G.J.; Fowler, J.S.; Logan, J.; Gatley, S.J.; Gifford, A.; Hitzemann, R.; Ding, Y.S.; Pappas, N. Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *Am. J. Psychiatry* **1999**, *156*, 1440–1443.
228. Volkow, N.D.; Wang, G.-J.; Fowler, J.S.; Hitzemann, R.; Angrist, B.; Gatley, S.J.; Logan, J.; Ding, Y.-S.; Pappas, N. Association of Methylphenidate-Induced Craving with Changes in Right Striato-orbitofrontal Metabolism in Cocaine Abusers: Implications in Addiction. *Am. J. Psychiatry* **1999**, *156*, 19–26. [CrossRef]
229. Eagle, D.M.; Tufft, M.R.A.; Goodchild, H.L.; Robbins, T.W. Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology* **2007**, *192*, 193–206. [CrossRef] [PubMed]
230. Chamberlain, S.R.; Hampshire, A.; Müller, U.; Rubia, K.; Del Campo, N.; Craig, K.; Regenthal, R.; Suckling, J.; Roiser, J.P.; Grant, J.E.; et al. Atomoxetine Modulates Right Inferior Frontal Activation During Inhibitory Control: A Pharmacological Functional Magnetic Resonance Imaging Study. *Biol. Psychiatry* **2009**, *65*, 550–555. [CrossRef]
231. Mückschel, M.; Roessner, V.; Beste, C. Task experience eliminates catecholaminergic effects on inhibitory control—A randomized, double-blind cross-over neurophysiological study. *Eur. Neuropsychopharmacol.* **2020**, *35*, 89–99. [CrossRef]
232. Xing, B.; Li, Y.-C.; Gao, W.-J. Norepinephrine versus dopamine and their interaction in modulating synaptic function in the prefrontal cortex. *Brain Res.* **2016**, *1641*, 217–233. [CrossRef]
233. Belanoff, J.K.; Kalehzan, M.; Sund, B.; Fleming, F.S.; Schatzberg, A.F. Cortisol activity and cognitive changes in psychotic major depres- sion. *Am. J. Psychiatry* **2001**, *158*, 1612–1616. [CrossRef] [PubMed]
234. Scholz, U.; La Marca, R.; Nater, U.M.; Aberle, I.; Ehlert, U.; Hornung, R.; Martin, M.; Kliegel, M. Go no-go performance under psychosocial stress: Beneficial effects of implementation intentions. *Neurobiol. Learn. Mem.* **2009**, *91*, 89–92. [CrossRef] [PubMed]
235. Arnsten, A.F.T.; Wang, M.; Paspalas, C.D. Dopamine's Actions in Primate Prefrontal Cortex: Challenges for Treating Cognitive Disorders. *Pharmacol. Rev.* **2015**, *67*, 681–696. [CrossRef] [PubMed]
236. Godsil, B.P.; Kiss, J.P.; Spedding, M.; Jay, T.M. The hippocampal–prefrontal pathway: The weak link in psychiatric disorders? *Eur. Neuropsychopharmacol.* **2013**, *23*, 1165–1181. [CrossRef]
237. Cummings, J.L. Anatomic and Behavioral Aspects of Frontal-Subcortical Circuits. *Ann. N. Y. Acad. Sci.* **1995**, *769*, 1–14. [CrossRef]
238. Volk, D.W.; Lewis, D.A. Impaired prefrontal inhibition in schizophrenia: Relevance for cognitive dysfunction. *Physiol. Behav.* **2002**, *77*, 501–505. [CrossRef]
239. Lewis, D.A.; Volk, D.W.; Hashimoto, T. Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: A novel target for the treatment of working memory dysfunction. *Psychopharmacology* **2003**, *174*, 143–150. [CrossRef]

240. Hayes, D.J.; Jupp, B.; Sawiak, S.J.; Merlo, E.; Caprioli, D.; Dalley, J.W. Brain γ-aminobutyric acid: A neglected role in impulsivity. *Eur. J. Neurosci.* **2014**, *39*, 1921–1932. [CrossRef] [PubMed]
241. Averill, L.A.; Purohit, P.; Averill, C.L.; Boesl, M.A.; Krystal, J.H.; Abdallah, C.G. Glutamate dysregulation and glutamatergic therapeutics for PTSD: Evidence from human studies. *Neurosci. Lett.* **2017**, *649*, 147–155. [CrossRef]
242. Ousdal, O.T.; Milde, A.M.; Craven, A.R.; Ersland, L.; Endestad, T.; Melinder, A.; Huys, Q.J.; Hugdahl, K. Prefrontal glutamate levels predict altered amygdala–prefrontal connectivity in traumatized youths. *Psychol. Med.* **2018**, *49*, 1822–1830. [CrossRef] [PubMed]
243. Sheth, C.; Prescot, A.P.; Legarreta, M.; Renshaw, P.F.; McGlade, E.; Yurgelun-Todd, D. Reduced gamma-amino butyric acid (GABA) and glutamine in the anterior cingulate cortex (ACC) of veterans exposed to trauma. *J. Affect. Disord.* **2019**, *248*, 166–174. [CrossRef] [PubMed]
244. Rodriguez, C.I.; Kegeles, L.S.; Levinson, A.; Ogden, R.T.; Mao, X.; Milak, M.S.; Vermes, D.; Xie, S.; Hunter, L.; Flood, P.; et al. In vivo effects of ketamine on glutamate-glutamine and gamma-aminobutyric acid in obsessive-compulsive disorder: Proof of concept. *Psychiatry Res. Neuroimaging* **2015**, *233*, 141–147. [CrossRef]
245. Yang, A.C.; Tsai, S.-J. New Targets for Schizophrenia Treatment beyond the Dopamine Hypothesis. *Int. J. Mol. Sci.* **2017**, *18*, 1689. [CrossRef]
246. Vlček, P.; Polák, J.; Brunovský, M.; Horáček, J. Role of Glutamatergic System in Obsessive-Compulsive Disorder with Possible Therapeutic Implications. *Pharmacopsychiatry* **2018**, *51*, 229–242. [CrossRef] [PubMed]
247. Wilkinson, S.T.; Sanacora, G. A new generation of antidepressants: An update on the pharmaceutical pipeline for novel and rapid-acting therapeutics in mood disorders based on glutamate/GABA neurotransmitter systems. *Drug Discov. Today* **2019**, *24*, 606–615. [CrossRef] [PubMed]
248. Duman, R.S.; Sanacora, G.; Krystal, J.H. Altered Connectivity in Depression: GABA and Glutamate Neurotransmitter Deficits and Reversal by Novel Treatments. *Neuron* **2019**, *102*, 75–90. [CrossRef] [PubMed]
249. Lavretsky, H.; Laird, K.T.; Krause-Sorio, B.; Heimberg, B.F.; Yeargin, J.; Grzenda, A.; Wu, P.; Thana-Udom, K.; Ercoli, L.M.; Siddarth, P. A Randomized Double-Blind Placebo-Controlled Trial of Combined Escitalopram and Memantine for Older Adults With Major Depression and Subjective Memory Complaints. *Am. J. Geriatr. Psychiatry* **2020**, *28*, 178–190. [CrossRef]
250. Ketenci, S.; Acet, N.G.; Sarıdoğan, G.E.; Aydın, B.; Cabadak, H.; Gören, M.Z. The Neurochemical Effects of Prazosin Treatment on Fear Circuitry in a Rat Traumatic Stress Model. *Clin. Psychopharmacol. Neurosci.* **2020**, *18*, 219. [CrossRef]
251. Foland-Ross, L.C.; Gotlib, I.H. Cognitive and Neural Aspects of Information Processing in Major Depressive Disorder: An Integrative Perspective. *Front. Psychol.* **2012**, *3*, 489. [CrossRef]
252. Gvozdanovic, G.; Stämpfli, P.; Seifritz, E.; Rasch, B. Structural brain differences predict early traumatic memory processing. *Psychophysiology* **2019**, *57*, e13354. [CrossRef]
253. Andrewes, D.G.; Jenkins, L.M. The Role of the Amygdala and the Ventromedial Prefrontal Cortex in Emotional Regulation: Implications for Post-traumatic Stress Disorder. *Neuropsychol. Rev.* **2019**, *29*, 220–243. [CrossRef]
254. Davidson, R.J.; Pizzagalli, D.; Nitschke, J.B.; Putnam, K. Depression: Perspectives from Affective Neuroscience. *Annu. Rev. Psychol.* **2002**, *53*, 545–574. [CrossRef]
255. Wolf, O.T. The influence of stress hormones on emotional memory: Relevance for psychopathology. *Acta Psychol.* **2008**, *127*, 513–531. [CrossRef]
256. Itzhaky, L.; Levin, Y.; Fingerhut, H.; Solomon, Z. Depression and PTSD Co-Morbidity: What are We Missing? *J. Depress. Anxiety* **2014**, *3*, 1–6.
257. Malhi, G.S.; Berk, M. Does dopamine dysfunction drive depression? *Acta Psychiatr. Scand.* **2007**, *115*, 116–124. [CrossRef]
258. Gören, M.Z.; Cabadak, H. Noradrenaline and Post-traumatic Stress Disorder. In *Comprehensive Guide to Post-Traumatic Stress Disorder*; Metzler, J.B., Ed.; Springer: Cham, Switzerland, 2015; pp. 1–16.
259. Dulawa, S.C.; Janowsky, D.S. Cholinergic regulation of mood: From basic and clinical studies to emerging therapeutics. *Mol. Psychiatry* **2019**, *24*, 694–709. [CrossRef]
260. Hasselmo, M.E.; Stern, C.E. Mechanisms underlying working memory for novel information. *Trends Cogn. Sci.* **2006**, *10*, 487–493. [CrossRef] [PubMed]
261. Holmes, E.A.; Bourne, C. Inducing and modulating intrusive emotional memories: A review of the trauma film paradigm. *Acta Psychol.* **2008**, *127*, 553–566. [CrossRef]
262. Maurer, S.V.; Williams, C.L. The Cholinergic System Modulates Memory and Hippocampal Plasticity via Its Interactions with Non-Neuronal Cells. *Front. Immunol.* **2017**, *8*, 1489. [CrossRef] [PubMed]
263. Christodoulou, T.; Messinis, L.; Papathanasopoulos, P.; Frangou, S. The impact of familial risk for schizophrenia or bipolar disorder on cognitive control during episodic memory retrieval. *Psychiatry Res.* **2012**, *197*, 212–216. [CrossRef]
264. Gotlib, I.H.; Joormann, J. Cognition and Depression: Current Status and Future Directions. *Annu. Rev. Clin. Psychol.* **2010**, *6*, 285–312. [CrossRef] [PubMed]
265. Dreifus, L.; Engler, H.; Kissler, J. Retrieval-induced forgetting under psychosocial stress: No reduction by delayed stress and beta-adrenergic blockade. *Neurobiol. Learn. Mem.* **2014**, *110*, 35–46. [CrossRef] [PubMed]
266. Robbins, T.W.; Vaghi, M.M.; Banca, P. Review obsessive-compulsive disorder: Puzzles and prospects. *Neuron* **2019**, *102*, 27–47. [CrossRef] [PubMed]

267. Tritsch, N.X.; Sabatini, B.L. Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. *Neuron* **2012**, *76*, 33–50. [CrossRef] [PubMed]
268. Glahn, D.C.; Knowles, E.E.M.; Pearlson, G.D. Genetics of cognitive control: Implications for Nimh's research domain criteria initiative. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **2015**, *171*, 111–120. [CrossRef]
269. Iacono, W.G.; Malone, S.M.; Vrieze, S.I. Endophenotype best practices. *Int. J. Psychophysiol.* **2017**, *111*, 115–144. [CrossRef]
270. James, E.L.; Lau-Zhu, A.; Clark, I.A.; Visser, R.M.; Hagenaars, M.A.; Holmes, E.A. The trauma film paradigm as an experimental psychopathology model of psychological trauma: Intrusive memories and beyond. *Clin. Psychol. Rev.* **2016**, *47*, 106–142. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

Genetic Variants Allegedly Linked to Antisocial Behaviour Are Equally Distributed Across Different Populations

Stefania Zampatti [1], Michele Ragazzo [2], Carlo Fabrizio [1], Andrea Termine [1], Giulia Campoli [1], Valerio Caputo [2], Claudia Strafella [1], Raffaella Cascella [1,3], Carlo Caltagirone [4] and Emiliano Giardina [1,2,*]

1 Genomic Medicine Laboratory UILDM, IRCCS Fondazione Santa Lucia, 00179 Rome, Italy; s.zampatti@hsantalucia.it (S.Z.); carlo.fabrizio217@gmail.com (C.F.); andreatermine544@gmail.com (A.T.); giuliacampoli90@gmail.com (G.C.); claudia.strafella@gmail.com (C.S.); raffaella.cascella@gmail.com (R.C.)
2 Department of Biomedicine and Prevention, Tor Vergata University of Rome, 00133 Rome, Italy; michele.ragazzo@uniroma2.it (M.R.); v.caputo91@gmail.com (V.C.)
3 Department of Biomedical Sciences, Catholic University Our Lady of Good Counsel, 1000 Tirana, Albania
4 Department of Clinical and Behavioral Neurology, IRCCS Fondazione Santa Lucia, 00179 Rome, Italy; c.caltagirone@hsantalucia.it
* Correspondence: emiliano.giardina@uniroma2.it

Abstract: Human behaviour is determined by a complex interaction of genetic and environmental factors. Several studies have demonstrated different associations between human behaviour and numerous genetic variants. In particular, allelic variants in SLC6A4, MAOA, DRD4, and DRD2 showed statistical associations with major depressive disorder, antisocial behaviour, schizophrenia, and bipolar disorder; BDNF polymorphic variants were associated with depressive, bipolar, and schizophrenia diseases, and TPH2 variants were found both in people with unipolar depression and in children with attention deficit-hyperactivity disorder (ADHD). Independent studies have failed to confirm polymorphic variants associated with criminal and aggressive behaviour. In the present study, a set of genetic variants involved in serotoninergic, dopaminergic, and neurobiological pathways were selected from those previously associated with criminal behaviour. The distribution of these genetic variants was compared across worldwide populations. While data on single polymorphic variants showed differential distribution across populations, these differences failed to be significant when a comprehensive analysis was conducted on the total number of published variants. The lack of reproducibility of the genetic association data published to date, the weakness of statistical associations, the heterogeneity of the phenotype, and the massive influence of the environment on human behaviour do not allow us to consider these genetic variants as undoubtedly associated with antisocial behaviour. Moreover, these data confirm the absence of ethnic predisposition to aggressive and criminal behaviour.

Keywords: genetic variants; criminal behaviour; frequency data

Citation: Zampatti, S.; Ragazzo, M.; Fabrizio, C.; Termine, A.; Campoli, G.; Caputo, V.; Strafella, C.; Cascella, R.; Caltagirone, C.; Giardina, E. Genetic Variants Allegedly Linked to Antisocial Behaviour Are Equally Distributed Across Different Populations. *J. Pers. Med.* **2021**, *11*, 213. https://doi.org/10.3390/jpm11030213

Academic Editor: Marco Costanzi

Received: 19 February 2021
Accepted: 12 March 2021
Published: 16 March 2021

1. Introduction

Human behaviour is determined by a complex interaction of genetic and environmental factors [1]. Although the first studies on human behaviour and genetics started in the 1800s with Sir Francis Galton, a rigorous scientific approach on the genetics of human behaviour started in the 1960s [2]. To date, several genetic studies have been performed to elucidate the mechanisms involved in the development of human behaviour [3]. Human behaviour is influenced by several genetic and environmental factors. Although several studies have been performed to decode the role of genetics in human behaviour, a systematic approach is complicated by different issues: (i) the extremely heterogeneous forms of behaviour disorders, (ii) the complex interplay between genetic and environmental factors, and (iii) challenges in standardizing environmental influences for statistical purposes [4,5]. Generally, environmental factors have been divided into two different groups: external and

internal [6]. The external features involve family, friends, home, stress, workplace, and life experiences. The internal features involve nutrition and dietary intake, hormones, viruses, bacteria, toxins, and molecules that can modify growth in pre- and post-natal life [6].

Different associations have been found between behaviour sub-phenotypes and genetics (i.e., neuropsychiatric disorders). In fact, allelic variants in genes involved in the neurotransmitter pathways have been associated with a differential susceptibility to neuropsychiatric disorders. Polymorphic variants in SLC6A4, MAOA, DRD4, and DRD2 have shown statistical associations with major depressive disorder, anti-social behaviour, schizophrenia, and bipolar disorder. Many of these disorders share similar genetic patterns of susceptibility. BDNF polymorphic variants have been associated with depressive, bipolar, and schizophrenia diseases. [7] Similarly, *TPH2* variants have been found both in people with unipolar depression and in children with attention deficit hyperactivity disorder (ADHD) [8,9]. The aetiology of neuropsychiatric disorders shares some pathways with so-called "criminal" behaviour. In general, criminal behaviour can be defined as behaviour with tendencies to take actions contravening criminal laws [10]. Over the years, genetic and environmental factors have been described as being involved in the development of criminal behaviour. Although neuropsychiatric disorders share some genetic association with criminal and aggressive behaviour, these diseases are clinically recognizable through DSM-5 criteria [11]. It is intriguing that many features of criminal behaviour can be also found in neuropsychiatric disorders (i.e., violent, antisocial, and aggressive behaviour) [12].

Technological evolution in genetic studies has enabled the sequencing of the entire human genome in a few days. Despite the proliferation of technologies, many studies have failed to confirm polymorphic variants associated with criminal and aggressive behaviour. Polymorphic variants in serotoninergic, dopaminergic, and neurobiological systems were selected after considering the studies focused on genetic influences in aggressive behaviour. [13–16]. In this paper, we investigate the genetic distribution, among different populations, of these variations.

To this extent, we implemented a multiple associations study with a case-control design to assess differences between SNP allelic frequencies among different populations.

2. Materials and Methods

2.1. Selection of Variants

In this work, we selected 24 polymorphisms in genes related to human behaviour previously associated with criminal behaviour [13–15,17]. We considered several genetic variants included in dopaminergic and serotoninergic pathways and other variants involved, for example, in association studies with Alzheimer's disease and in glucocorticoid receptors [13–15,17].

The selected variants are summarized in Table 1. In particular, the third column shows associated alleles selected for the calculation of weighted average number of genetic variants.

Table 1. Polymorphisms details. Abbreviations: African (AFR), American (AMR), East Asian (EAS), European (EUR), Toscani in Italy (TSI) and South Asian (SAS), not considered (n.c.) [13–15,18].

			Frequency Data					
Gene	**Variant**	**Associated Allele**	**AFR**	**AMR**	**EAS**	**EUR**	**TSI**	**SAS**
TPH1	rs1800532	G	G: 84% T: 16%	G: 63% T: 37%	G: 52% T: 48%	G: 61% T: 39%	G: 62% T: 38%	G: 73% T: 27
TPH1	rs1799913	T	G: 84% T: 16%	G: 63% T: 37%	G: 52% T: 48%	G: 61% T: 39%	G: 62% T: 38%	G: 73% T: 27%
TPH2	rs4570625	T	G: 63% T: 37%	G: 66% T: 34%	G: 45% T: 55%	G: 79% T: 21%	G: 77% T: 23%	G: 72% T: 28%
TPH2	rs6582071	A	G: 47% A: 53%	G: 64% A: 36%	G: 45% A: 55%	G: 78% A: 22%	G: 77% A: 23%)	G: 72% A: 28%

Table 1. *Cont.*

			Frequency Data					
Gene	**Variant**	**Associated Allele**	**AFR**	**AMR**	**EAS**	**EUR**	**TSI**	**SAS**
SLC6A4	rs25531	C	T: 78% C: 22%	T: 95% C: 5%	T: 87% C: 13%	T: 91% C: 9%	T: 93% C: 7%	T: 86% C: 14%
COMT	rs4680	A	G: 72% A: 28%	G: 62% A: 38%	G: 72% A: 28%	G: 50% A: 50%	G: 55% A: 45%	G: 56% A: 44%
COMT	rs6269	G	A: 63% G: 37%	A: 69% G: 31%	A: 66% G: 34%	A: 59% G: 41%	A: 51% G: 49%	A: 67% G: 33%
COMT	rs4818	G	C: 83% G: 17%	C: 70% G: 30%	C: 66% G: 34%	C: 60% G: 40%	C: 53% G: 47%	C: 69% G: 31%
MAOA	rs1346551029	n.c.	ACCG . . . : 99% ACCG . . . : 1%	ACCG . . . : 100% ACCG . . . : 0%	ACCG . . . : 100% ACCG . . . : 0%	ACCG . . . : 99% ACCG . . . : 1%	NA	ACCG . . . : 100% ACCG . . . : 0%
DRD4	rs761010487	n.c.	CGCC . . . : 100% CGCC . . . : 0%	CGCC . . . : 100% CGCC . . . : 0%	CGCC . . . : 100% CGCC . . . : 0%	CGCC . . . : 100% CGCC . . . : 0%	NA	CGCC . . . : 100% CGCC . . . : 0%
HTR1B	rs6296	G	C: 76% G: 24%	C: 60% G: 40%	C: 49% G: 51%	C: 74% G: 26%	C: 78% G: 22%	C: 68% G: 32%
HTR1B	rs130058	A	T: 97% A: 3%	T: 72% A: 28%	T: 91% A: 9%	T: 66% A: 34%	T: 63% A: 37%	T: 74% A: 26%
HTR1B	rs13212041	C	C: 56% T: 44%	C: 17% T: 83%	C: 23% T: 77%	C: 19% T: 81%	C: 13% T: 87%	C: 16% T: 84%
HTR2B	rs79874540	A	G: 100%	G: 100%	G: 100%	G: 100% A: 0%	G: 100%	G: 100%
HTR2A	rs6313	G	G: 61% A: 39%	G: 65% A: 35%	G: 41% A: 59%	G: 56% A: 44%	G: 50% A: 50%	G: 58% A: 42%
HTR2A	rs6311	C	C: 59% T: 41%	C: 64% T: 36%	C: 41% T: 59%	C: 56% T: 44%	C: 50% T: 50%	C: 60% T: 40%
HTR2A	rs7322347	A	T: 32% A: 68%	T: 60% A: 40%	T: 79% A: 21%	T: 56% A: 44%	C: 49% T: 51%	T: 67% A: 33
SLC6A3	rs28363170	n.c.	NA					
BDNF	rs6265	C	C: 99% T: 1%	C: 85% T: 15%	C: 51% T: 49%	C: 80% T: 20%	C: 76% T: 24%	C: 80% T: 20%
ApoE	rs7412 (A > G ApoE epsylon4 Variant)	T	C: 90% T: 10%	C: 95% T: 5%	C: 90% T: 10%	C: 94% T: 6%	C: 95% T: 5%	C: 96% T: 4%
ApoE	rs429358 (A > G ApoE epsylon4 Variant)	T	T: 73% C: 27%	T: 90% C: 10%	T: 91% C: 9%	T: 84% C: 16%	T: 90% C: 10%	T: 91% C: 9%
NR3C2	rs2070951	C	G: 84% C: 16%	G: 45% C: 55%	G: 24% C: 76%	G: 51% C: 49%	G: 57% C: 43%	G: 32% C: 68%
MAOA	rs6323	G	G: 14% T: 86%	G: 29% T: 71%	G: 57% T: 43%	G: 29% T: 71%	G: 27% T: 73%	G: 65% T: 35%
MAOA	rs1137070	T	T: 36% C: 64%	T: 39% C: 61%	T: 58% C: 42%	T: 29% C: 71%	T: 28 C: 72%	T: 65% C: 35%

2.2. Statistical Analysis

Genotype and frequency data relating to the African (AFR), American (AMR), East Asian (EAS), European (EUR), Toscani in Italy (TSI), and South Asian (SAS) populations of the 1000 Genomes Project, available on the Ensembl genome browser, were used [17–19].

A multiple associations study implementing a case-control design was conducted to assess the differences between the selected SNP allelic frequencies in all of the meaningful comparisons between populations (AMR–AFR; EAS–AFR; EUR–AFR; TSI–AFR; SAS–AFR; EAS–AMR; EUR–AMR; TSI–AMR; SAS–AMR; EUR–EAS; TSI–EAS; SAS–EAS; TS–EUR; SAS–EUR; SAS–TSI; AMR–AFR; EAS–AFR; EUR–AFR; TSI–AFR; SAS–AFR; EAS–AMR; EUR–AMR; TSI–AMR; SAS–AMR; EUR–EAS; TSI–EAS; SAS–EAS; TSI–EUR; SAS–EUR; SAS–TSI) [20,21]. The population samples were analysed using several Two-Sided Fisher's Exact Tests [22]. Alleles and genotypes odds ratio (OR) with 95% confidence intervals were also estimated. The significance threshold was set at $p < 0.05$ and multiple correction methods were computed: Benjamini–Hochberg Procedure, q-values, and the Sidak, Bonferroni, Holm corrections [23–26]. Differences in allelic frequencies were deemed significant using the most conservative method (Bonferroni).

Based on the Hardy–Weinberg frequencies, the weighted average number of genetic variants associated with criminal behaviour in six populations were evaluated. It is assumed that genotype frequencies in each population were distributed according to the Hardy–Weinberg equilibrium. Based on this assumption, people can be heterozygous or homozygous for the selected associated variants (Table 1). The average number of associated alleles present in the population was calculated according to the frequencies expected for each population.

To compare differences between the number of associated alleles by population, multiple Two-Sided T-Tests and Wilcoxon Tests for parametric/non-parametric data were performed [27,28]. All of the biostatistical analyses were carried out using the R 4.0.3 software [29].

3. Results

Genotype data from the 1000 Genomes Projects on AFR, AMR, EAS, EUR, TSI, and SAS populations were used to assess the differences between the selected SNP allelic frequencies.

Associated variants of selected genes showed a differential distribution across the populations. Statistical analyses carried out with Fisher's Exact Tests predictably revealed significant differences across populations (Table 1). Distant populations showed significant differences for a large number of polymorphisms when compared with neighbouring populations (Figure 1).

In this analysis, rs1346551029, rs761010487, and rs28363170 were not included because the frequencies of alleles associated with criminal behaviour were not available for the populations considered. These results confirmed the known genetic distance between populations across the world.

It should be noted that the simple differential distribution of single genetic polymorphisms in the various populations does not imply that there are differences in genetic susceptibility.

To assess whether some populations had, on average, a greater number of variants associated with antisocial behaviour, it is necessary to calculate the average number of "antisocial susceptibility variants" expected for each population.

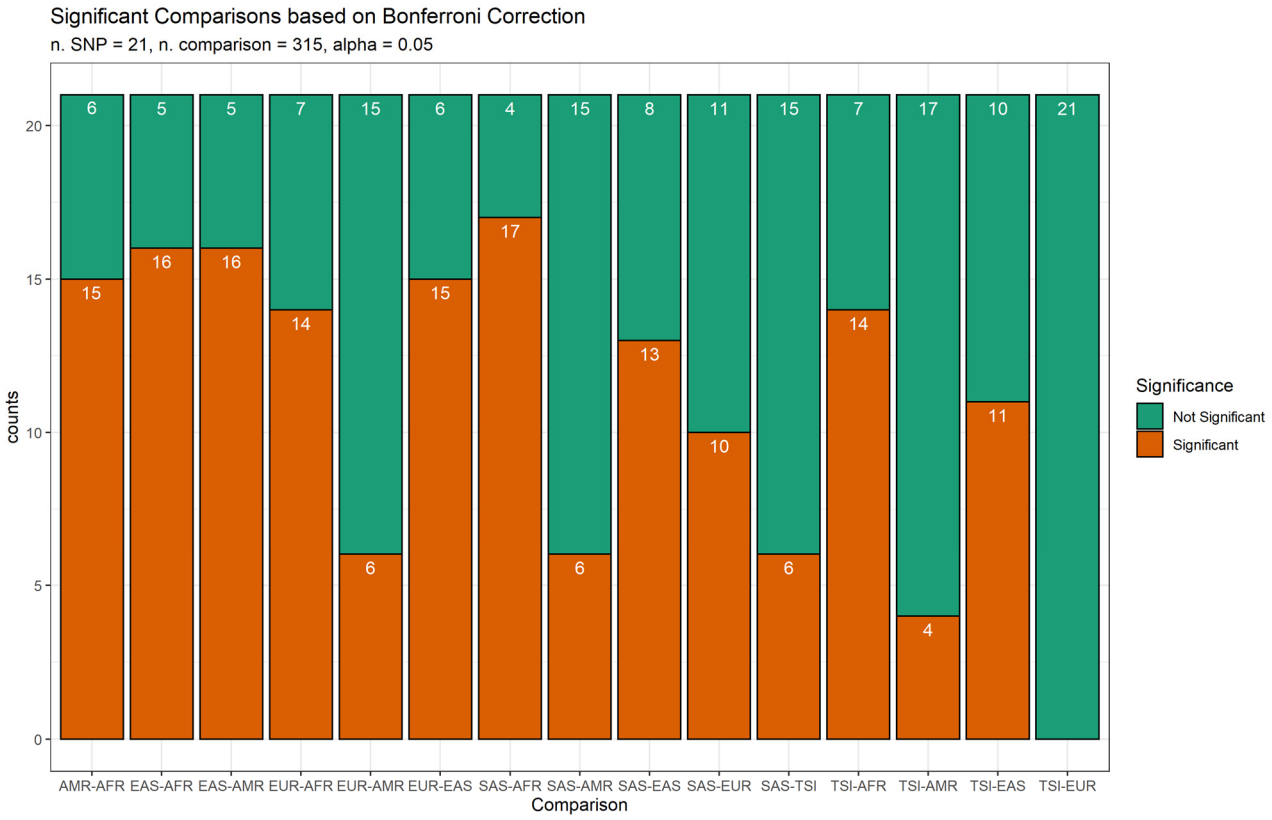

Figure 1. Comparison of variants frequency across populations. Bars indicate the overall number of variants with significant (orange) and not-significant (green) differences in frequencies between populations. Abbreviations: African (AFR), American (AMR), East Asian (EAS), European (EUR), Toscani in Italy (TSI) and South Asian (SAS).

The weighted average number of genetic variants associated with criminal behaviour in six populations was calculated assuming the Hardy–Weinberg equilibrium. The frequencies expected for each population allowed the determination that each population carries an average number of genetic alleles between 15.7 and 17.3. As expected, although single polymorphic variants show differential distribution across populations, these statistical differences, assessed by means of Two-Sided T-Tests and Wilcoxon Tests were not confirmed when a comprehensive analysis was conducted on the total number of published variants. In fact, some variants could be more frequent in a population than in another, but the overall number of "alleged antisocial susceptibility variants" is very similar in different populations (Table 2).

Table 2. Comparison of mean load of "antisocial susceptibility variants" across populations.

SNP (Count)	Comparison	*p*-Value (*t*-Test)	*p*-Value (Wilcoxon)
21	AMR–AFR	0.95	0.67
21	EAS–AFR	0.63	0.61
21	EUR–AFR	0.96	0.74
21	TSI–AFR	0.97	0.84
21	SAS–AFR	0.67	0.57
21	EAS–AMR	0.64	0.55
21	EUR–AMR	0.90	0.97
21	TSI–AMR	0.91	0.99
21	SAS–AMR	0.69	1.00

Table 2. *Cont.*

SNP (Count)	Comparison	*p*-Value (*t*-Test)	*p*-Value (Wilcoxon)
21	EUR–EAS	0.54	0.40
21	TSI–EAS	0.56	0.36
21	SAS–EAS	0.97	0.96
21	TSI–EUR	0.98	0.90
21	SAS–EUR	0.59	0.76
21	SAS–TSI	0.61	0.70

These data support the absence of significant ethnic differences in molecular pathways that have been associated with aggressive and criminal behaviour.

4. Discussion

The technological advances in genetics have allowed researchers to generate considerable genetic data in a short time and with reduced costs. The current challenge involves the accurate interpretation of genetic data and the translation of research data into useful instruments for forensic purposes. Many studies performed in the past decades demonstrated the complex interplay between genetics, environment, and epigenetic factors. Although technological innovations make unlimited genetic data available, their interpretation is challenging due to an unexpected number of complexity levels and functional adjustments. The interaction network between genes, gene expression factors (microRNAs, methylation, etc.), and environment (exposure to toxic agents, life experiences, etc.) varies with respect to time and tissues. These aspects make the evaluation of penetrance of single genetic variants on individual phenotypes challenging. Despite these limitations, genetic analyses for the prediction of human criminal behaviour have been used in judiciary practice. Scientific evidence shows that genotyping analyses cannot predict criminal and aggressive behaviour [30].

Here, we report an evaluation of the polymorphic distribution of genetic variants that have been associated with aggressive and criminal behaviour across populations. The analysis considered several variants involved in different pathways and six different populations.

While data on single polymorphic variants showed differential distribution across populations, these statistical differences were not confirmed when a comprehensive analysis was conducted on the total number of published variants. These data confirm the scientific assumption of the absence of biological races, even from a criminalistic point of view [31].

When human behaviour is considered as a phenotype, the lack of reproducibility of the genetic association data published so far, the weakness of statistical associations, the heterogeneity of the phenotype, and the massive influence of the environment on human behaviour do not allow us to consider these genetic variants as clearly associated with antisocial behaviour.

As demonstrated by GWAS studies performed to date on both pathological and physiological human phenotypes, no single variant is able of significantly modifying a specific phenotype. In addition, even when we assume that the susceptibility conferred by these variants is demonstrated, the distribution of these variants, considered as a whole, does not show statistically significant differences between the various populations. Altogether, these data support the absence of significant ethnic differences in molecular pathways that have been associated with aggressive and criminal behaviour.

In fact, clinical experience in all fields shows that a useful test has to reach high levels of utility and validity. To date, the impossibility of fully decoding a single phenotype became evident when considering brain complexity. As shown, prenatal exposure to risk factors (i.e., maternal smoking, maternal dietary insufficiency, alcohol abuse), childhood experiences (i.e., violence, sexual abuse, maternal separation), drug and alcohol abuse,

lifetime stress, and psychiatric disorders can modulate the risk of developing aggressive and criminal behaviour. At the same time, these environmental factors can modify epigenetic mechanisms that regulate gene expression. As largely shown in the past, every single person is not only the product of these genes, but every facet of them is a result of the interplay of genes and environment.

In particular, violent behaviour manifests itself in many different ways, such as anger, unaffectivity, sexual crimes, etc. We believe that no person is born violent. Every person is born emotionally healthy and acquires the possibility of developing violent behaviour following exposure to environmental triggers. Based on this evidence, the Italian Society of Human Genetics (SIGU) disclosed its scientific opinion with a position statement on forensic use of susceptibility genetic tests on aggressive behaviour. The SIGU does not recognize any scientific validity of susceptibility genetic tests for behavioural traits. This position is particularly strong in forensics, as no susceptibility genetic test for behaviour shows any practical utility. It is believed that these tests are useless, invalid, and scientifically unsuitable for achieving the purposes for which they are performed. More studies have to be conducted to understand the complex mechanisms that underlie individual differences in behaviour. However, the current knowledge landscape is insufficient to design a technical analysis able to predict either a personal behavioural profile or a behavioural trajectory.

Furthermore, the evaluation of allelic distribution by means of multiple Two-Sided T-Tests and Wilcoxon Tests in populations shows no significant differences. These data confirm the absence of genotypic differences between ethnic groups [31]. Even when limited to the polymorphic variants associated with differences in behaviour, the allelic distribution does not vary between populations. These data further confirm the absence of ethnic predisposition to aggressive and criminal behaviour. Therefore, no genetic discrimination (positive or negative) should be conducted on ethnic background for two main scientific truths: (i) the absence of significant ethnic genome diversity and (ii) the absence of reproducible genetic susceptibility to crime.

Author Contributions: Conceptualization, S.Z., M.R., and E.G.; methodology, S.Z., M.R., and E.G.; software, C.F., A.T., and V.C.; validation, S.Z., M.R., and R.C.; formal analysis, S.Z. and M.R.; data curation, S.Z., M.R., G.C., C.S., and R.C.; writing—original draft preparation, S.Z. and M.R.; writing—review and editing, S.Z., M.R., C.C., and E.G.; supervision, C.C. and E.G. All authors have read and agreed to the published version of the manuscript.

Funding: This work is also funded by the Italian Ministry of Health (5XMille—2018) and the National Research Council CNR (project "A multifactorial intervention for successful aging").

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated in the present study are included within the manuscript.

Acknowledgments: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ebstein, R.P.; Israel, S. Molecular Genetics of Personality: How Our Genes can Bring Us to a Better Understanding of Why We Act the Way We Do. In *Handbook of Behavior Genetics*, 1st ed.; Yong-Kyu, K., Ed.; Springer: New York, NY, USA, 2009; pp. 243–244. [CrossRef]
2. Loehlin, J.C. History of Behavior Genetics. In *Handbook of Behavior Genetics*, 1st ed.; Yong-Kyu, K., Ed.; Springer: New York, NY, USA, 2009; pp. 3–11. [CrossRef]
3. O'Mahony, C.; de Paor, A. The use of behavioural genetics in the criminal justice system: A disability & human rights perspective. *Int. J. Law Psychiatry* **2017**, *54*, 16–25. [CrossRef]
4. van der Gronde, T.; Kempes, M.; van El, C.; Rinne, T.; Pieters, T. Neurobiological correlates in forensic assessment: A systematic review. *PLoS ONE* **2014**, *9*, e110672. [CrossRef]
5. Manchia, M.; Comai, S.; Pinna, M.; Pinna, F.; Fanos, V.; Denovan-Wright, E.; Carpiniello, B. Biomarkers in aggression. *Adv. Clin. Chem.* **2019**, *93*, 169–237. [CrossRef]

6. Baker, C. What Is Behavioral Genetics? In *Behavioral Genetics an Introduction to How Genes and Environments Interact through Development to Shape Differences in Mood, Personality, and Intelligence*, 1st ed.; American Association for the Advancement of Science Directorate for Science & Policy Programs: Washington, DC, USA, 2004; pp. 1–7.
7. Assary, E.; Vincent, J.P.; Keers, R.; Pluess, M. Gene-environment interaction and psychiatric disorders: Review and future directions. *Semin. Cell Dev. Biol.* **2018**, *77*, 133–143. [CrossRef] [PubMed]
8. Zhang, X.; Gainetdinov, R.R.; Beaulieu, J.-M.; Sotnikova, T.D.; Burch, L.H.; Williams, R.B.; Schwartz, D.A.; Krishnan, K.R.R.; Caron, M.G. Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. *Neuron* **2005**, *45*, 11–16. [CrossRef] [PubMed]
9. McKinney, J.; Johansson, S.; Halmøy, A.; Dramsdahl, M.; Winge, I.; Knappskog, P.M.; Haavik, J. A loss-of-function mutation in tryptophan hydroxylase 2 segregating with attention-deficit/hyperactivity disorder. *Mol. Psychiatry* **2008**, *13*, 365–367. [CrossRef]
10. Kamaluddin, M.; Shariff, N.S.; Nurfarliza, S.; Othman, A.; Ismail, K.H.; Mat Saat, G.A. Psychological traits underlying different killing methods among Malaysian male murderers. *Malays. J. Pathol.* **2014**, *36*, 41–50.
11. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*, 5th ed.; American Psychiatric Association Publisher: Washington, DC, USA, 2013; ISBN 9780890425558/0890425558.
12. Rosell, D.R.; Siever, L.J. The neurobiology of aggression and violence. *CNS Spectr.* **2015**, *20*, 254–279. [CrossRef] [PubMed]
13. Waltes, R.; Chiocchetti, A.G.; Freitag, C.M. The neurobiological basis of human aggression: A review on genetic and epigenetic mechanisms. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2016**, *171*, 650–675. [CrossRef] [PubMed]
14. Cupaioli, F.A.; Zucca, F.A.; Caporale, C.; Lesch, K.P.; Passamonti, L.; Zecca, L. The neurobiology of human aggressive behavior: Neuroimaging, genetic, and neurochemical aspects. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **2021**, *106*, 110059. [CrossRef] [PubMed]
15. Kolla, N.J.; Bortolato, M. The role of monoamine oxidase A in the neurobiology of aggressive, antisocial, and violent behavior: A tale of mice and men. *Prog. Neurobiol.* **2020**, *194*, 101875. [CrossRef]
16. Asherson, P.; Cormand, B. The genetics of aggression: Where are we now? *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **2016**, *171*, 559–561. [CrossRef]
17. Ragazzo, M.; Puleri, G.; Errichiello, V.; Manzo, L.; Luzzi, L.; Potenza, S.; Strafella, C.; Peconi, C.; Nicastro, F.; Caputo, V.; et al. Evaluation of OpenArray™ as a Genotyping Method for Forensic DNA Phenotyping and Human Identification. *Genes* **2021**, *12*, 221. [CrossRef]
18. Yates, A.D.; Achuthan, P.; Akanni, W.; Allen, J.; Allen, J.; Alvarez-Jarreta, J.; Amode, M.R.; Armean, I.M.; Azov, A.G.; Bennett, R.; et al. Ensembl 2020. *Nucleic Acids Res.* **2020**, *48*, D682–D688. [CrossRef] [PubMed]
19. 1000 Genomes Project Consortium; Auton, A.; Brooks, L.D.; Durbin, R.M.; Garrison, E.P.; Kang, H.M.; Korbel, J.O.; Marchini, J.L.; McCarthy, S.; McVean, G.A.; et al. A global reference for human genetic variation. *Nature* **2015**, *526*, 68–74. [CrossRef] [PubMed]
20. Strafella, C.; Caputo, V.; Termine, A.; Barati, S.; Gambardella, S.; Borgiani, P.; Caltagirone, C.; Novelli, G.; Giardina, E.; Cascella, R. Analysis of ACE2 Genetic Variability among Populations Highlights a Possible Link with COVID-19-Related Neurological Complications. *Genes* **2020**, *11*, 741. [CrossRef]
21. Strafella, C.; Caputo, V.; Termine, A.; Barati, S.; Caltagirone, C.; Giardina, E.; Cascella, R. Investigation of Genetic Variations of IL6 and IL6R as Potential Prognostic and Pharmacogenetics Biomarkers: Implications for COVID-19 and Neuroinflammatory Disorders. *Life* **2020**, *10*, 351. [CrossRef] [PubMed]
22. Clarke, L.; Zheng-Bradley, X.; Smith, R.; Kulesha, E.; Xiao, C.; Toneva, I.; Vaughan, B.; Preuss, D.; Leinonen, R.; Shumway, M.; et al. 1000 Genomes Project Consortium. The 1000 Genomes Project: Data management and community access. *Nat. Methods* **2012**, *9*, 459–462. [CrossRef] [PubMed]
23. Abdi, H. The Bonferroni and Sidak corrections for multiple comparisons. In *Encyclopedia of Measurement and Statistics*; Salkind, N.J., Ed.; Sage: Thousand Oaks, CA, USA, 2007.
24. Simes, R.J. An improved Bonferroni procedure for multiple tests of significance. *Biometrika* **1986**, *50*, 813–820. [CrossRef]
25. Storey, J.D.; Bass, A.J.; Dabney, A.; Robinson, D. qvalue: *Q-value* Estimation for False Discovery Rate Control. R package version 2.18.0. 2019. Available online: http://github.com/jdstorey/qvalue (accessed on 2 February 2021).
26. Thissen, D.; Steinberg, L.; Kuang, D. Quick and easy implementation of the Benjamini-Hochberg procedure for controlling the false positive rate in multiple comparisons. *J. Educ. Behav. Stat.* **2002**, *27*, 77–83. [CrossRef]
27. Ruxton, G.D. The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test. *Behav. Ecol.* **2006**, *17*, 688–690. [CrossRef]
28. Wilcox, R.R. *Introduction to Robust Estimation and Hypothesis Testing*, 3rd ed.; Academic Press: Cambridge, MA, USA, 2011.
29. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019. Available online: https://www.R-project.org/ (accessed on 2 February 2021).
30. Forzano, F.; Borry, P.; Cambon-Thomsen, A.; Hodgson, S.V.; Tibben, A.; de Vries, P.; van El, C.; Cornel, M. Italian appeal court: A genetic predisposition to commit murder? *Eur. J. Hum. Genet.* **2010**, *18*, 519–521. [CrossRef] [PubMed]
31. Barbujani, G.; Ghirotto, S.; Tassi, F. Nine things to remember about human genome diversity. *Tissue Antigens.* **2013**, *82*, 155–164. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Article

Emotional Dysregulation, Temperament and Lifetime Suicidal Ideation among Youths with Mood Disorders

Delfina Janiri [1,2,†], Lorenzo Moccia [1,3,†], Eliana Conte [1,4], Laura Palumbo [1], Daniela Pia Rosaria Chieffo [5], Giulia Fredda [5], Renato Maria Menichincheri [4], Andrea Balbi [4], Georgios D. Kotzalidis [6], Gabriele Sani [1,3,*] and Luigi Janiri [1,3]

1 Department of Neuroscience, Section of Psychiatry, Università Cattolica del Sacro Cuore, 00168 Rome, Italy; delfina.janiri@uniroma1.it (D.J.); lorenzo.moccia@unicatt.it (L.M.); eliana.conte@aslroma3.it (E.C.); laura.palumbo01@icatt.it (L.P.); luigi.janiri@unicatt.it (L.J.)
2 Department of Human Neuroscience, Sapienza University of Rome, 00185 Rome, Italy
3 Department of Psychiatry, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, 00168 Rome, Italy
4 Early Intervention Unit, ASL Roma 3, 00152 Rome, Italy; renatomaria.menichincheri@aslroma3.it (R.M.M.); andrea.balbi@aslroma3.it (A.B.)
5 Clinical Psychology Unit, Fondazione Policlinico Gemelli IRCCS, 00168 Rome, Italy; danielapiarosaria.chieffo@policlinicogemelli.it (D.P.R.C.); giulia.fredda@guest.policlinicogemelli.it (G.F.)
6 Department of Neurosciences, Mental Health and Sensory Organs (NESMOS), Sapienza University of Rome, 00189 Rome, Italy; giorgio.kotzalidis@uniroma1.it
* Correspondence: gabriele.sani@unicatt.it; Tel.: +39-0630155807; Fax: +39-0630155767
† Co-first authors.

Abstract: *Background*: Psychopathological dimensions contributing to suicidal ideation in young age are poorly understood. We aimed to investigate the involvement of emotional dysregulation and temperament in suicide risk in a sample of accurately selected young patients with mood disorders and a matched sample of healthy controls (HC). *Methods*: We assessed 50 young patients (aged 14–25 years) with DSM-5 bipolar or depressive disorders for clinical and psychopathological characteristics and 82 age and sex, educational level, and smoking habits-matched HC. Emotional dysregulation and temperament were assessed using the Difficulties in Emotion Regulation Scale (DERS) and the Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire (TEMPS-A-39), respectively. We tested their associations with suicidal ideation, using standard univariate/bivariate methods, preceded by overall multivariate analysis. *Results*: In the group of patients, 24 (48%) reported lifetime suicide ideation (LSI). Patients with LSI scored higher on emotional dysregulation ($p < 0.001$) and cyclothymic ($p < 0.001$), irritable ($p = 0.01$), and hyperthymic temperaments ($p = 0.003$) than HC. Patients with LSI specifically presented with more emotional dysregulation ($p < 0.001$) and cyclothymic temperament ($p = 0.001$), than patients without LSI (N = 26). *Conclusions*: Temperamental features, in particular cyclothymic temperament, and emotion dysregulation may represent independent factors for increased vulnerability to lifetime suicidal ideation in young adults with mood disorders.

Keywords: emotional dysregulation; affective temperaments; depressive disorders; bipolar disorders; youth

Citation: Janiri, D.; Moccia, L.; Conte, E.; Palumbo, L.; Chieffo, D.P.R.; Fredda, G.; Menichincheri, R.M.; Balbi, A.; Kotzalidis, G.D.; Sani, G.; et al. Emotional Dysregulation, Temperament and Lifetime Suicidal Ideation among Youths with Mood Disorders. *J. Pers. Med.* **2021**, *11*, 865. https://doi.org/10.3390/jpm11090865

Academic Editor: Marco Costanzi

Received: 3 August 2021
Accepted: 29 August 2021
Published: 30 August 2021

1. Introduction

In adolescents and young adults, suicide rates have been steadily increasing over the last decade [1]. The number of hospitalizations and emergency visits for suicidal ideation and suicide attempts in this age group has doubled over time [2]. Suicidal ideas in young age predict adult psychiatric morbidity and may serve as a marker of vulnerability to psychopathology [3]. Prompt identification of suicidal ideation in youth allows intervention planning aiming at better functional outcomes in mental health [4].

Emotion dysregulation is conceptualized as difficulty in several areas, including the ability to monitor and evaluate emotional experiences, adapt to their intensity and duration, and modulate emotional reactions in order to meet situational demands [5]. It could also be defined as difficulty in regulating the rapid oscillations of intense affects [6]. Emotional dysregulation is associated with increased psychiatric morbidity, particularly mood disorders. The DSM-IV had grouped bipolar and depressive disorders under the heading of mood disorders, but the DSM-5 has split them in two different categories. Nevertheless, both major depressive and bipolar disorders present with emotional dysregulation, which may be considered a shared feature among mood disorders [7,8]. Emotional dysregulation may impact the clinical course of both major depressive and bipolar disorders. In particular, it is shown to constitute a biological determinant of suicide risk in both adults [9] and adolescents [10].

Temperament identifies stable, early-appearing characteristics in behavioral tendencies that have a constitutional and biological basis. Premorbid affective temperament types refer to individual activity levels, rhythms, mood and related cognitions, [11] and have an important role in the clinical evolution of mood disorders [12]. In particular, differences in temperament traits are differently associated with suicide risk in adult patients with mood disorders [13].

In light of the above observations, we decided to investigate whether there is a specific relationship between emotional dysregulation, temperament, and suicide risk in a sample of accurately selected young patients with a bipolar or a depressive disorder, and a matched sample of healthy individuals.

2. Material and Methods

2.1. Participants

We consecutively assessed 50 young outpatients who had been diagnosed with a DSM-5 [14] bipolar (N = 21) or depressive disorder (N = 29). Patients were enrolled at the Early Intervention for Mood Disorders Unit at Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome, Italy. Patients were screened by trained staff for DSM-5 disorders, and clinical diagnoses were confirmed, using the Structured Clinical Interview for DSM-5–Research Version [15]. In addition to a diagnosis of mood disorder, inclusion criteria were as follows: (i) age between 14 and 25 years, (ii) stable phase of illness according to psychometric evaluation (Hamilton Depression Rating Scale, HAM-D $\leq$ 7; Young Mania Rating Scale $\leq$ 12), (iii) fluency in Italian, and (iv) at least five years of school education. Exclusion criteria wereas follows: (i) traumatic head injury with loss of consciousness; (ii) lifetime history of major medical or neurological disorders; (iii) suspected cognitive impairment based on a Mini-Mental State Examination (MMSE) [16] score lower than 24; (iv) recent (past six weeks) changes in any psychotropic medication; (v) current use of stimulant medications; and (vi) a history of psychosis unrelated to the primary mood disorder. We also recruited 82 healthy controls (HC), matched for age, sex, smoking status, and educational level, from the same geographical area. All HC were screened for current or lifetime history of DSM-5 disorders. For the aims of this study, they were also interviewed to determine their suicidal behavior potential; not one of them reported lifetime suicidal behavior. Participants were interviewed to assess whether any first-degree relative was affected by mood disorders or schizophrenia. If they had a positive family history, they were excluded. Other exclusion criteria were the same as those for the patient group. The study was approved and undertaken in accordance with the guidelines of the Fondazione Policlinico Universitario Agostino Gemelli Ethics Committee and in accordance with the Principles of Human Rights, as adopted by the World Medical Association at the 18th WMA General Assembly, Helsinki, Finland, June 1964 and subsequently amended at the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. All participants gave their written informed consent to participate in the study after they had received a complete explanation of the study procedures.

2.2. Assessment

To assess deficits in emotion regulation, we used the Difficulties in Emotion Regulation Scale (DERS) [5], a 36-item self-report measure that assesses individuals' typical levels of emotion dysregulation. Participants rate each item, using a 5-point Likert-type scale (ranging from 1 = almost never, to 5 = almost always). Higher scores indicate greater difficulties regulating emotions. In prior studies, the DERS demonstrated convergent validity with other established measures of emotion dysregulation, good test-retest reliability, excellent internal consistency and adequate predictive validity of several behavioral outcomes associated with emotion dysregulation [5].

Affective temperaments (cyclothymic, depressive, irritable, hyperthymic, and anxious) were assessed through the short, 39-item version of the validated Italian Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire (TEMPS-A-39) [17]. This instrument is widely used in research and has demonstrated good psychometric properties and optimal factor structure [18].

Clinical characteristics were collected during a clinical interview. Lifetime suicidal ideation was assessed with a semi-structured questionnaire consisting of two parts, one related to the past 6 months, and the other, lifetime. Each part included two questions: (1) "Have you ever seriously thought about committing suicide?" (2) "Have you ever made a plan for committing suicide?" Respondents had to answer only "Yes" or "No". The semi-structured questionnaire has not been yet validated, but it was already used previously by our group [11].

2.3. Statistical Analyses

We compared the three groups' (i.e., patients with and without lifetime suicidal ideation, and HC) sociodemographic and clinical characteristics on the basis of the chi-squared (χ^2) test for nominal variables and one-way analysis of variance (ANOVA1way) followed by post hoc Bonferroni tests for continuous variables and by pairwise post hoc analyses for nominal variables.

For the aims of this study, we focused on the distribution patterns of temperament and emotional dysregulation in the three groups. Accordingly, we conducted a series of one-way ANOVAs, followed by Bonferroni post hoc tests, to compare means among groups. The level of significance was set at $p < 0.05$ for the ANOVA comparative measurements. To minimize the likelihood of type I errors, the ANOVAs were preceded by overall multivariate analysis of variance (MANOVA) using all of the continuous variables considered in each of the analyses as dependent variables.

3. Results

The sociodemographic and clinical characteristics of the sample are shown in Table 1. In the total group of the 50 mood disorder patients, 24 (48%) reported lifetime suicide ideation (LSI). Regarding clinical characteristics, LSI patients reported more family history of psychiatric disorders (83.3%), more use of lithium (45.8%) and antipsychotic medications (50.0%) than patients without LSI (NoLSI) (Table 1). Furthermore, in the suicidal ideation group, most participants (70.8%) reported psychotherapy treatment (Table 1). There were no differences in belonging to the LSI or NoLSI groups among the diagnoses.

Regarding the distribution patterns of temperament and emotional dysregulation, a preliminary MANOVA revealed a significant global effect (Wilks' Lambda = 0.56, F = 6.73, df = 12, $p < 0.0001$) of all the variables of interest on the three groups (i.e., patients with and without lifetime suicidal ideation, and HC). Factorial ANOVAs indicated a main effect of diagnosis on emotional dysregulation, cyclothymic, irritable, and hyperthymic temperaments (Table 2). In particular, a series of Bonferroni post hoc tests clarified that LSI patients scored higher on emotional dysregulation and cyclothymic, irritable, and hyperthymic temperaments than HC, whereas NoLSI patients scored higher than HC only on the irritable temperament. At the direct comparison, patients with and without LSI differed in emotional dysregulation and cyclothymic temperament. In particular, patients

with LSI presented with higher emotional dysregulation and higher endorsement of the cyclothymic temperament.

Table 1. Sociodemographic and clinical characteristics of LSI, NoLSI and HC.

Characteristics	LSI (n = 24)	NoLSI (n = 26)	HC (n = 82)	F or χ^2	df	p
Age (years): mean ± (SD)	18.42 (3.61)	19.12 (3.98)	19.29 (3.90)	0.48	2	0.622
Females: n (%)	20 (83.3%)	19 (73.1%)	64 (78.0%)	0.77	2	0.682
Educational level (years): mean ± (SD)	11.75 (2.21)	11.92 (2.43)	12.33 (3.64)	0.38	2	0.686
Smokers: n (%)	9 (37.5%)	5 (19.2%)	29 (35.4%)	2.66	2	0.264
Family history of psychiatric disorders: n (%)	20 (83.3%)	15 (57.7%)	-	3.91	1	0.048
Age at onset (years): mean ± (SD)	13.75 (2.86)	14.69 (4.60)	-	0.74	1	0.394
Hospitalization: n (%)	9 (37.5%)	5 (19.2%)	-	2.07	1	0.151
Substance use: n (%)	4 (16.7%)	6 (23.1%)	-	0.32	1	0.571
Drugs:						
Antidepressants: n (%)	8 (33.3%)	8 (30.8%)	-	0.04	1	0.846
Antiepileptics: n (%)	17 (70.8%)	12 (46.2%)	-	3.12	1	0.077
Antipsychotics: n (%)	12 (50.0%)	6 (23.1%)	-	3.93	1	0.048
Lithium: n (%)	11 (45.8%)	1 (3.8%)	-	12.06	1	0.001
Benzodiazepines: n (%)	8 (33.3%)	5 (19.2%)	-	1.29	1	0.256
Diagnoses:						
Major depressive disorder: n (%)	9 (37.5%)	12 (46.2%)	-	0.83	2	0.65
Bipolar disorder: n (%)	14 (58.3%)	12 (46.2%)	-			
Persistent depressive disorder: n (%)	1 (4.2%)	2 (7.6%)	-			
Psychotherapy: n (%)	17 (70.8%)	10 (38.5%)	-	5.27	1	0.022

Abbreviations: df = degrees of freedom; HC = healthy controls; LSI = patients with lifetime suicidal ideation; NoLSI = patients without lifetime suicidal ideation; SD = standard deviation.

Table 2. Distribution patterns of emotional dysregulation and TEMPS-A-39 affective temperaments in LSI (N = 24), NoLSI (N = 26) and HC (N = 82).

	LSI Mean ± (SD)	NoLSI Mean ± (SD)	HC Mean ± (SD)	F	df	p	HC vs. LSI * (p)	HC vs. NoLSI * (p)	LSI vs. NoLSI * (p)
DERS total	89.38 (18.73)	66.73 (21.95)	64.94 (17.76)	16.07	2	<0.0001	<0.0001	1.000	<0.0001
Cyclothymic	7.79 (2.47)	5.08 (2.53)	5.10 (2.50)	11.43	2	<0.0001	<0.0001	1.000	0.001
Depressive	3.71 (1.78)	3.96 (2.07)	4.26 (2.21)	0.69	2	0.502	0.798	1.000	1.000
Irritable	6.04 (2.07)	6.12 (1.97)	4.42 (2.74)	6.82	2	0.002	0.018	0.010	1.000
Hyperthymic	5.71 (1.92)	4.92 (2.13)	4.11 (2.00)	6.38	2	0.002	0.003	0.225	0.512
Anxious	1.38 (1.17)	1.42 (0.99)	1.38 (1.10)	0.02	2	0.982	1.000	1.000	1.000

Abbreviations: DERS, Difficulties in Emotion Regulation Scale; df = Degrees of freedom; HC = Healthy controls; LSI = Patients with lifetime suicidal ideation; NoLSI = Patients without lifetime suicidal ideation; SD = Standard deviation; TEMPS-A-39 = 39-item Temperament Evaluation of Memphis, Pisa, Paris and San Diego-Autoquestionnaire. * Bonferroni post hoc test.

4. Discussion

Suicide is the second leading cause of death in young adults and adolescents with psychiatric morbidity, including mood disorders. Suicide attempts during adolescence are related to a 7-fold increase of the odds of a subsequent suicide attempt during young adulthood [19]. The present findings highlight that impaired emotion regulation abilities along with cyclothymic, irritable, and hyperthymic temperament differentiate young patients with a diagnosis of a mood disorder and LSI from HC. Furthermore, when directly comparing patients with and without LSI, young individuals with a diagnosis of mood disorders and LSI reported more use of lithium and antipsychotic medications, higher rates of family history of psychiatric disorders, and psychotherapy, as well as increased emotion dysregulation and cyclothymic temperament.

Contemporary conceptualizations of emotion dysregulation have moved from a dichotomous framework, in which an individual is either successful or unsuccessful at inhibiting or controlling affect, particularly negative emotions, to a multidimensional model of emotion regulation. The latter encompasses awareness, comprehension, and acceptance of emotions, the ability to engage in goal-directed behaviors, and to refrain

from acting hastily when experiencing distressful affects, as well as the perception of one's ability to effectively adopt emotion regulation strategies when situationally challenged [5]. Emotion dysregulation occurs when any of these self-regulatory domains is impaired [20]. A growing body of evidence suggests a role of emotion dysregulation in the onset and maintenance of mood disorders [21–24]. Similarly, studies examining the role of emotion dysregulation in suicide also observed that individuals who perceive themselves as incapable of exerting effective emotion regulation strategies when extremely distressed, are at increased risk of suicidal ideation, independently from mood symptoms [25,26]. According to escape theories of suicide [27], individuals wish to die when they feel overwhelmed by acute and unbearable affects that prevent them from adopting any adaptive regulation strategies. This intolerable emotional state, which is perceived as uncontrollable, leads patients to think of suicide as an effective way to escape these feelings.

While research on suicide risk has so far focused on individuals with mood disorders, the influence of temperamental features as an independent risk factor has only been partially investigated. Consistent with previous studies [13,28,29], distinct affective temperaments including cyclothymic, irritable, and hyperthymic, were associated with lifetime suicidal ideation in young patients with mood disorders. However, in our sample, only cyclothymic temperament significantly discriminated between LSI and NoLSI patients with mood disorders. Affective instability, including increased mood reactivity and lability, consistently proved to contribute to suicidal ideation [6,30]. Accordingly, cyclothymic temperament, which is characterized by abrupt shifts in mood, behavior, and rapidly changing thinking [31] may therefore represent a specific vulnerability marker for suicidal ideation in individuals with mood disorders.

The biological underpinnings of suicidal ideation are currently unclear. However, it appears that genetic factors together with environmental influences explain most of variance [32] and may relate to decreased network strength and efficiency, which differentiate people with suicidal ideation and those free from such ideation [33]. In particular, suicidal ideation is conceived to be stress-related [34], and this is witnessed by blunted responses to dexamethasone in adolescents [35,36]. Furthermore, persons with suicidal ideation show reduced performance in emotional regulation tasks as witnessed by their inability to increase late positive potentials in response to stimuli [37]. These data point to the existence of a loop between stress, emotional dysregulation, and suicidal ideation, which matches our results.

As for the influence of affective temperaments on suicidal ideation, cyclothymic and depressive affective temperaments were found to be higher in individuals with prominent psychological distress and this effect was mediated by the lack of impulse control and lack of clarity dimensions of emotional dysregulation [38]. In another study, the cyclothymic temperament was found to be predisposed to the consequences of emotional dysregulation in an attention deficit/hyperactivity disorder [39]. Emotional dysregulation in turn was found to moderate the link between mental pain and suicidal ideation [40]. These results point to bidirectional influences between affective temperaments, emotional dysregulation, and suicidal ideation.

Before drawing conclusions, we have to acknowledge some potential limitations. First, the cross-sectional nature of our study does not allow us to generalize our results to the entire mood disorder population and is not fit for establishing causal relationships. Second, we assessed history of LSI, using a not yet validated, semi-structured questionnaire, so it is possible that it is not sufficiently sensitive for detecting suicidality. In particular, it did not provide a quantitative measure of suicidal risk. Furthermore, we did not stratify our sample, according to mood disorder diagnosis. However, the need to split the sample according to diagnosis was set off by the fact that all included diagnoses were not in a clinically active episode. Moreover, we found no differences in belonging to the LSI or NoLSI groups among diagnoses. Finally, the reliability of self-administered questionnaires may be partially biased. On the other hand, our study has some strengths, including the

investigation of the heretofore poorly investigated connection between LSI and emotional dysregulation and LSI and temperament.

In conclusion, our data highlight that temperamental features and emotion dysregulation may represent independent factors for increased vulnerability to lifetime suicidal ideation in adolescents and young adults with mood disorders while in their euthymic or "asymptomatic" phase. To confirm this association, and thus shed light on the pathway leading to suicide risk in young adults suffering from mental disorders, longitudinal studies are desirable for establishing causal relationships.

Author Contributions: Conceptualization, D.J. and L.M.; methodology, E.C., L.P. and G.D.K.; software, D.J., L.M. and L.P.; validation, G.S., L.J. and G.D.K.; formal Analysis, D.J. and L.M.; investigation, L.P., D.P.R.C., G.F. and A.B.; data curation, D.J., L.M., E.C., L.P., R.M.M., A.B. and G.D.K.; writing—D.J., L.M. and E.C.; writing-review and editing, G.S., L.J. and G.D.K.; supervision, G.S. and L.J.; project administration, E.C., A.B., R.M.M., G.S. and L.J. All authors qualified for authorship and contributed substantially to the work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the Fondazione Policlinico Universitario Agostino Gemelli Ethics Committee.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. The paper contains no information allowing to identify participants.

Data Availability Statement: Data will be available from the corresponding author upon reasonable request without restriction.

Acknowledgments: We are grateful to Angela Gonsalez del Castillo for critically reviewing our manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Naghavi, M. Global Burden of Disease Self-Harm Collaborators Global, regional, and national burden of suicide mortality 1990 to 2016: Systematic analysis for the Global Burden of Disease Study 2016. *BMJ* **2019**, *364*, l94. [CrossRef]
2. Burstein, B.; Agostino, H.; Greenfield, B. Suicidal Attempts and Ideation among Children and Adolescents in US Emergency Departments, 2007–2015. *JAMA Pediatr.* **2019**, *173*, 598–600. [CrossRef] [PubMed]
3. Copeland, W.E.; Shanahan, L.; Hinesley, J.; Chan, R.F.; Aberg, K.A.; Fairbank, J.A.; van den Oord, E.J.C.G.; Costello, E.J. Association of Childhood Trauma Exposure With Adult Psychiatric Disorders and Functional Outcomes. *JAMA Netw. Open* **2018**, *1*, e184493. [CrossRef] [PubMed]
4. Janiri, D.; Doucet, G.E.; Pompili, M.; Sani, G.; Luna, B.; Brent, D.A.; Frangou, S. Risk and protective factors for childhood suicidality: A US population-based study. *Lancet Psychiatry* **2020**, *7*, 317–326. [CrossRef]
5. Gratz, K.L.; Roemer, L. Multidimensional Assessment of Emotion Regulation and Dysregulation: Development, Factor Structure, and Initial Validation of the Difficulties in Emotion Regulation Scale. *J. Psychopathol. Behav. Assess.* **2004**, *26*, 41–54. [CrossRef]
6. Marwaha, S.; He, Z.; Broome, M.; Singh, S.P.; Scott, J.; Eyden, J.; Wolke, D. How is affective instability defined and measured? A systematic review. *Psychol. Med.* **2014**, *44*, 1793–1808. [CrossRef]
7. Eskander, N.; Emamy, M.; Saad-Omer, S.M.; Khan, F.; Jahan, N. The impact of impulsivity and emotional dysregulation on comorbid bipolar disorder and borderline personality disorder. *Cureus* **2020**, *12*, e9581. [CrossRef]
8. Kim, M.K.; Kim, J.S.; Park, H.I.; Choi, S.W.; Oh, W.J.; Seok, J.H. Early life stress, resilience and emotional dysregulation in major depressive disorder with comorbid borderline personality disorder. *J. Affect. Disord.* **2018**, *236*, 113–119. [CrossRef]
9. Wiebenga, J.X.M.; Dickhoff, J.; Mérelle, S.Y.M.; Eikelenboom, M.; Heering, H.D.; Gilissen, R.; van Oppen, P.; Penninx, B.W.J.H. Prevalence, course, and determinants of suicide ideation and attempts in patients with a depressive and/or anxiety disorder: A review of NESDA findings. *J. Affect. Disord.* **2021**, *283*, 267–277. [CrossRef]
10. Pan, L.A.; Hassel, S.; Segreti, A.M.; Nau, S.A.; Brent, D.A.; Phillips, M.L. Differential patterns of activity and functional connectivity in emotion processing neural circuitry to angry and happy faces in adolescents with and without suicide attempt. *Psychol. Med.* **2013**, *43*, 2129–2142. [CrossRef]
11. Von Zerssen, D.; Akiskal, H.S. Personality factors in affective disorders: Historical developments and current issues with special reference to the concepts of temperament and character. *J. Affect. Disord.* **1998**, *51*, 1–5.

12. Luciano, M.; Janiri, D.; Fiorillo, A.; Sani, G. Clinical Picture, Temperament, and Personality of Patients with Mixed States. *Psychiatr. Clin. N. Am.* **2020**, *43*, 15–26. [CrossRef]
13. Janiri, D.; De Rossi, P.; Kotzalidis, G.D.; Girardi, P.; Koukopoulos, A.E.; Reginaldi, D.; Dotto, F.; Manfredi, G.; Jollant, F.; Gorwood, P.; et al. Psychopathological characteristics and adverse childhood events are differentially associated with suicidal ideation and suicidal acts in mood disorders. *Eur. Psychiatry* **2018**, *53*, 31–36. [CrossRef]
14. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*; American Psychiatric Association Publishing, Inc.: Arlington, VA, USA, 2013; ISBN 9780890425541.
15. First, M.B.; Williams, J.B.W.; Karg, R.S.; Spitzer, R.L. *Structured Clinical Interview for DSM-5 Research Version*; American Psychiatric Publishing, Inc.: Arlington, VA, USA, 2015.
16. Folstein, M.F.; Folstein, S.E.; McHugh, P.R. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* **1975**, *12*, 189–198. [CrossRef]
17. Preti, A.; Vellante, M.; Zucca, G.; Tondo, L.; Akiskal, K.; Akiskal, H. The Italian version of the validated short TEMPS-A: The temperament evaluation of Memphis, Pisa, Paris and San Diego. *J. Affect. Disord.* **2010**, *120*, 207–212. [CrossRef]
18. Elias, L.R.; Köhler, C.A.; Stubbs, B.; Maciel, B.R.; Cavalcante, L.M.; Vale, A.; Gonda, X.; Quevedo, J.; Hyphantis, T.N.; Soares, J.C.; et al. Measuring affective temperaments: A systematic review of validation studies of the Temperament Evaluation in Memphis Pisa and San Diego (TEMPS) instruments. *J. Affect. Disord.* **2017**, *212*, 25–37. [CrossRef]
19. Clarke, M.C.; Coughlan, H.; Harley, M.; Connor, D.; Power, E.; Lynch, F.; Fitzpatrick, C.; Cannon, M. The impact of adolescent cannabis use, mood disorder and lack of education on attempted suicide in young adulthood. *World Psychiatry* **2014**, *13*, 322–323. [CrossRef]
20. Moccia, L.; Conte, E.; Ambrosecchia, M.; Janiri, D.; Di Pietro, S.; De Martin, V.; Di Nicola, M.; Rinaldi, L.; Sani, G.; Gallese, V.; et al. Anomalous self-experience, body image disturbance, and eating disorder symptomatology in first-onset anorexia nervosa. *Eat. Weight Disord.* **2021**. [CrossRef]
21. Joormann, J.; Quinn, M.E. Cognitive processes and emotion regulation in depression. *Depress. Anxiety* **2014**, *31*, 308–315. [CrossRef]
22. Fitzgerald, J.M.; Klumpp, H.; Langenecker, S.; Phan, K.L. Transdiagnostic neural correlates of volitional emotion regulation in anxiety and depression. *Depress. Anxiety* **2019**, *36*, 453–464. [CrossRef]
23. Moccia, L.; Janiri, D.; Giuseppin, G.; Agrifoglio, B.; Monti, L.; Mazza, M.; Caroppo, E.; Fiorillo, A.; Sani, G.; Di Nicola, M.; et al. Reduced hedonic tone and emotion dysregulation predict depressive symptoms severity during the covid-19 outbreak: An observational study on the Italian general population. *Int. J. Environ. Res. Public Health* **2021**, *18*, 255. [CrossRef] [PubMed]
24. Janiri, D.; Moccia, L.; Dattoli, L.; Pepe, M.; Molinaro, M.; De Martin, V.; Chieffo, D.; Di Nicola, M.; Fiorillo, A.; Janiri, L.; et al. Emotional dysregulation mediates the impact of childhood trauma on psychological distress: First Italian data during the early phase of COVID-19 outbreak. *Aust. N. Z. J. Psychiatry* **2021**. [CrossRef] [PubMed]
25. Rajappa, K.; Gallagher, M.; Miranda, R. Emotion Dysregulation and Vulnerability to Suicidal Ideation and Attempts. *Cognit. Ther. Res.* **2012**, *36*, 833–839. [CrossRef]
26. Miranda, R.; Tsypes, A.; Gallagher, M.; Rajappa, K. Rumination and Hopelessness as Mediators of the Relation Between Perceived Emotion Dysregulation and Suicidal Ideation. *Cognit. Ther. Res.* **2013**, *37*, 786–795. [CrossRef]
27. Baumeister, R.F. Suicide as escape from self. *Psychol. Rev.* **1990**, *97*, 90–113. [CrossRef]
28. Skala, K.; Kapusta, N.D.; Schlaff, G.; Unseld, M.; Erfurth, A.; Lesch, O.M.; Walter, H.; Akiskal, K.K.; Akiskal, H.S. Suicidal ideation and temperament: An investigation among college students. *J. Affect. Disord.* **2012**, *141*, 399–405. [CrossRef]
29. Baldessarini, R.J.; Vázquez, G.H.; Tondo, L. Affective temperaments and suicidal ideation and behavior in mood and anxiety disorder patients. *J. Affect. Disord.* **2016**, *198*, 78–82. [CrossRef]
30. Law, K.C.; Khazem, L.R.; Anestis, M.D. The role of emotion dysregulation in suicide as considered through the ideation to action framework. *Curr. Opin. Psychol.* **2015**, *3*, 30–35. [CrossRef]
31. Akiskal, K.K.; Akiskal, H.S. The theoretical underpinnings of affective temperaments: Implications for evolutionary foundations of bipolar disorder and human nature. *J. Affect. Disord.* **2005**, *85*, 231–239. [CrossRef]
32. Maciejewski, D.F.; Creemers, H.E.; Lynskey, M.T.; Madden, P.A.; Heath, A.C.; Statham, D.J.; Martin, N.G.; Verweij, K.J. Overlapping genetic and environmental influences on nonsuicidal self-injury and suicidal ideation: Different outcomes, same etiology? *JAMA Psychiatry* **2014**, *71*, 699–705. [CrossRef]
33. Liao, W.; Li, J.; Duan, X.; Cui, Q.; Chen, H.; Chen, H. Static and dynamic connectomics differentiate between depressed patients with and without suicidal ideation. *Hum. Brain Mapp.* **2018**, *39*, 4105–4118. [CrossRef]
34. McEwen, B.S. The Brain on Stress: Toward an Integrative Approach to Brain, Body, and Behavior. *Perspect. Psychol. Sci.* **2013**, *8*, 673–675. [CrossRef]
35. Beauchaine, T.P.; Crowell, S.E.; Hsiao, R.C. Post-dexamethasone cortisol, self-inflicted injury, and suicidal ideation among depressed adolescent girls. *J. Abnorm. Child Psychol.* **2015**, *43*, 619–632. [CrossRef]
36. Shalev, A.; Porta, G.; Biernesser, C.; Zelazny, J.; Walker-Payne, M.; Melhem, N.; Brent, D. Cortisol response to stress as a predictor for suicidal ideation in youth. *J. Affect. Disord.* **2019**, *257*, 10–16. [CrossRef]
37. Albanese, B.J.; Macatee, R.J.; Stanley, I.H.; Bauer, B.W.; Capron, D.W.; Bernat, E.; Joiner, T.E.; Schmidt, N.B. Differentiating suicide attempts and suicidal ideation using neural markers of emotion regulation. *J. Affect. Disord.* **2019**, *257*, 536–550. [CrossRef]

38. Janiri, D.; Kotzalidis, G.D.; Giuseppin, G.; Molinaro, M.; Modica, M.; Montanari, S.; Terenzi, B.; Carfì, A.; Landi, F.; Sani, G. Gemelli Against COVID-19 Post-acute Care Study Group. Psychological distress after Covid-19 recovery: Reciprocal effects with temperament and emotional dysregulation. An exploratory study of patients over 60 years of age assessed in a post-acute care service. *Front. Psychiatry* **2020**, *11*, 590135. [CrossRef]
39. Quaranta, G.; Barbuti, M.; Pallucchini, A.; Colombini, P.; Moriconi, M.; Gemmellaro, T.; Tripodi, B.; Palagini, L.; Schiavi, E.; Perugi, G. Relationships among delayed sleep phase disorder, emotional dysregulation, and affective temperaments in adults with attention deficit hyperactivity disorder and cyclothymia. *J. Nerv. Ment. Dis.* **2020**, *208*, 857–862. [CrossRef]
40. Shelef, L.; Fruchter, E.; Hassidim, A.; Zalsman, G. Emotional regulation of mental pain as moderator of suicidal ideation in military settings. *Eur. Psychiatry* **2015**, *30*, 765–769. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

The Relationship between Alexithymia, Dysmorphic Concern, and Exercise Addiction: The Moderating Effect of Self-Esteem

Alessio Gori [1,2,*], Eleonora Topino [3], Caterina Pucci [1] and Mark D. Griffiths [4]

1 Department of Health Sciences, University of Florence, Via di San Salvi 12, Pad. 26, 50135 Florence, Italy; caterina.pucci@stud.unifi.it
2 Integrated Psychodynamic Psychotherapy Institute (IPPI), via Ricasoli 32, 50122 Florence, Italy
3 Department of Human Sciences, LUMSA University of Rome, Via della Traspontina 21, 00193 Rome, Italy; eleonora.topino@gmail.com
4 Psychology Department, Nottingham Trent University, 50 Shakespeare Street, Nottingham NG1 4FQ, UK; mark.griffiths@ntu.ac.uk
* Correspondence: Correspondence: alessio.gori@unifi.it

Abstract: As with other addictions, exercise addiction can severely impact individuals' lives and have significant psychophysical consequences. Consequently, the study of the mechanisms involved in this psychopathological condition has great clinical and practical relevance. Therefore, the goal of the present study was to explore the risk factors and protective factors for exercise addiction, with a particular focus on the roles of alexithymia, body image concerns, and self-esteem. A sample of 288 regular exercisers (mean age = 28.35 years, SD = 8.26; 72% females, 18% males) completed the Exercise Addiction Inventory, 20-Item Toronto Alexithymia Scale, Body Image Concern Inventory, and Rosenberg Self-Esteem Scale. Data were analyzed by implementing a moderated mediation model. Results showed a significant and positive association between alexithymia and exercise addiction, totally mediated by body image concerns. Furthermore, self-esteem showed a relevant moderating effect, such that at high levels of self-esteem the effect of alexithymia on body image concerns became insignificant. Such data have important implications, highlighting some core variables on which it might be useful to keep a focus in order to elaborate tailored interventions, from both preventive and treatment perspectives.

Keywords: exercise addiction; alexithymia; emotional dysregulation; body image; self-esteem

Citation: Gori, A.; Topino, E.; Pucci, C.; Griffiths, M.D. The Relationship between Alexithymia, Dysmorphic Concern, and Exercise Addiction: The Moderating Effect of Self-Esteem. *J. Pers. Med.* **2021**, *11*, 1111. https://doi.org/10.3390/jpm11111111

Academic Editor: Marco Costanzi

Received: 11 October 2021
Accepted: 28 October 2021
Published: 29 October 2021

1. Introduction

A large body of research has shown that physical activity—defined as any body movement that requires energy expenditure [1]—has positive effects on physical and mental health [2]. Recommendations for the minimum level of physical activity that is needed for beneficial health effects have been provided by the World Health Organization [3], with parameters based on age and physical condition. Specifically, the recommendation for people aged 18–64 years without chronic conditions or disabilities, and not in a pre/postpartum state, is at least 150–200 min of moderate-intensity aerobic physical activity, at least 75–150 min of vigorous-intensity aerobic physical activity, or an equivalent combination of these activities, per week [3]. However, when exercise becomes excessive, it can lead to the implementation of compulsive training patterns that can evolve into a pathology—so-called "exercise addiction" [4].

Some authors define exercise addiction as a morbid behavior in which individuals gradually lose control over their exercise habits, act compulsively, and experience negative consequences—both physically, and in their social and/or professional lives [5,6]. Physical damage is manifested predominantly through long-term risks such as musculoskeletal injuries and psychological damage (typically expressed through immediate changes in mood, such as the feeling of depression when the individual cannot exercise) [6,7]. More

specifically, the symptoms and consequences of exercise addiction have been characterized by six common components of addiction: salience, mood modification, tolerance, withdrawal symptoms, personal conflict, and relapse [8,9]. In the event of addiction, the negative consequences are ignored, and do not prevent individuals from continuing to exercise [9,10]. Investigations of these factors have been helpful to the field from both preventive and treatment perspectives [11,12]. Therefore, the present study explores the interaction of risk factors and protective factors in pathways towards exercise addiction among regular exercisers, with a particular focus on the roles of alexithymia, body image concerns, and self-esteem.

Alexithymia, a construct originally introduced by Sifneos [13], is a specific form of emotional dysregulation that is defined by a difficulty in identifying, describing, and verbalizing emotions, as well as difficulty by individuals in discriminating their own emotional experiences from underlying physiological activation, which is also characterized by constricted imaginary processes [14–16]. It appears that psychic suffering resulting from affective dysregulation can lead those with alexithymia to regulate their negative emotions through behaviors that may be a risk to their health (e.g., drug use, engaging in risky sports, eating disorders) [17–19]. Therefore, it is not surprising that many scientific studies have shown a relationship between alexithymia and substance dependence [17,20] and behavioral addictions [21–23], including that related to exercise addiction.

The relationship between alexithymia and exercise addiction has been examined in different populations of exercisers, such as those attending fitness centers [24], swimmers [25], and sports university students [26]. Some research has suggested that the use of physical activity could be a means by which these individuals try to suppress their unmentalized emotional states, as a form of dissociation from painful experiences [17,24]. An interesting point of view is provided by some research arguing that the inability to symbolize emotional experiences—as well as the resulting undifferentiated and dysregulated affect—may also lead to body image distortion [27]. In other words, the inability of an individual to discriminate between emotional states and bodily sensations can increase dissatisfaction with their body, and could lead to a wrong interpretation of the perceptual and behavioral aspects of their body image [28]. Such inability could indeed arouse an emotional void that leads individuals to focus excessively on the details of their own body. This, in turn, can result in the use of maladaptive strategies (including excessive exercise) in order to control the body and physical appearance [26], as found in some cases of body dysmorphic disorder (BDD) [27].

On this basis, it is important to emphasize the associations between body concerns and exercise addiction. Several studies (e.g., [29,30]) have found different psychopathological conditions that co-occur with exercise addiction; among these, BDD is a severe psychiatric condition characterized by a recurring and persistent concern with an imagined or minor defect in physical appearance, with a focus on a specific body part [29]. In fact, individuals showing excessive concern over body image and weight are among those most likely to experience exercise addiction [31]. Some other studies have suggested that preoccupation with body image may be a driving force underlying exercise addiction [32]. Indeed, this dissatisfaction may lead to the search for a transformation of the perceived body image into an "ideal body image", through inadequate nutritional planning and excessive physical exercise. Consequently, physical exercise could become a vehicle to improve body image, as opposed to being motivated by the desire for increased health and wellbeing [29].

Within this framework, self-esteem could also be a relevant factor, since lower levels of self-esteem can influence the ways individuals perceive their own bodies [33,34]. Scientific literature agrees that self-esteem plays a central role in individuals' mental health, and it is more likely that a positive self-image and a strong sense of self-esteem help individuals to become more satisfied with their bodies [35]. In fact, higher self-esteem may protect the individual from the negative feelings related to their body weight, and from anxiety arising from the negative judgments of others [36–38], showing a beneficial influence on body-image-related preoccupation [35,39]. On the other hand, individuals with low self-esteem

appear to be more vulnerable to comments concerning their bodies, and more dissatisfied with physical aspects of their bodies over time [36,40]. Previous evidence suggests that increased self-esteem could play a protective role against body image concerns [41].

Given this evidence, the present study examined the role of alexithymia, body image concerns, and self-esteem in exercise addiction, by testing a moderated mediation model among a sample of regular exercisers. More specifically, it was hypothesized that body image concerns would mediate the relationship between alexithymia and exercise addiction, with self-esteem moderating the relationship between alexithymia and body image concerns (see Figure 1A).

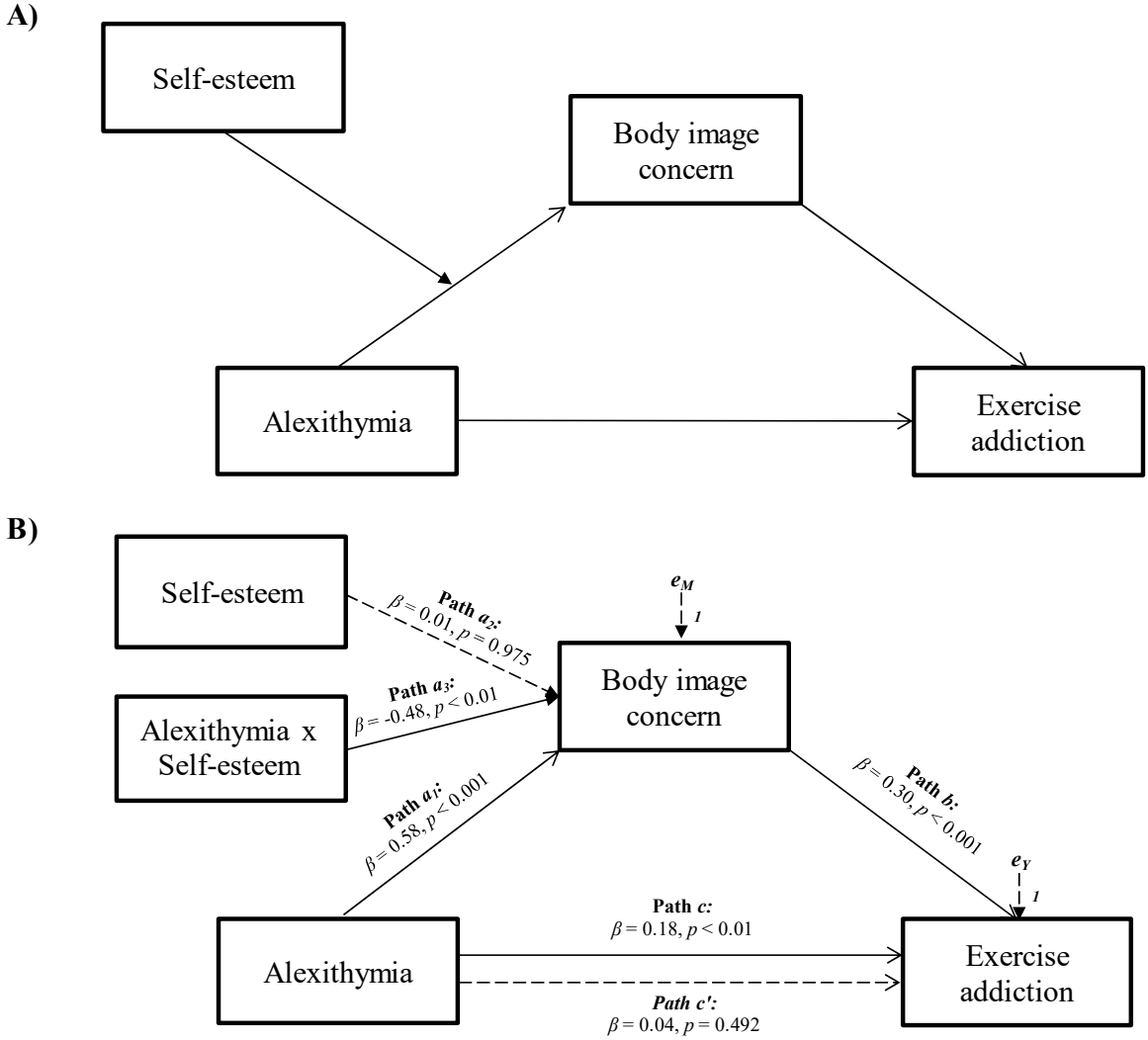

Figure 1. Statistical (**A**) and conceptual (**B**) forms of the moderated mediation model involving alexithymia, body image concerns, self-esteem, and exercise addiction.

2. Materials and Methods

2.1. Participants, Procedure, and Ethics

The sample comprised 288 Italian participants who declared that they regularly engaged in exercise (i.e., at least three times per week for a minimum of 30 min each session). Their age ranged from 19 to 53 years (M_{age} = 28.35 years, *SD* = 8.26), and they were predominantly females (72%). As shown in Table 1, most of the participants declared that they were single (75%), had a high school diploma (46%), and were students (35%).

All of the participants were recruited online. The study was advertised on the authors' various social networks, with a recruitment message that included an anonymous link to the survey. Therefore, the survey was further distributed utilizing a snowball sampling

method. The participants voluntarily took part in the study by completing a self-report survey hosted on the *Google Forms* platform, after they had been informed about the general aims of the research and provided informed consent electronically. The protocol of the present study was approved by the Ethical Committee of the Integrated Psychodynamic Psychotherapy Institute (IPPI) (ethical approval number 004/2021).

Table 1. Demographic characteristics of the sample (N = 288).

Characteristics		$M \pm SD$	n	%
Age (years)		28.4 ± 8.26		
Sex				
	Females		206	71.5
	Males		82	28.5
Marital Status				
	Single		215	74.7
	Married		25	8.7
	Cohabiting		39	13.5
	Separated		4	1.4
	Divorced		4	1.4
	Widowed		1	0.3
Education				
	Middle school diploma		8	2.8
	High school diploma		132	45.8
	University degree		86	29.9
	Master's degree		43	14.9
	Post-lauream specialization		19	6.6
Occupation				
	Student		98	34.0
	Working student		58	20.1
	Employee		78	27.1
	Freelance		18	6.3
	Entrepreneur		12	4.2
	Trader		8	2.8
	Artisan		3	1.0
	Armed forces		1	0.3
	Unemployed		11	3.8
	Retired		1	0.3

2.2. Measures

Exercise Addiction Inventory (EAI): The EAI [9,42] is a self-report measure that assesses the risk of exercise addiction. The six items (e.g., "*If I have to miss an exercise session, I feel moody and irritable*") were developed using the components model of behavioral addiction (Griffiths, 1996), and comprise the dimensions of salience, mood modification, tolerance, withdrawal symptoms, conflict, and relapse. Items are scored on a five-point Likert scale from 1 (*strongly disagree*) to 5 (*strongly agree*). The total scores range from 6 to 30, with higher scores indicating more problematic exercise for the individual. A cutoff score for individuals considered at risk of exercise addiction is 24, while a score of 13–23 indicates a symptomatic individual, and a score of 0–12 suggests an asymptomatic individual [9]. Cronbach's alpha value for the Italian version [43] used in the present study was α = 0.71.

Twenty-Item Toronto Alexithymia Scale (TAS-20): The TAS-20 [14,15] is a self-report measure that assesses alexithymia. The 20 items of the TAS-20 are scored on a five-point Likert scale from 1 ("*strongly disagree*) to 5 (*strongly agree*) and comprising three subscales: difficulty identifying feelings (e.g., "*I am often confused about what emotion I am feeling*"), difficulty describing feelings (e.g., "*It is difficult for me to find the right words for my feelings*"), and externally oriented thinking (e.g., "*I prefer to analyze problems rather than just describe them*"). Cronbach's alpha value for the Italian version [44] used in the present study was α = 0.75 for the total scale.

Body Image Concern Inventory (BICI): The BICI [45] is a self-report measure that assesses dysmorphic body image concerns. The 19 items of the BICI are scored on a five-point Likert scale from 1 (*never*) to 5 (*always*) and comprising two subscales: dysmorphic symptoms (e.g., "*I am dissatisfied with some aspect of my appearance*"), and symptom interference (e.g., "*I have missed social activities because of my appearance*"). Cronbach's alpha value for the Italian version [46] used in the present study was $\alpha = 0.91$ for the total scale.

Rosenberg Self-Esteem Scale (RSES): The RSES [47] is a self-report measure that assesses self-esteem. The 10 items of the RSES are scored on a four-point Likert scale from 0 (*strongly disagree*) to 3 (*strongly agree*). Cronbach's alpha value for the Italian version [48] used in the present study was $\alpha = 0.84$.

2.3. Data Analysis

Data were analyzed using SPSS for Windows (v. 21). A two-sided value of $p < 0.01$ was the level of statistical significance in the present study. There were no missing values in the dataset because the online platform used did not allow the submission of surveys unless all items were answered. Descriptive statistics for the sample and the study measures were carried out. A Pearson's *r* correlation analysis was performed to investigate the associations between the variables, together with the coefficient of determination (R^2). According to Cohen [49], values of 0.25, 0.09, and 0.01 correspond to large, moderate, or small relationships, respectively. The hypothesized moderated mediation model was tested through the macro-program PROCESS 3.4 [50], by performing Model 7. For completeness, the potential confounding role of age was also explored in the model. The 95% confidence interval (CI) was calculated for each regression coefficient, such that when the 95% CI (from LLCI to ULCI) does not contain the zero, the effect should be considered significant. The conditional indirect effect was evaluated following Wayne et al.'s [51] procedure, by analyzing the index of the moderated relationship at three different levels of the moderator (−1DS, Mean, +1DS). Furthermore, a bootstrapping procedure with 95% CI at 5000 samples was used to confirm the statistical significance of the moderation effect. When the bootstrapped confidence interval (from boot LLCI to boot ULCI) does not contain the zero, the effect should be considered significant.

3. Results

Descriptive statistics are reported in Tables 1 and 2. Pearson's *r* analysis (see Table 2) showed that the highest correlation was between exercise addiction and body image concern ($r = 0.317$, $p < 0.01$), explaining 30% of the variance. Furthermore, there were significant positive correlations between exercise addiction and age ($r = 0.153$, $p < 0.01$) and exercise addiction and alexithymia ($r = 0.178$, $p < 0.01$). There was a significant negative correlation between exercise addiction and self-esteem ($r = -0.152$, $p < 0.01$). In turn, self-esteem was significantly negatively correlated with body image concerns ($r = -0.608$, $p < 0.01$) and alexithymia ($r = -0.512$, $p < 0.01$) scores. Body image concerns and alexithymia were significantly positively correlated ($r = 0.454$, $p < 0.01$).

The moderated mediation analysis showed that body image concerns totally mediated the relationship between alexithymia and exercise addiction, and the association between alexithymia and body image concerns was moderated by self-esteem (see Figure 1).

More specifically, the total effect of alexithymia on exercise addiction was significant and positive (Path *c* in Figure 1B; $\beta = 0.18$, $p < 0.01$, LLCI = 0.0217 − ULCI = 0.0997). Alexithymia was also significantly and positively associated with body image concerns, the mediator variable (Path a_1 in Figure 1B; $\beta = 0.58$, $p < 0.001$, LLCI = 0.3846 − ULCI = 1.1129). Body image concerns showed a significant and positive relationship with exercise addiction (Path *b* in Figure 1B; $\beta = 0.30$, $p < 0.001$, LLCI = 0.0458–ULCI = 0.1115) and, when included in the model, totally mediated the association between alexithymia and exercise addiction (see Model 1a in Table 3), which became insignificant (Path *c'* in Figure 1B; $\beta = 0.04$, $p = 0.492$, LLCI = −0.0275 − ULCI = 0.0570). Furthermore, self-esteem was found to be a significant moderator in the relationship between alexithymia and body image concerns

(Path a_3 in Figure 1B; $\beta = -0.48$, $p < 0.01$, LLCI = −0.0413 − ULCI = −0.0079): $\Delta R^2 = 0.017$, $F(1, 285) = 6.810$, $p < 0.01$ (index of moderated mediation = −0.0019, Boot LLCI = −0.0037 − Boot ULCI = −0.0007).

Table 2. Pearson's correlation, means, and standard deviations of the study variables.

	1	2	3	4	5	6	7	8	9
1. EAI	1								
2. TAS-20	**0.178** **	1							
(R^2)	(0.032)								
3. TAS-20 (F1)	**0.163** **	**0.803** **	1						
(R^2)	(0.027)	(0.645)							
4. TAS-20 (F2)	**0.214** **	**0.844** **	**0.556** **	1					
(R^2)	(0.046)	(0.712)	(0.309)						
5. TAS-20 (F3)	0.006	**0.585** **	**0.279** **	**0.211** **	1				
(R^2)	(0.000)	(0.342)	(0.078)	(0.045)					
6. RSES	**−0.152** **	**−0.512** **	**−0.397** **	**−0.559** **	**−0.137** *	1			
(R^2)	(0.023)	(0.262)	(0.158)	(0.312)	(0.019)				
7. BICI	**0.317** **	**0.454** **	**0.331** **	**0.538** **	0.091	**−0.608** **	1		
(R^2)	(0.100)	(0.206)	(0.110)	(0.289)	(0.008)	(0.370)			
8. BICI (F1)	**0.305** **	**0.449** **	**0.343** **	**0.531** **	0.078	**−0.598** **	**0.990** **	1	
(R^2)	(0.093)	(0.202)	(0.118)	(0.282)	(0.006)	(0.358)	(0.980)		
9. BICI (F2)	**0.298** **	**0.382** **	**0.218** **	**0.457** **	**0.126** *	**−0.525** **	**0.837** **	**0.751** **	1
(R^2)	(0.089)	(0.146)	(0.048)	(0.209)	(0.016)	(0.276)	(0.701)	(0.564)	
10. Age	**0.153** **	**−0.175** **	**−0.151** *	**−0.173** **	−0.073	**0.191** **	−0.104	**−0.128** *	0.012
(R^2)	(0.023)	(0.031)	(0.023)	(0.030)	(0.005)	(0.036)	(0.011)	(0.016)	(0.000)
M	17.510	47.656	13.736	16.618	35.014	20.406	47.781	40.903	6.878
SD	4.446	13.061	5.091	6.924	4.401	7.006	16.795	13.927	3.595

Note: Bold values indicate significant *p*-values. **: Correlation is significant at the $p < 0.01$ level (2-tailed); *: correlation is significant at the $p < 0.05$ level (2-tailed). EAI: Exercise Addiction Inventory; TAS-20: 20-Item Toronto Alexithymia Scale; TAS-20 (F1): difficulty describing feelings (20-Item Toronto Alexithymia Scale); TAS-20 (F2): difficulty identifying feelings (20-Item Toronto Alexithymia Scale); TAS-20 (F3): externally oriented thinking (20-Item Toronto Alexithymia Scale); RSES: Rosenberg Self-Esteem Scale; BICI: Body Image Concern Inventory; BICI (F1): dysmorphic symptoms (Body Image Concern Inventory); BICI (F2): symptom interference (Body Image Concern Inventory).

The conditional indirect effect was evaluated by analyzing the index of the moderated relationship at three different levels of the moderator (−1DS, Mean, +1DS). The association between alexithymia and body image concerns was slightly stronger at low levels of self-esteem (estimate = 0.419[0.10], $p < 0.001$, LLCI = 0.2425 − ULCI = 0.5958) than at average levels (estimate = 0.246[0.07], $p < 0.001$, LLCI = 0.1131 − ULCI = 0.3806), and became insignificant at high levels (estimate = 0.075[0.09], $p = 0.822$, LLCI = −0.1039 − ULCI = 0.2529). Therefore, when participants reported higher levels of self-esteem, the positive indirect effect of alexithymia on exercise addiction via body image concerns weakened to become insignificant: effect = 0.0059(0.0068), BootLLCI = −0.0085- BootULCI = 0.0183 (see Figure 2).

Finally, the statistical significance of the moderation effect was confirmed via the bootstrapping procedure, since the bootstrapped confidence interval did not contain the zero: Boot LLCI = −0.0421 − Boot ULCI = −0.0098.

The potential confounding of age was also examined. Age showed a significant covariant effect on exercise addiction for both the indirect effect of alexithymia on exercise addiction via the mediation of body image concern, and the moderation of body image concern ($\beta = 0.20$, $p < 0.001$, Boot LLCI = 0.0547 − Boot ULCI = 0.1594), as well as for the total effect ($\beta = 0.19$, $p < 0.01$, Boot LLCI =0.0444–Boot ULCI = 0.1621). Moreover, controlling for age, both the total effect of alexithymia on exercise addiction ($\beta = 0.21$, $p < 0.001$, LLCI = 0.0331 − ULCI = 0. 1110) and the moderated mediation model (see Model 1b in Table 3) remained statistically significant.

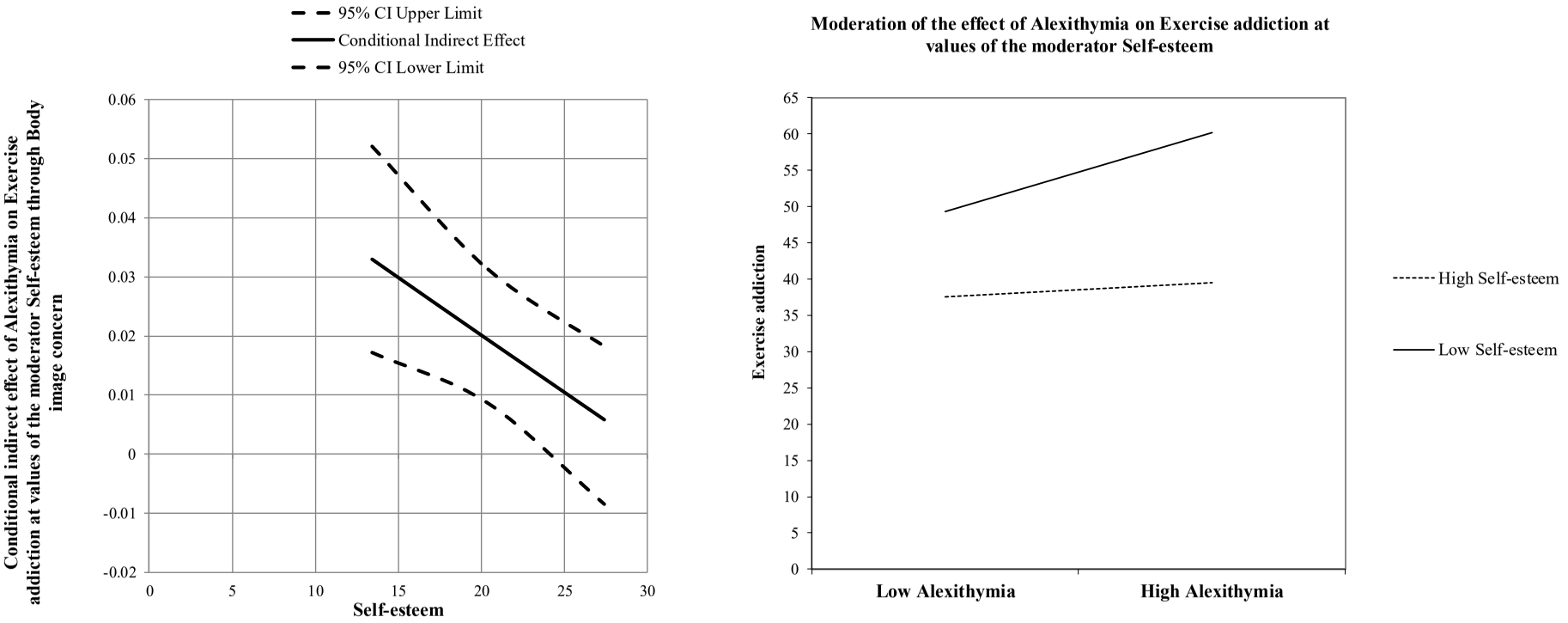

Figure 2. Graphical representation of the moderation effect.

Table 3. Coefficients of the models.

Model 1a										
	Consequent									
	M (Body image concern)					Y (Exercise addiction)				
Antecedent		Coeff.	SE	p	95% CI		Coeff.	SE	p	95% CI
X (Alexithymia)	a_1	0.749	0.185	0.001	[0.385, 1.113]	c'	0.015	0.022	0.492	[−0.028, 0.057]
M (Body image concern)		-	-	-	-	b	0.079	0.017	< 0.001	[0.046, 0.112]
W (Self-esteem)	a_2	0.014	0.443	0.975	[−0.859, 0.886]		-	-	-	-
X * W	a_3	−0.025	0.009	0.004	[−0.041, −0.008]		-	-	-	-
Constant	i_M	34.590	19.389	0.004	[14.141, 55.040]	i_Y	13.051	1.001	< 0.001	[11.081, 15.021]
				$R^2 = 0.415$					$R^2 = 0.102$	
				$F(3, 284) = 67.190$, $p < 0.001$					$F(2, 285) = 16.154$, $p < 0.001$	
Model 1b										
	Consequent									
	M (Body image concern)					Y (Exercise addiction)				
Antecedent		Coeff.	SE	p	95% CI		Coeff.	SE	p	95% CI
X (Alexithymia)	a_1	0.749	0.185	0.001	[0.385, 1.113]	c'	0.015	0.022	0.492	[−0.016, 0.068]
M (Body image concern)		-	-	-	-	b_1	0.080	0.016	< 0.001	[0.048, 0.112]
W (Self-esteem)	a_2	−0.002	0.445	0.975	[−0.878, 0.874]		-	-	-	-
X * W	a_3	−0.024	0.009	0.004	[−0.041, −0.008]		-	-	-	-
C (Age)	a_4	0.050	0.095	0.601	[−0.137; 0.236]	b_2	0.106	0.030	< 0.001	[0.048; 0.112]
Constant	i_M	33.363	10.663	0.020	[12.373, 54.353]	i_Y	9.440	1.416	< 0.001	[6.653, 12.227]
				$R^2 = 0.416$					$R^2 = 0.140$	
				$F(4, 283) = 50.332$, $p < 0.001$					$F(3, 284) = 15.375$, $p < 0.001$	

Note: Model 1a: the relationship between alexithymia and exercise addiction, with body image concern as mediator and self-esteem as moderator; Model 1b: the relationship between alexithymia and exercise addiction, with body imageconcern as mediator, self-esteem as moderator, and age as covariate.

4. Discussion

The physical, psychological, aesthetic, and social benefits of regular exercise activity are well documented [52–54], both for the adult population [54] and in the pre-adult developmental phase [55]. However, evidence is emerging in the literature that for a small minority of individuals, excessive physical exercise can acquire the features of an addiction [12,56], characterized by feelings of loss of control, overtraining problems such as fatigue and sleep disturbances, and withdrawal symptoms such as restlessness, sadness, and irritability [57]. In light of these considerations, and the psychophysical damage associated with this condition, the study of the mechanisms involved in the development and maintenance of this unhealthy form of exercise acquires great clinical and practical relevance. Given this framework, the present research analyzed the interaction between alexithymia, body image concerns, and self-esteem in contributing to exercise addiction among regular exercisers.

First, our results showed a significant and positive influence of alexithymia on exercise addiction, concurring with previous research [58,59]. This is consistent with other evidence highlighting the role of alexithymia and, more generally, emotional dysregulation in facilitating addictive behaviors, which may become dysfunctional strategies to cope with painful emotions (see Morie et al. [18] for a review). Indeed, given their lack of emotional awareness, individuals with high levels of alexithymia tend to have difficulty in managing their affect [14,60,61], and engaging in addictive behavior may become a dysfunctional strategy to cope with painful emotion [62,63]. Consistently, the theory of affect regulation [64] suggests that physical activity may lead to improvements in positive moods and decreases in negative ones (anxiety, irritability, and guilt). Therefore, some individuals may consider exercise as a means of coping with stress, to the point of becoming addicted to it [56,64–66]. Furthermore, the findings of the present study showed a significant positive association between alexithymia and body image concerns.

Consistently, previous evidence has found that poor emotional expression is related to higher levels of body dissatisfaction, among both clinical [27] and nonclinical samples [28]. One of the characteristics of alexithymia is that individuals have difficulty in understanding their own affective experiences and/or the association between emotional states and somatic manifestations. This may lead to an excessive focus on physical components and body image distortions so as to avoid contact with the emotional experiences [28,67]. Our results also highlighted the significant and positive influence of body image concerns on exercise addiction, determining a total mediation in the relationship between alexithymia and exercise addiction. Consistent with these data, significant and positive associations between negative body image and pathological exercise behaviors have previously been found [42,68].

This can be understood in light of the great potential that exercise has to modify the characteristics of the body (see Marques et al. [69] for a review). Therefore, body dissatisfaction may lead to morbid exercise, both through negative reinforcement (e.g., guilt by individuals for wasting opportunities to improve their appearance when skipping workouts) and positive reinforcement (e.g., a more toned body) [70]. Therefore, by integrating and enriching the existing evidence, data from the present research suggested that the total effect of alexithymia on exercise addiction did not occur directly, but manifested itself indirectly through the increase in dissatisfaction related to an individual's own body image, which resulted in morbid exercise, plausibly interpretable as a dysfunctional coping strategy [65]. However, the results also showed the relevant influence of self-esteem in this indirect path, so that as the score of self-esteem increased, the effect of alexithymia on body image concerns diminished to become insignificant. Such data are consistent with a previous study that highlighted an inverse relationship between self-esteem and negative perceptions of body image [28], and further corroborate the existing evidence relating to the core role of self-esteem in psychological wellbeing (e.g., [71]) and as a protective factor for mental health (e.g., [72]).

Additionally, age was found to be a significant confounding variable in the model, given its effect on exercise addiction. More specifically, older participants reported higher levels of morbid physical activity. These data add to the extant literature that currently reports conflicting results, sometimes identifying higher levels of exercise addiction among young people, while other studies report no difference based on age (e.g., [73,74]). Further studies are needed in order to investigate this aspect.

The present study has some limitations, which should be noted when interpreting its findings. First, the cross-sectional design of this research hinders the inference of causal links between the variables under examination. Furthermore, the implementation of the moderated mediation model did not consider the bidirectionality of the associations between the variables. Although the hypothesized links were based on a solid body of pre-existing literature, the present data only provide preliminary support for the observed relationships. Future longitudinal research is needed in order to test the relationships empirically, as well as considering the possibility of bidirectionality in the association between the variables, and further enriching the model by exploring the roles of other promising factors in the field of addiction and mental health, such as attachment (e.g., [75]), family functioning (e.g., [76]), and dissociation (e.g., [61]), to name but a few. Furthermore, data were collected online, and this could limit the generalizability of the study (for example, with respect to exercisers who did not have internet access). The relatively small sample size should also be noted when interpreting the study's outcomes. Moreover, the imbalance concerning some demographic variables (e.g., gender, relationship status, occupation) did not allow reliable evaluation of their relationship with the risk/protective factors included in the model, or of the relationships between them. Therefore, a more in-depth study utilizing a larger and more balanced sample, recruited with a more extensive and representative sampling method, is needed for future research, in order to provide a more complete picture of these results. In addition, no information was collected on the level of physical activity (e.g., recreational or competitive). Future research could

explore the influence of this variable on the hypothesized moderated mediation model. Finally, self-report measures were used, and this exposed the possibility of some bias (e.g., social desirability). Integration of other kinds of measure (e.g., structural interviews) following a multimethod approach could be important in future research to overcome this limitation. Therefore, in light of these limitations, the results must be generalized with caution, and studies with more representative samples of the national population, with a better distribution for demographic variables (e.g., gender, occupational status, relational status, etc.), are necessary. Further research is also needed examining the level/mode of sport (competitive/non-competitive; individual/team sport), with a longitudinal design and an integrated multimethod collection of further variables of interest, in order to obtain a clearer picture. On the other hand, the present research offers preliminary data that provide useful indications concerning the protective value of high levels of self-esteem against exercise addiction, as well as the importance of considering the potential risks that could be associated with alexithymia and body image concern.

5. Conclusions

As with other addictions, exercise addiction influences individuals in their daily lives, resulting in a loss of control and psychophysical damage [70]. Given the significant impairment in the lives of affected individuals, increasing research has focused on risk factors and antecedents for exercise addiction (e.g., [77]). Within this framework, the present study explored the positive relationships between alexithymia, body image concerns, and exercise addiction, but also confirmed the important protective role of self-esteem. These findings provide wider knowledge and insight regarding the variables associated with exercise addiction, and may have important clinical implications—for example, by orienting preventive activities among regular exercisers, as well as addressing tailored treatments for addicted individuals.

Author Contributions: Conceptualization, A.G.; methodology, A.G. and E.T.; formal analysis, A.G. and E.T.; data curation, A.G. and C.P.; writing—original draft preparation, A.G., E.T., C.P. and M.D.G.; writing—review and editing, A.G., E.T., C.P. and M.D.G.; supervision, A.G. and M.D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Integrated Psychodynamic Psychotherapy Institute (IPPI; protocol code 004/2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available, for reasons of privacy.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. WHO Guidelines on Physical Activity and Sedentary Behavior. 2020. Available online: https://www.who.int/publications/i/item/9789240015128 (accessed on 6 October 2021).
2. Reiner, M.; Niermann, C.; Jekauc, D.; Woll, A. Long-term health benefits of physical activity—A systematic review of longitudinal studies. *BMC Public Health* **2013**, *13*, 2–9. [CrossRef] [PubMed]
3. World Health Organization. *Physical Activity*; World Health Organization: Geneva, Switzerland, 2020. Available online: https://www.who.int/news-room/fact-sheets/detail/physical-activity (accessed on 6 October 2021).
4. Venturella, F.; Cancellieri, G.; Liga, A. Rilevazione statistica della diffusione dei fenomeni di binge drinking, drunkoressia e gambling tra gli adolescenti palermitani. *Mission* **2017**, *48*, 47–51. [CrossRef]
5. Szabo, A.; Griffiths, M.D.; de La Vega Marcos, R.; Mervó, B.; Demetrovics, Z. Methodological and conceptual limitations in exercise addiction research. *Yale J. Biol. Med.* **2015**, *88*, 303–308. [PubMed]
6. Lichtenstein, M.B.; Melin, A.K.; Szabo, A.; Holm, L. The prevalence of exercise addiction symptoms in a sample of national level elite athletes. *Front. Sports Act. Living* **2021**, *3*, 635418. [CrossRef] [PubMed]
7. Landolfi, E. Exercise addiction. *Sports Med.* **2012**, *43*, 111–119. [CrossRef]

8. Griffiths, M. A 'components' model of addiction within a biopsychosocial framework. *J. Subst. Use* **2005**, *10*, 191–197. [CrossRef]
9. Terry, A.; Szabo, A.; Griffiths, M. The exercise addiction inventory: A new brief screening tool. *Addict. Res. Theory* **2004**, *12*, 489–499. [CrossRef]
10. Veale, D. Does primary exercise dependence really exist? In *Exercise Addiction: Motivation for Participation in Sport and Exercise*; Annett, J., Cripps, B., Steinberg, H., Eds.; British Psychological Society: Leicester, UK, 1995; pp. 1–5.
11. Freimuth, M.; Moniz, S.; Kim, S.R. Clarifying exercise addiction: Differential diagnosis, co-occurring disorders, and phases of addiction. *Int. J. Environ. Res. Public Health* **2011**, *8*, 4069–4081. [CrossRef]
12. Griffiths, M. Exercise addiction: A case study. *Addict. Res.* **1997**, *5*, 161–168. [CrossRef]
13. Sifneos, P.E. The prevalence of 'alexithymic' characteristics in psychosomatic patients. *Psychother. Psychosom.* **1973**, *22*, 255–262. [CrossRef]
14. Bagby, R.M.; Parker, J.D.; Taylor, G.J. The twenty-item Toronto Alexithymia Scale—I. Item selection and cross-validation of the factor structure. *J. Psychosom. Res.* **1994**, *38*, 23–32. [CrossRef]
15. Bagby, R.M.; Taylor, G.J.; Parker, J.D. The twenty-item Toronto Alexithymia Scale—II. Convergent, discriminant, and concurrent validity. *J. Psychosom. Res.* **1994**, *38*, 33–40. [CrossRef]
16. Taylor, G.J. Recent developments in alexithymia theory and research. *Can. J. Psychiatry* **2000**, *45*, 134–142. [CrossRef]
17. Dell'Arte, S.; Lenzo, V. Personality, exercise addiction and orthorexia. A research contribution. *J. Clin. Child. Psychol.* **2021**, *3*, 63–83. [CrossRef]
18. Morie, K.P.; Yip, S.W.; Nich, C.; Hunkele, K.; Carroll, K.M.; Potenza, M.N. Alexithymia and addiction: A review and preliminary data suggesting neurobiological links to reward/loss processing. *Curr. Addict. Rep.* **2016**, *3*, 239–248. [CrossRef] [PubMed]
19. Lafollie, D. Detection of high-risk personalities in risky sports. *L'encephale* **2007**, *33*, 135–141. [CrossRef]
20. Hamidi, S.; Rostami, R.; Farhoodi, F.; Abdolmanafi, A. A study and comparison of Alexithymia among patients with substance use disorder and normal people. *Procedia-Soc. Behav. Sci.* **2010**, *5*, 1367–1370. [CrossRef]
21. de Berardis, D.; D'Albenzio, A.; Gambi, F.; Sepede, G.; Valchera, A.; Conti, C.M.; Fulcheri, M.; Cavuto, M.; Ortolani, C.; Salerno, R.M.; et al. Alexithymia and its relationships with dissociative experiences and Internet addiction in a nonclinical sample. *Cyberpsychol. Behav. Soc. Netw.* **2009**, *12*, 67–69. [CrossRef] [PubMed]
22. Parker, J.D.; Wood, L.M.; Bond, B.J.; Shaughnessy, P. Alexithymia in young adulthood: A risk factor for pathological gambling. *Psychother. Psychosom.* **2004**, *74*, 51–55. [CrossRef] [PubMed]
23. Orsolini, L. Unable to describe my feelings and emotions without an addiction: The interdependency between alexithymia and addictions. *Front. Psychiatry* **2020**, *11*, 543346. [CrossRef] [PubMed]
24. Manfredi, P.; Gambarini, A. Exercise addiction and alexithymia. *J. Psychol. Behav. Sci.* **2015**, *3*, 61–70. [CrossRef]
25. Allegre, B.; Souville, M.; Noel-Jorand, M.C.; Pellegrin, L.; Therme, P. Intensive physical activity and alexithymia: Results from swimmers' discourse analysis. *Psychol. Rep.* **2007**, *100*, 1129–1139. [CrossRef] [PubMed]
26. Iacolino, C.; Pellerone, M.; Formica, I.; Concetta Lombardo, E.M.; Tolini, G. Alexithymia, body perception and dismorphism: A study conducted on sportive and non-sportive subjects. *Clin. Neuropsychiatry* **2017**, *14*, 400–406.
27. Fenwick, A.S.; Sullivan, K.A. Potential link between body dysmorphic disorder symptoms and alexithymia in an eating-disordered treatment-seeking sample. *Psychiatry Res.* **2011**, *189*, 299–304. [CrossRef]
28. de Berardis, D.; Serroni, N.; Campanella, D.; Carano, A.; Gambi, F.; Valchera, A.; Conti, C.; Sepede, G.; Caltabiano, M.; Pizzorno, A.M.; et al. Alexithymia and its relationships with dissociative experiences, body dissatisfaction and eating disturbances in a non-clinical female sample. *Cognit. Ther. Res.* **2009**, *33*, 471–479. [CrossRef]
29. Corazza, O.; Simonato, P.; Demetrovics, Z.; Mooney, R.; van de Ven, K.; Roman-Urrestarazu, A.; Rácmolnár, L.; de Luca, I.; Cinosi, E.; Santacroce, R.; et al. The emergence of exercise addiction, body dysmorphic disorder, and other image-related psychopathological correlates in fitness settings: A cross sectional study. *PLoS ONE* **2019**, *14*, e0213060. [CrossRef] [PubMed]
30. Fortes, L.D.S.; Neves, C.M.; Filgueiras, J.F.; Almeida, S.S.; Ferreira, M.E.C. Body dissatisfaction, psychological commitment to exercise and eating behavior in young athletes from aesthetic sports. *Rev. Bras. Cineantropometria Desempenho Hum.* **2013**, *15*, 695–704.
31. Klein, D.A.; Bennett, A.S.; Schebendach, J.; Foltin, R.W.; Devlin, M.J.; Walsh, B.T. Exercise "addiction" in anorexia nervosa: Model development and pilot data. *CNS Spectr.* **2004**, *9*, 531–537. [CrossRef]
32. Blaydon, M.J.; Linder, K.J.; Kerr, J.H. Metamotivational characteristics of exercise dependence and eating disorders in highly active amateur sport participants. *Pers. Individ. Differ.* **2004**, *36*, 1419–1432. [CrossRef]
33. Gilbert, N.; Meyer, C. Fear of negative evaluation and the development of eating psychopathology: A longitudinal study among nonclinical women. *Int. J. Eat. Disord.* **2005**, *37*, 307–312. [CrossRef] [PubMed]
34. Grilo, C.M.; Masheb, R.M. Correlates of body image dissatisfaction in treatment-seeking men and women with binge eating disorder. *Int. J. Eat. Disord.* **2005**, *38*, 162–166. [CrossRef] [PubMed]
35. O'Dea, J.A. Body image and self-esteem. In *Encyclopedia of Body Image and Human Appearance*; Cash, T.F., Ed.; Elsevier Academic Press: Norfolk, VA, USA, 2012; pp. 141–147.
36. Ahadzadeh, A.S.; Rafik-Galea, S.; Alavi, M.; Amini, M. Relationship between body mass index, body image, and fear of negative evaluation: Moderating role of self-esteem. *Health Psychol. Open* **2018**, *5*, 205510291877425. [CrossRef] [PubMed]
37. Dunaev, J.L.; Schulz, J.L.; Markey, C.N. Cosmetic surgery attitudes among midlife women: Appearance esteem, weight esteem, and fear of negative appearance evaluation. *J. Health Psychol.* **2018**, *23*, 59–66. [CrossRef] [PubMed]

38. Junghans-Rutelonis, A.N.; Suorsa, K.I.; Tackett, A.P. Self-esteem, self-focused attention, and the mediating role of fear of negative evaluation in college students with and without asthma. *J. Am. Coll. Health* **2015**, *63*, 554–562. [CrossRef]
39. Jankauskiene, R.; Baceviciene, M. Body image concerns and body weight overestimation do not promote healthy behaviour: Evidence from adolescents in Lithuania. *Int. J. Environ. Res.* **2019**, *16*, 864. [CrossRef]
40. Paxton, S.J.; Eisenberg, M.E.; Neumark-Sztainer, D. Prospective predictors of body dissatisfaction in adolescent girls and boys: A five-year longitudinal study. *Dev. Psychol.* **2006**, *42*, 888–899. [CrossRef] [PubMed]
41. Gori, A.; Topino, E.; Griffiths, M.D. Protective and risk factors in exercise addiction: A series of moderated mediation analyses. *Int. J. Environ. Res. Public Health* **2021**, *18*, 9706. [CrossRef] [PubMed]
42. Griffiths, M.D.; Szabo, A.; Terry, A. The exercise addiction inventory: A quick and easy screening tool for health practitioners. *Br. J. Sports Med.* **2005**, *39*, e30. [CrossRef] [PubMed]
43. Gori, A.; Topino, E.; Griffiths, M. A screening tool for exercise addiction: The psychometric properties of the Italian Exercise Addiction Inventory. *Int. J. Ment. Health Addict.* **2021**, in press.
44. Bressi, C.; Taylor, G.; Parker, J.; Bressi, S.; Brambilla, V.; Aguglia, E.; Allegranti, I.; Bongiorno, A.; Giberti, F.; Bucca, M.; et al. Cross validation of the factor structure of the 20-item Toronto Alexithymia Scale: An Italian multicenter study. *J. Psychosom. Res.* **1996**, *41*, 551–559. [CrossRef]
45. Littleton, H.L.; Axsom, D.; Pury, C.L. Development of the Body Image Concern Inventory. *Behav. Res. Ther.* **2005**, *43*, 229–241. [CrossRef]
46. Luca, M.; Giannini, M.; Gori, A.; Littleton, H. Measuring dysmorphic concern in Italy: Psychometric properties of the Italian Body Image Concern Inventory (I-BICI). *Body Image* **2011**, *8*, 301–305. [CrossRef] [PubMed]
47. Rosenberg, M. Rosenberg Self-Esteem Scale (RSE). *PsycTESTS Dataset* **1965**, *61*, 52.
48. Prezza, M.; Trombaccia, F.R.; Armento, L. La scala dell'autostima di Rosenberg: Traduzione e validazione Italiana [The Rosenberg Self-Esteem Scale: Italian translation and validation]. *Giunti Organ. Spec.* **1997**, *223*, 35–44.
49. Cohen, J. *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed.; Lawrence Erlbaum: Hillsdale, NJ, USA, 1988.
50. Hayes, A.F. *Introduction to Mediation, Moderation, and Conditional Process Analysis Second Edition: A Regression-Based Approach*; Guilford Press: New York, NY, USA, 2018.
51. Wayne, S.J.; Lemmon, G.; Hoobler, J.M.; Cheung, G.W.; Wilson, M.S. The ripple effect: A spillover model of the detrimental impact of work–family conflict on job success. *J. Organ. Behav.* **2017**, *38*, 876–894. [CrossRef]
52. Biddle, S.J.; Mutrie, N.; Gorely, T.; Faulkner, G. *Psychology of Physical Activity: Determinants, Well-Being and Interventions*; Routledge: New York, NY, USA, 2021. [CrossRef]
53. Warburton, D.E.; Bredin, S.S. Reflections on physical activity and health: What should we recommend? *Can. J. Cardiol.* **2016**, *32*, 495–504. [CrossRef]
54. Warburton, D.E.; Nicol, C.W.; Bredin, S.S. Health benefits of physical activity: The evidence. *CMAJ* **2006**, *174*, 801–809. [CrossRef]
55. Piko, B.F.; Keresztes, N. Physical activity, psychosocial health and life goals among youth. *J. Community Health* **2006**, *31*, 136–145. [CrossRef]
56. Szabo, A. *Exercise Addiction: A Symptom or a Disorder*; Nova Science Publishers: Hauppauge, NY, USA, 2010.
57. Hausenblas, H.A.; Schreiber, K.; Smoliga, J.M. Addiction to exercise. *BMJ* **2017**, *357*, j1745. [CrossRef]
58. Woodman, T.; Cazenave, N.; Le Scanff, C. Skydiving as emotion regulation: The rise and fall of anxiety is moderated by alexithymia. *J. Sport Exerc. Psychol.* **2008**, *30*, 424–433. [CrossRef] [PubMed]
59. Van Landeghem, C.; Jakobson, L.S.; Keough, M.T. Risk of exercise dependence in university students: A subtyping study utilizing latent profile analysis. *Psychol. Sport Exerc.* **2019**, *45*, 101575. [CrossRef]
60. Pellerone, M.; Cascio, M.I.; Costanzo, G.; Gori, A.; Pace, U.; Craparo, G. Alexithymia and psychological symptomatology: Research conducted on a non-clinical group of Italian adolescents. *Int. J. Cult. Ment.* **2017**, *10*, 300–309. [CrossRef]
61. Gori, A.; Topino, E.; Craparo, G.; Bagnoli, I.; Caretti, V.; Schimmenti, A. A comprehensive model for gambling behaviors: Assessment of the factors that can contribute to the vulnerability and maintenance of gambling disorder. *J. Gambl. Stud.* **2021**. Online first. [CrossRef]
62. Craparo, G.; Magnano, P.; Zapparrata, M.V.; Gori, A.; Costanzo, G.; Pace, U.; Pellerone, M. Coping, attachment style and resilience: The mediating role of alexithymia. *Mediterr. J. Clin. Psychol.* **2018**, *6*, 1–30. [CrossRef]
63. Caretti, V.; Gori, A.; Craparo, G.; Giannini, M.; Iraci-Sareri, G.; Schimmenti, A. A new measure for assessing substance-related and addictive disorders: The Addictive Behavior Questionnaire (ABQ). *J. Clin. Med.* **2018**, *7*, 194. [CrossRef]
64. Hamer, M.; Karageorghis, C.I. Psychobiological mechanisms of exercise dependence. *Sports Med.* **2007**, *37*, 477–484. [CrossRef]
65. Berczik, K.; Szabó, A.; Griffiths, M.D.; Kurimay, T.; Kun, B.; Urbán, R.; Demetrovics, Z. Exercise addiction: Symptoms, diagnosis, epidemiology, and etiology. *Subst. Use Misuse* **2012**, *47*, 403–417. [CrossRef]
66. Szabo, A. The impact of exercise deprivation on well-being of habitual exercises. *Aust. J. Sci. Med. Sport* **1995**, *27*, 68–75. [PubMed]
67. Montebarocci, O.; Codispoti, M.; Surcinelli, P.; Franzoni, E.; Baldaro, B.; Rossi, N. Alexithymia in female patients with eating disorders. *Eat. Weight Disord.* **2006**, *11*, 14–21. [CrossRef]
68. Tornero-Quiñones, I.; Sáez-Padilla, J.; Castillo Viera, E.; Garcia Ferrete, J.J.; Sierra Robles, A. Risk of dependence on sport in relation to body dissatisfaction and motivation. *Sustainability* **2019**, *11*, 5299. [CrossRef]
69. Marques, A.; Peralta, M.; Sarmento, H.; Loureiro, V.; Gouvei, A.É.R.; de Matos, M.G. Prevalence of risk for exercise dependence: A systematic review. *Sports Med.* **2019**, *49*, 319–330. [CrossRef] [PubMed]
70. Szabo, A.; Demetrovics, Z.; Griffiths, M.D. Morbid exercise behavior: Addiction or psychological escape? In *The Exercise Effect on Mental Health: Neurobiological Mechanisms*; Budde, H., Wegner, M., Eds.; Routledge: New York, NY, USA, 2018; pp. 277–311.

71. Pandey, R.; Tiwari, G.K.; Parihar, P.; Rai, P.K. Positive, not negative, self-compassion mediates the relationship between self-esteem and well-being. *Psychol. Psychother.* **2021**, *94*, 1–15. [CrossRef] [PubMed]
72. Brausch, A.M.; Decker, K.M. Self-esteem and social support as moderators of depression, body image, and disordered eating for suicidal ideation in adolescents. *J. Abnorm. Child. Psychol.* **2014**, *42*, 779–789. [CrossRef]
73. Reche, C.; de Francisco, C.; Martínez-Rodríguez, A.; Ros-Martínez, A. Relationship among sociodemographic and sport variables, exercise dependence, and burnout: A preliminary study in athletes. *Ann. Psychol.* **2018**, *34*, 398–404. [CrossRef]
74. Mayolas-Pi, C.; Simon-Grima, J.; Peñarrubia-Lozano, C.; Munguía-Izquierdo, D.; Moliner- Urdiales, D.; Legaz-Arrese, A. Exercise addiction risk and health in male and female amateur endurance cyclists. *J. Behav. Addict.* **2016**, *6*, 74–83. [CrossRef]
75. Giannini, M.; Gori, A.; De Sanctis, E.; Schuldberg, D. Attachment in psychotherapy: Psychometric properties of the Psychological Treatment Inventory Attachment Styles Scale (PTI-ASS). *J. Psychother. Integr.* **2011**, *21*, 363–381. [CrossRef]
76. Cacioppo, M.; Barni, D.; Correale, C.; Mangialavori, S.; Danioni, F.; Gori, A. Do attachment styles and family functioning predict adolescents' problematic internet use? A relative weight analysis. *J. Child. Fam. Stud.* **2019**, *28*, 1263–1271. [CrossRef]
77. Bircher, J.; Griffiths, M.D.; Kasos, K.; Demetrovics, Z.; Szabo, A. Exercise addiction and personality: A two-decade systematic review of the empirical literature (1995–2015). *Balt. J. Sport Health Sci.* **2017**, *3*, 19–33. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

The Link between Attachment and Gambling in Adolescence: A Multiple Mediation Analysis with Developmental Perspective, Theory of Mind (Friend) and Adaptive Response

Grazia Terrone [1,*], Alessio Gori [2], Eleonora Topino [3], Alessandro Musetti [4], Alessia Scarinci [5], Camilla Guccione [3] and Vincenzo Caretti [3]

[1] Department of History, Cultural Heritage, Education and Society, University of Rome Tor Vergata, 00133 Rome, Italy
[2] Department of Human Sciences, University of Florence, 50100 Florence, Italy; gori.alessio@gmail.com
[3] Department of Human Sciences, Lumsa University of Rome, 00185 Rome, Italy; eleonora.topino@gmail.com (E.T.); c.guccione@lumsa.it (C.G.); vincenzocaretti@gmail.com (V.C.)
[4] Department of Humanities, Social Sciences and Cultural Industries, University of Parma, 43121 Parma, Italy; alessandro.musetti@unipr.it
[5] Department of Education Sciences, Psychology, Communication, University of Bari, 70121 Bari, Italy; alessia.scarinci@uniba.it
* Correspondence: grazia.terrone@uniroma2.it

Abstract: Introduction: Several studies have supported the evidence that attachment styles are a central factor in adolescent gambling problems. On this theoretical basis, the aim of the present study is to analyze a hypothesized mediation model exploring both the direct and indirect effects of insecure attachment on gambling disorder by investigating the role of the developmental perspective, theory of mind (friend) and adaptive response in that relationship. **Method**: The sample consists of 178 adolescents who underwent the *Measures: South Oaks Gambling Screen—Revised for Adolescents* and *Friends and Family Interview*. **Result**: The mediation analysis was conducted following Hayes' (2018) procedure, using Model 6. The results showed a significant association between insecure attachment and gambling disorder ($\beta = 0.669$; $p < 0.001$). The findings also highlighted a significant chained mediation model in which insecure attachment negatively influenced the developmental perspective ($\beta = -0.742$; $p < 0.001$), which affected the theory of mind toward one's own best friend ($\beta = 0.352$; $p < 0.001$). **Conclusions**: The results highlighted a significant role of insecure attachment in predicting the symptomatic expression of gambling among adolescents, specifically impacting the development perspective, theory of mind toward one's best friend and adaptive response to stress, which were linked to each other by a sequential influence. Therefore, our results showed that a poor developmental self-vision predicted a dysfunctional theory of mind toward the best friend. This could hinder the formation of positive peer relationships, which are crucial for the development of one's identity.

Keywords: gambling disorder; attachment; adolescence; friend and family interview

Citation: Terrone, G.; Gori, A.; Topino, E.; Musetti, A.; Scarinci, A.; Guccione, C.; Caretti, V. The Link between Attachment and Gambling in Adolescence: A Multiple Mediation Analysis with Developmental Perspective, Theory of Mind (Friend) and Adaptive Response. *J. Pers. Med.* **2021**, *11*, 228. https://doi.org/10.3390/jpm11030228

Academic Editor: Marco Costanzi

Received: 28 January 2021
Accepted: 13 March 2021
Published: 22 March 2021

1. Introduction

Research has shown that gambling is a popular conduct among adolescents, with high rates of problematic and pathological gambling [1]; they can indulge in classic and popular types of gambling, but authors have highlighted a steady increase in novel forms of gambling via the Internet [2–4], with greater local availability and accessibility [5]. Adolescent gambling may lead to negative consequences such as problematic relationships, delinquent and aggressive behavior [6], depression symptoms [7], increased risk of attempted suicide, increased risk of comorbidity with other forms of addiction [8] and general health problems [9–12].

Several studies have supported the evidence that attachment styles could play a key role in adolescent gambling problems [13–16]. Indeed, a growing body of research analyzed the relationship between adolescent gambling and attachment styles and found a higher incidence of insecure relationships with caregiver in gamblers and also links between alexithymia, attachment, and gambling disorder [17–19]. More specifically, insecure attachment hinders the development of adequate regulation skills and this predisposes one to emotional maladjustment [20]; therefore, addictive behaviors can be seen as an attachment disorder [15,21,22] and as an attempt at self-medication [23–25]. Indeed, previous research showed that gambling behaviours may act as external regulators of internal emotional states [17,26–28] and insecure attachment could be a vulnerability factor for its onset [29,30].

According to this framework, the aim of this present study was to investigate the impact of insecure attachment on gambling disorders in adolescence and to analyze the mediating role of several related variables. Indeed, the internal working models modeled in early childhood will influence aspects that are still being defined in this delicate and important life stage, such as the temporal perspective [31], the quality of relationships with peers [32] and the ability to provide adaptive responses to distress [33].

During adolescence, one acquires a greater awareness of his or her identity, taking up and creating his or her own memories of the past. At the same time, greater importance is put on the future, including the realization of one's aspirations and projects [34–36]. This developmental self-vision, which is linked to one's entire past, present and future axes, is extremely influenced by relationships with one's caregivers. For example, if caregivers were not available for or responsive to the child's needs, the child will perceive himself or herself as unworthy of being loved, and this negative vision will structure the child's expectations of the future [37–39]. On the contrary, when parents represent a secure base [40], the adolescent will be able to lean on it, which will help the adolescent to imagine his or her present, past and future in much more optimistic and hopeful terms, favoring better psychological adaptation and a better ability to have trusting and supportive peer relationships [41,42].

In particular, adolescence is the period of differentiation from one's caregivers in favor of peer relationships [43–45], although caregivers remain an important internal and external reference point [46] through the indirect influence that they have on one's beliefs about appropriate social behaviors and relationship models based on attachment experiences [47]. In this regard [48], argued that when social information is likely to cause psychological pain, insecure individuals will be more likely than confident ones to exclude or defensively suppress this information from further processing, because insecure individuals are less likely to have had experiences with an attachment figure in which their painful emotions were understood and elaborated. This will also influence the level of "theory of the mind", defined as the ability to interpret others' behaviors within a mentalistic structure in order to understand how oneself and others think, feel, perceive, imagine, react, attribute, infer and so on [49].

Finally, in addition to relationships, attachment also influences coping strategies aimed at dealing with stressful situations [50,51]. Taken together, recent research suggests that successful coping has important implications for the severity of gambling among young people.

The results also revealed that heavier players used more maladaptive forms of coping than others, whether oriented toward emotions or distraction [52,53]. This evidence fits well within [54,55], which suggests that pathological gamblers exhibit various psychological vulnerabilities that leave them ill-equipped, compared to others, to cope with stress. In this context, gambling, akin to other addictive behaviors, is aimed at negotiating negative or stressful experiences when the subject is lacking the resources to find more adequate answers [56].

On these theoretical bases, the present study aims to analyze a hypothesized mediation model exploring both the direct and indirect effects of insecure attachment on gambling

disorder by investigating the roles of the developmental perspective, theory of mind (friend) and adaptive responses in that relationship.

2. Method

2.1. Participants and Procedure

The sample consisted of 178 adolescents (42.1% male and 57.9% female), with a mean age of 17.51 years (*SD* = 0.818), ranging from 16 to 22. The participants were recruited from several secondary schools in Rome. The interview and questionnaire were administered in person in a one-to-one setting by one of the researchers.

Informed consent was obtained from both adolescents and their parents prior to participation in the study. The subjects did not receive any form of payment for participating and were free to leave the study at any time.

2.2. Measures

South Oaks Gambling Screen—Revised for Adolescents (SOGS-RA).

The *South Oaks Gambling Screen—Revised for Adolescents* (SOGS-RA; [57]) is a self-report questionnaire used to assess gambling behaviors and gambling-related problems in adolescents. It is made up of 12 dichotomously scored items and other unscored ones investigating the frequency of participation in different gambling activities, the largest amount of money gambled in a day, and parental involvement in problematic gambling.

The SOGS-RA scale identifies three categories: nonproblem gambler (score of 0 or 1), at-risk gambler (score of 2 or 3) and problem gambler (score of 4 or more). For the present study, the Italian version [58] was used. The SOGS demonstrated high internal consistency, with a Cronbach's alpha coefficient of 0.84.

2.3. Friends and Family Interview (FFI)

The *Friends and Family Interview* (FFI; [59,60]) is a semistructured interview designed to assess the attachment representations of adolescents, focusing on oneself, peers (one's best friend), siblings and parents. It lasts around 45 min and consists of 27 questions about the adolescents and their most significant relationships, with scores ranging from 1 (*"no evidence"*) to 4 (*"marked evidence"*) and including half-points.

The FFI coding system comprises both attachment classifications (*secure-autonomous, insecure-dismissing, insecure-preoccupied* and *insecure-disorganized*) and dimensional scores across numerous domains: (1) Firstly, the coherence of answers is evaluated based on the entire interview, based on their *truth* (based on the presence of convincing evidence), *economy* (based on the amount of given information), *relation* (based on the relevance of the examples provided) and *manner* (based on the maintenance of age-appropriate levels of attention, politeness and interest). (2) Another domain concerns reflective functioning (RF), which includes one's *developmental perspective* (the ability to relate one's own present views, feeling and thoughts with past and future attitudes), *theory of mind* (the ability to assume others' mental perspectives), *diversity of feelings* (the ability to discuss negative and positive affections that could be linked to oneself and significant relationships) and *internal working models* (the availability of a secure base from the subjects' mothers and fathers, emerging from their narrative). (3) An evaluation of the child's self-esteem is also given, comprising *social competence*, *school competence* and *self-regard*. (4) Peer relations are explored, in terms of both *frequency of contact* and *quality* of one's best friendship. (5) Sibling relations are investigated in terms of *warmth*, *hostility* and *rivalry*. (6) The FFI captures affective regulation strategies, in terms of both *defensive response* (idealization, role reversal, anger and derogation) and *adaptive response* to distress. (7) Finally, the differentiation of parental representation is examined by observing the participant's ability to compare and contrast the quality of one's relationships with each caregiver. For the present study, the Italian version by [61] was used.

2.4. Data Analysis

All of the data analyses were performed with the SPSS statistical software (IBM-SPSS version 25.0, IBM, Armonk, NY, USA) for Windows. Descriptive statistics were calculated. Pearson's *r* correlations were used to investigate the associations between the variables. Then, the SPSS macroprogram PROCESS 3.4 [62] was used to verify the hypnotized multiple-mediation model. Bootstrapping with 5000 samples and a 95% confidence interval was performed to test the significance of the indirect effect.

3. Results

Table 1 shows the descriptive statistics for both the sample and the measures.

Table 1. Descriptive statistics.

	Means (SD)					
	Total (*N* = 178)	**Gender**		**Gambling Disease**		
		Boys (*N* = 75)	**Girls (*N* = 103)**	**Absent (*N* = 141)**	**Risk (*N* = 24)**	**Pathological (*N* = 13)**
Age	17.51 (0.82)	17.52 (0.88)	17.50 (0.78)	17.38 (0.651)	18.12 (1.30)	17.77 (0.83)
Measures						
SOGS	0.95 (1.81)	1.71 (2.41)	0.40 (0.88)	0.20 (0.40)	2.58 (0.50)	6.08 (2.40)
FFI Attachment Patterns						
Secure-autonomous	2.74 (0.81)	2.62 (0.84)	2.84 (0.76)	2.90 (0.73)	2.10 (0.83)	2.36 (0.81)
Insecure-dismissing	1.95 (0.88)	2.09 (0.92)	1.83 (0.84)	1.80 (0.79)	2.62 (0.97)	2.17 (1.03)
Insecure-preoccupied	1.45 (0.67)	1.46 (0.76)	1.44 (0.60)	1.43 (0.63)	1.50 (0.72)	1.58 (1.00)
Disorganized	1.26 (0.63)	1.24 (0.65)	1.27 (0.63)	1.23 (0.59)	1.33 (0.82)	1.46 (0.67)
Coherence						
Truth	2.87 (0.72)	2.81 (0.76)	2.92 (0.68)	2.98 (0.69)	2.39 (0.58)	2.69 (0.86)
Economy	2.79 (0.65)	2.71 (0.71)	2.84 (0.61)	2.91 (0.61)	2.33 (0.57)	2.38 (0.77)
Relation	2.69 (0.80)	2.53 (0.85)	2.80 (0.73)	2.82 (0.76)	2.17 (0.72)	2.31 (0.75)
Manner	3.25 (0.73)	3.20 (0.69)	3.29 (0.76)	3.36 (0.69)	2.79 (0.83)	3.00 (0.58)
Overall coherence	2.74 (0.76)	2.62 (0.88)	2.83 (0.67)	2.85 (0.65)	2.74 (1.10)	2.75 (0.50)
Reflective Functioning						
Developmental perspective	2.77 (0.93)	2.74 (0.89)	2.79 (0.95)	2.85 (0.91)	2.35 (0.86)	2.73 (1.01)
Theory of mind						
Mother	2.68 (0.80)	2.61 (0.84)	2.73 (0.77)	2.75 (0.76)	2.48 (0.85)	2.31 (1.03)
Father	2.58 (0.83)	2.31 (0.87)	2.60 (0.78)	2.54 (0.82)	2.26 (0.81)	2.23 (0.93)
Friend	2.43 (0.91)	2.26 (0.90)	2.55 (0.90)	2.54 (0.89)	1.96 (0.88)	2.23 (0.93)
Sibling	2.42 (0.84)	2.27 (0.88)	2.52 (0.80)	2.57 (0.77)	1.82 (0.88)	1.78 (0.83)
Teacher	2.59 (0.75)	2.58 (0.66)	2.60 (0.80)	2.64 (0.73)	2.25 (0.91)	2.69 (0.48)
Diversity of feelings						
Self	2.73 (0.97)	2.68 (0.95)	2.76 (0.99)	2.83 (0.99)	2.38 (0.82)	2.36 (0.81)
Mother	2.48 (1.06)	2.37 (1.04)	2.56 (1.07)	2.60 (1.04)	2.09 (1.04)	2.00 (1.04)
Father	2.54 (0.80)	2.51 (0.76)	2.56 (0.84)	2.60 (0.81)	2.43 (0.79)	2.17 (0.72)
Friend	2.46 (0.93)	2.33 (0.87)	2.54 (0.96)	2.53 (0.96)	2.08 (0.78)	2.46 (0.78)
Sibling	2.60 (0.83)	2.56 (0.78)	2.62 (0.88)	2.72 (0.79)	2.12 (0.89)	2.20 (0.79)
Secure base/safe haven						
Mother	2.52 (0.83)	2.38 (0.93)	2.63 (0.73)	2.60 (0.80)	2.23 (0.92)	2.25 (0.87)
Father	2.17 (0.73)	2.13 (70)	2.20 (0.76)	2.21 (0.72)	1.96 (0.81)	2.15 (0.69)
Self-esteem						
Social competence	2.86 (68)	2.79 (0.72)	2.92 (0.64)	2.56 (0.53)	2.54 (0.72)	2.77 (0.83)
School competence	2.90 (0.57)	2.81 (0.60)	2.97 (0.54)	2.93 (0.64)	2.71 (0.55)	3.00 (0.95)
Self-regard	2.61 (0.67)	2.67 (0.61)	2.57 (0.69)	2.67 (0.64)	2.30 (0.77)	0.62 (0.51)
Friend relationship						
Frequency of contact	2.63 (1.01)	2.66 (1.03)	2.61 (0.99)	2.69 (0.99)	2.70 (0.97)	1.92 (1.04)
Quality of relation	2.77 (0.78)	2.65 (0.82)	2.85 (0.73)	2.85 (0.75)	2.46 (0.78)	2.54 (0.88)
Sibling relationship						
Warmth	2.83 (0.82)	2.67 (0.84)	2.94 (0.80)	2.93 (0.80)	2.29 (0.77)	2.60 (0.84)
Hostility	1.41 (0.64)	1.54 (0.72)	1.33 (0.57)	1.36 (0.62)	1.56 (0.63)	1.80 (0.79)
Rivalry	1.13 (0.33)	1.15 (0.37)	1.11 (0.32)	1.11(0.32)	1.12 (0.33)	1.30 (0.48)

Table 1. *Cont.*

	Means (SD)					
	Total (*N* = 178)	Gender		Gambling Disease		
		Boys (*N* = 75)	Girls (*N* = 103)	Absent (*N* = 141)	Risk (*N* = 24)	Pathological (*N* = 13)
Affective regulation Idealization						
Self	1.19 (0.43)	1.20 (0.44)	1.19 (0.42)	1.17 (0.40)	1.29 (0.55)	1.23 (0.44)
Mother	1.76 (0.72)	1.83 (0.77)	1.72 (0.68)	1.70 (0.64)	2.09 (1.00)	1.83 (0.84)
Father	1.68 (0.68)	1.64 (0.70)	1.71 (0.67)	1.65 (0.65)	1.83 (0.89)	1.69 (0.63)
Role reversal						
Mother	1.25 (0.50)	1.29 (0.52)	1.22 (0.50)	1.22 (0.50)	1.35 (0.49)	1.38 (0.51)
Father	1.15 (0.42)	1.15 (0.44)	1.15 (0.41)	1.14 (0.39)	1.29 (0.62)	1.00 (0.00)
Anger						
Mother	1.24 (0.52)	1.30 (0.57)	1.19 (0.47)	1.23 (0.51)	1.38 (0.65)	1.08 (0.28)
Father	1.19 (0.44)	1.31 (0.55)	1.11 (0.31)	1.17 (0.38)	1.25 (0.53)	1.31 (0.75)
Derogation						
Self	1.17 (0.44)	1.21 (0.45)	1.14 (0.43)	1.18 (0.42)	1.08 (0.41)	1.23 (0.60)
Mother	1.10 (0.41)	1.17 (0.45)	1.05 (0.37)	1.06 (0.37)	1.21 (0.51)	1.31 (0.48)
Father	1.12 (0.37)	1.16 (0.44)	1.10 (0.30)	1.09 (0.32)	1.13 (0.34)	1.42 (0.67)
Adaptive Response	2.69 (0.80)	2.56 (0.84)	2.78 (0.76)	2.83 (0.77)	2.17 (0.72)	2.23 (0.73)
Differentiation of parental representations	3.05 (0.73)	3.04 (0.75)	3.06 (0.71)	3.15 (0.67)	2.67 (0.96)	2.85 (0.56)

The association patterns between the SOGS scores, FFI attachment classifications and other FFI domains are presented in Table 2.

Table 2. Correlation matrix between South Oaks Gambling Screen (SOGS), Friends and Family Interview (FFI) attachment patterns and FFI domains.

	FFI Attachment Patterns					SOGS
	4-Way				2-Way	
	Secure-Autonomous	Insecure-Dismissing	Insecure-Preoccupied	Disorganized-Disoriented	Secure/Insecure	
SOGS	−0.263 **	0.186 *	0.046	0.051	0.311 **	1
FFI Coeherence						
Truth	0.791 **	−0.658 **	0.002	−0.378 **	−0.592 **	−0.156 *
Economy	0.728 **	−0.520 **	−0.134	−0.337 **	−0.592 **	−0.249 **
Relation	0.752 **	−0.482 **	−0.138	−0.351 **	−0.567 **	−0.229 **
Manner	0.578 **	−0.332 **	−0.197 *	−0.236 **	−0.396 **	−0.182 *
Overall coherence	0.733 **	−0.554 **	−0.075	−0.402 **	−0.530 **	−0.157
Reflective Functioning						
Developmental perspective	0.515 **	−0.499 **	0.115	−0.207 **	−0.320 **	−0.076
Theory of mind						
Mother	0.488 **	−0.381 **	0.079	−0.156 *	−0.249 **	−0.132
Father	0.442 **	−0.365 **	0.127	−0.153	−0.286 **	−0.126
Friend	0.514 **	−0.522 **	0.033	−0.224 **	−0.480 **	−0.148
Sibling	0.377 **	−0.391 **	−0.108	−0.198 *	−0.371 **	−0.264 **
Teacher	0.415 **	−0.452 **	0.124	−0.152	−0.303 **	−0.035
Diversity of feelings						
Self	0.572 **	−0.536 **	0.067	−0.351 **	−0.429 **	−0.126
Mother	0.559 **	−0.459 **	0.129	−0.192 *	−0.390 **	−0.210 **
Father	0.520 **	−0.457 **	0.135	−0.059	−0.344 **	−0.149
Friend	−0.077	0.100	−0.055	−0.033	0.132	−0.044
Sibling	0.656 **	−0.538 **	−0.060	−0.324 **	−0.604 **	−0.186 *

Table 2. *Cont.*

	FFI Attachment Patterns					SOGS
	4-Way				2-Way	
	Secure-Autonomous	Insecure-Dismissing	Insecure-Preoccupied	Disorganized-Disoriented	Secure/Insecure	
Secure base/safe haven						
Mother	0.612 **	−0.402 **	−0.165 *	−0.261 **	−0.503 **	−0.123
Father	0.468 **	−0.345 **	−0.037	−0.201 *	−0.375 **	−0.077
Self-esteem						
Social competence	0.462 **	−0.411 **	−0.053	−0.296 **	−0.320 **	−0.100
School competence	0.374 **	−0.382 **	−0.040	−0.255 **	−0.343 **	0.003
Self-regard	0.423 **	−0.317 **	−0.074	−0.289 **	−0.304 **	−0.115
Friend relationship						
Frequency of contact	0.141	−0.220 **	0.037	−0.162 *	−0.140	−0.123
Quality of relation	0.585 **	−0.561 **	0.087	−0.248 **	−0.458 **	−0.107
Sibling relationship						
Warmth	0.398 **	−0.340 **	−0.034	−0.283 **	−0.333 **	−0.157
Hostility	−0.227 *	0.109	0.041	0.035	0.159	0.143
Rivalry	−0.043	−0.068	0.146	−0.013	0.049	0.143
Affective regulation						
Self	−0.135	0.183 *	−0.043	0.173 *	0.120	0.038
Mother	−0.429 **	0.574 **	−0.246 **	0.176 *	0.373 **	0.035
Father	−0.388 **	0.439 **	−0.205 **	0.154 *	0.342 **	−0.013
Role reversal						
Mother	−0.036	−0.201 *	0.263 **	−0.011	−0.014	0.124
Father	−0.030	0.019	0.160 *	0.216 **	0.049	−0.055
Anger						
Mother	−0.202 *	−0.055	0.328 **	0.150	0.209 **	0.003
Father	−0.118	−0.151	0.364 **	0.020	0.085	0.133
Derogation						
Self	−0.250 **	0.136	0.115	0.222 **	0.182 *	0.022
Mother	−0.360 **	0.212 **	0.122	0.084	0.326 **	0.136
Father	−0.208 *	−0.026	0.309 **	0.018	0.187 *	0.228 **
Adaptive Response	0.635 **	−0.391 **	−0.134	−0.261 **	−0.505 **	−0.290 **
Differentiation of parental representations	0.335 **	−0.441 **	0.210 **	−0.045	−0.312 **	−0.243 **

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

The mediation analysis was conducted following [62] procedure, using Model 6 (see Figure 1).

The results showed a significant association between insecure attachment and gambling disorder ($\beta = 0.669$, $p < 0.001$) when estimating path c in Figure 1. The findings also highlighted a significant chained mediation model in which insecure attachment negatively influenced the developmental perspective (path a^1 in Figure 1; $\beta = -0.742$, $p < 0.001$), which affected the theory of mind toward one's own best friend (path a^4 in Figure 1; $\beta = 0.352$, $p < 0.001$), which in turn predicted the adaptive response to distress (path b^3 in Figure 1; $\beta = 0.215$, $p < 0.05$), which ultimately impacted gambling disease levels (path b^5 in Figure 1; $\beta = -0.219$, $p < 0.05$). However, this finding did not suffer any direct effects from the first two mediators (path b^2 in Figure 1 with $\beta = 0.048$, $p = 0.592$ and path b^4 in Figure 1 with $\beta = 0.052$, $p = 0.590$, respectively). Insecure attachment also negatively and significantly predicted the theory of mind toward one's best friend (path a^2 in Figure 1; $\beta = -0.841$,

$p < 0.001$) and adaptive response (path a^3 in Figure 1; $\beta = -0.806$, $p < 0.001$), although its direct effect on gambling disorder was not significant (path c' in Figure 1; $\beta = 0.443$, $p = 0.055$), indicating a complete mediation after controlling the mediators ($R^2 = 0.115$, $F_{4,148} = 4.825$, $p = 0.001$) (see Table 3).

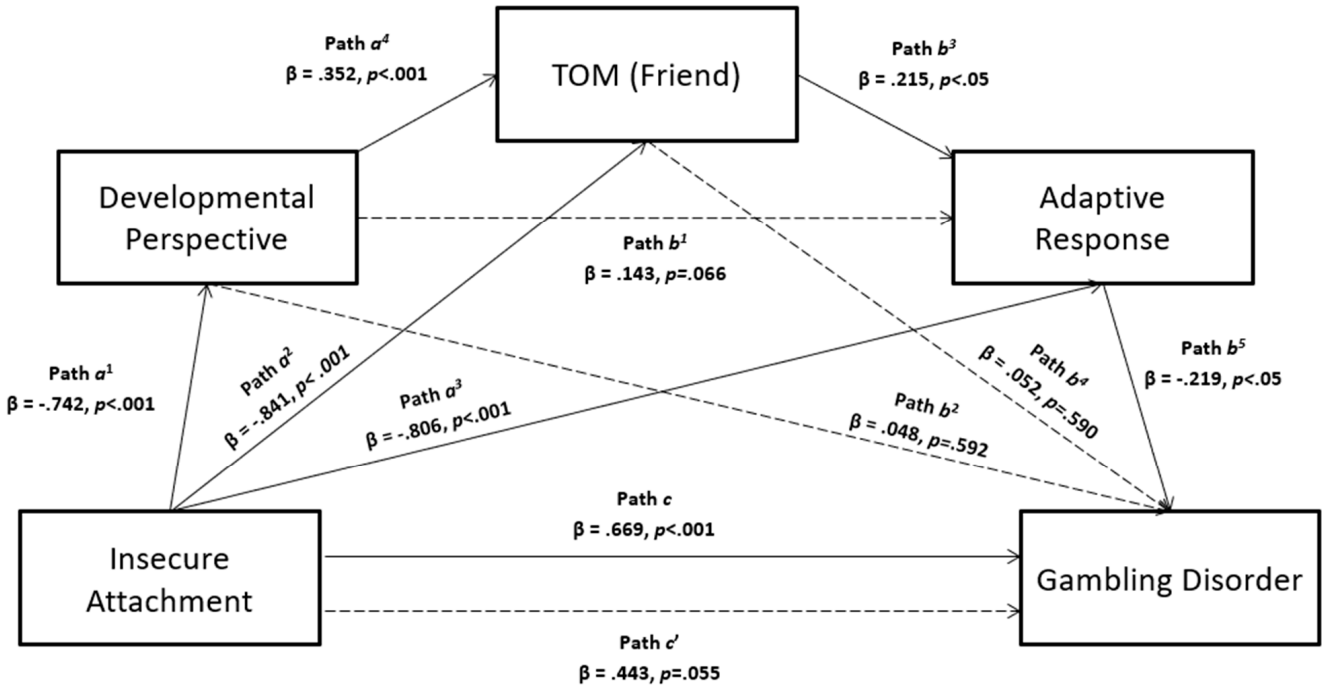

Figure 1. Chained multiple mediation model from insecure attachment to gambling disorder, through developmental perspective, theory of mind (friend) and adaptive response.

Table 3. Mediation model coefficients.

Antecedent	Consequent															
	M1				M2				M3				Y			
		Coeff.	SE	*p*		Coeff.	SE	*p*		Coeff.	SE	*p*		Coeff.	SE	*p*
X	a^1	−0.691	0.167	<0.001	a^2	−0.760	0.147	<0.001	a^3	−0.653	0.146	<0.001	c'	0.930	0.401	0.022
M1		-	-	-	a^4	0.341	0.068	<0.001	b^1	0.124	0.067	0.066	b^2	0.094	0.175	0.592
M2		-	-	-		-	-	-	b^3	0.193	0.075	0.011	b^4	0.106	0.196	0.590
M3		-	-	-		-	-	-		-	-	-	b^5	−0.494	0.211	0.021
Constant	i_{M1}	3.631	0.220	<0.001	i_{M2}	2.456	0.308	<0.001	i_{M2}	2.698	0.335	<0.001	i_Y	0.616	1.034	0.545
	$R^2 = 0.102$ $F(1,151) = 17.064$, $p < 0.001$				$R^2 = 0.336$ $F(2,150) = 37.863$, $p < 0.001$				$R^2 = 0.317$ $F(3,149) = 0.317$, $p < 0.001$				$R^2 = 0.115$ $F(4,148) = 4.825$, $p = 0.001$			

Note: X = Insecure attachment; M1 = developmental perspective; M2 = theory of mind (friend); M3 = adaptive response; Y = gambling disorder.

The bootstrapping procedure confirmed the statistical stability of this chained mediation model and the significance of its indirect effect (boot LLCI = 0.001, boot ULCI = 0.063; see Table 4).

Table 4. Model effect indices.

Total Effect	Direct Effect	Indirect Effect	Partial Standardized Indirect Effect	Bootstrapping 95% CI
1.22	0.93	0.02	0.01	(0.001, 0.063]

4. Discussion

Pathological gambling is a multifaceted phenomenon with numerous underlying factors in its development and maintenance [29,63]. On the other hand, adolescence is an extremely vulnerable phase [64], during which subjects are more inclined to be involved in risky behaviors [65]. Therefore, the early onset of this disorder can have a potentially devastating effect on the individual's development, which is strongly associated with a more serious and chronic course of the disease as well as with various comorbidities [66,67]. On this basis, we investigated the impacts of insecure attachment on gambling disorder in adolescence and also analyzed the mediating roles of developmental perspective, theory of mind (friend) and adaptive response to distress.

Consistent with field research [17–19], the results highlighted a significant role of insecure attachment in predicting the symptomatic expression of gambling in adolescents. However, our data also showed that this association was achieved through an indirect path, by influencing some core aspects in the adolescent's adjustment: insecure attachment negatively impacted the development perspective, theory of mind toward one's best friend and adaptive response to stress, which were linked to each other by a sequential influence. This is in line with evidence considering secure attachment as the starting point for the construction of a functional time perspective, a theory of mind and emotional regulation that will allow for adequate and adaptive self-development [68–71]. By contrast, chronically negative early relationships with caregivers are a risk factor for opposite effects, which could lead to psychopathology [50,72–74].

Moreover, the domination of several time categories may be responsible for limited psychosocial functioning [75,76], and adolescents who focus on the present and on the immediate future have a greater risk of engaging in high-risk behaviors such as substance abuse [76–78]. Therefore, our results showed that a poor developmental self-vision predicted a dysfunctional theory of mind toward one's best friend. This could hinder the formation of positive peer relationships, which are crucial for the development of one's identity [79,80]. The ability to interpret others' behaviors within a mentalistic structure to understand how oneself and others think, feel, perceive, imagine, react, attribute and infer [49] influences adaptation strategies for social interactions [45,72]. When relationships with peers are negative and problematic, an adolescent may experience dysfunctional responses such as delinquent and aggressive behaviors [12], symptoms of depression [7], increased risk of comorbidity [81], general health problems [10] and gambling disorders [8]. All of these factors provide an understandable explanation for the connection highlighted by the data between one's theory of the mind (friend) and adaptive responses to stress, which in turn affect gambling behaviors. Indeed, according to previous research [50,52–56], gambling disorder in adolescence, as with other addictive behaviors, could be interpreted as a dysfunctional response used to cope with stress and negative situations when the subject lacks the resources to find more adequate answers. Adolescents with insecure attachment tend to have maladaptive emotion-regulation strategies [20]. Based on this perspective, pathological gambling could be an attempt at self-medication [23,24].

5. Conclusions

Our study adds two main aspects. First, a multiple-mediation model was used to explore some latent psychological constructs in the pathological manifestation of gambling, specifically insecure attachment, deficits in the developmental perspective, a failed theory of the mind, and nonadaptive responses. Second, we used the Friends and Family Interview (FFI; [59,60]), a semistructured interview similar to the Adult Attachment Interview (AAI; [82]), which detects attachment representations among adolescents. Compared to the AAI, the FFI is focused on oneself and one's peers (best friend), siblings and parents, and it systematically investigates the adolescent's perspective, instead of comparing one's semantic and episodic memories of past experiences with attachment figures as the AAI does. Our results can be applied to psychological interventions based on restructuring

attachment patterns, developing theory of mind and reflective self-functioning, promoting adaptive coping strategies, and improving relationships with peers.

Importantly, this study has some limitations. Its cross-sectional nature limited the possibility of establishing inferences about the causal/directional relationships between the variables. Future longitudinal research may be important to consolidate the conclusions drawn from this study and to investigate results also in adult pathological gamblers. Additionally, gambling behaviors were analyzed using a self-report measure, which, although quick and easy to administer, exposes participants to the risk of bias such as social desirability biases. The measure's integration with different methods (e.g., interviews) could be useful in future studies. Finally, the different subtypes of gambling were not analyzed. The exploration of such data could be an important challenge for future research to delineate different profiles of pathological gamblers.

Author Contributions: Conceptualization, G.T.; Methodology, G.T. and A.G.; Formal analysis, A.G. and E.T.; Investigation, A.S. and C.G.; Writing original draft preparation, E.T. and C.G.; Writing review and editing G.T., A.G. and A.M.; Supervision, V.C. All authors have read and agreed to the published version of the manuscript.

Funding: No funding received for this publication.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of University Lumsa of Rome (Nº 3650/2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: All authors have no conflicts of interests to declare.

References

1. Wilber, M.K.; Potenza, M.N. Adolescent gambling: Research and clinical implications. *Psychiatry* **2006**, *3*, 40.
2. Derevensky, J.; Gupta, R. Adolescent gambling: Current knowledge, myths, assessment strategies and public policy implications. In *Research and Measurement Issues in Gambling Studies*; Smith, G., Hodgins, D., Williams, R., Eds.; Emerald: Bingley, UK, 2007; pp. 437–463.
3. Griffiths, M. Internet gambling: Issues, concerns, and recommendations. *Cyberpsychol. Behav.* **2003**, *6*, 557–568. [CrossRef]
4. Griffiths, M.D.; Parke, J. Adolescent gambling on the Internet: A review. *Int. J. Adolesc. Med. Health* **2010**, *22*, 59–75.
5. Floros, G.D. Gambling disorder in adolescents: Prevalence, new developments, and treatment challenges. *Adolesc. HealthMed. Ther.* **2018**, *9*, 43. [CrossRef]
6. Derevensky, J.L.; Gupta, R. The measurement of youth gambling problems: Current instruments, methodological issues and future directions. In *Gambling Problems in Youth: Theoretical and Applied Perspectives*; Derevensky, J., Gupta, R., Eds.; Kluwer Academic/Plenum Publishers: New York, NY, USA, 2004; pp. 121–143. [CrossRef]
7. Bonnaire, C.; Bungener, C.; Varescon, I. Subtypes of French pathological gamblers: Comparison of sensation seeking, alexithymia and depression scores. *J. Gambl. Stud.* **2009**, *25*, 455–471. [CrossRef]
8. Blinn-Pike, L.; Worthy, S.L.; Jonkman, J.N. Adolescent gambling: A review of an emerging field of research. *J. Adolesc. Health* **2010**, *47*, 223–236. [CrossRef] [PubMed]
9. Gupta, R.; Derevensky, J.L. Adolescent gambling behavior: A prevalence study and examination of the correlates associated with problem gambling. *J. Gambl. Stud.* **1998**, *14*, 319–345. [CrossRef] [PubMed]
10. Hanss, D.; Mentzoni, R.A.; Griffiths, M.D.; Pallesen, S. The impact of gambling advertising: Problem gamblers report stronger impacts on involvement, knowledge, and awareness than recreational gamblers. *Psychol. Addict. Behav.* **2015**, *29*, 483. [CrossRef]
11. Potenza, M.N.; Wareham, J.D.; Steinberg, M.A.; Rugle, L.; Cavallo, D.A.; Krishnan-Sarin, S.; Desai, R.A. Correlates of at-risk/problem internet gambling in adolescents. *J. Am. Acad. Child Adolesc. Psychiatry* **2011**, *50*, 150–159. [CrossRef]
12. Vitaro, F.; Brendgen, M.; Ladouceur, R.; Tremblay, R.E. Gambling, delinquency, and drug use during adolescence: Mutual influences and common risk factors. *J. Gambl. Stud.* **2001**, *17*, 171–190. [CrossRef]
13. Caretti, V.; Gori, A.; Craparo, G.; Giannini, M.; Iraci-Sareri, G.; Schimmenti, A. A new measure for assessing substance-related and addictive disorders: The addictive behavior questionnaire (ABQ). *J. Clin. Med.* **2018**, *7*, 194. [CrossRef]
14. Diamond, N.; Marrone, M. *Attachment and Intersubjectivity*; Whurr Publishers: London, UK, 2003.
15. Flores, P.J. *Addiction as an Attachment Disorder*; Jason Aronson: Lanham, MD, USA, 2004.
16. Terrone, G.; Musetti, A.; Raschielli, S.; Marino, A.; Costrini, P.; Mossi, P.; Salvatore, S.; Caretti, V. Attachment relationships and internalization and externalization problems in a group of adolescents with pathological gambling disorder. *Clin. Neuropsychiatry* **2018**, *15*, 66–74.

17. Di Trani, M.; Renzi, A.; Vari, C.; Zavattini, G.C.; Solano, L. Gambling disorder and affect regulation: The role of alexithymia and attachment style. *J. Gambl. Stud.* **2017**, *33*, 649–659. [CrossRef] [PubMed]
18. Magoon, M.E.; Ingersoll, G.M. Parental modeling, attachment, and supervision as moderators of adolescent gambling. *J. Gambl. Stud.* **2006**, *22*, 1–22. [CrossRef] [PubMed]
19. Pace, U.; Schimmenti, A.; Zappulla, C.; Di Maggio, R. Psychological variables characterizing different types of adolescent gamblers: A discriminant function analysis. *Clinical. Neuropsychiatry* **2013**, *10*, 253–259.
20. Beebe, B.; Lachmann, F. Organizing principles of interaction from infant research and the lifespan prediction of attachment: Application to adult treatment. *J. InfantChildAdolesc. Psychother.* **2002**, *2*, 61–89. [CrossRef]
21. Schimmenti, A.; Guglielmucci, F.; Barbasio, C.; Granieri, A. Attachment disorganization and dissociation in virtual worlds: A study on problematic Internet use among players of online roleplaying games. *Clin. Neuropsychiatry* **2012**, *9*, 195–202.
22. Schimmenti, A.; Musetti, A.; Costanzo, A.; Terrone, G.; Maganuco, N.R.; Rinella, C.A.; Gervasi, A.M. The unfabulous four: Maladaptive personality functioning, insecure attachment, dissociative experiences, and problematic internet use among young adults. *Int. J. Ment. Health Addict.* **2019**. [CrossRef]
23. Khantzian, E.J. Understanding addictive vulnerability: An evolving psychodynamic perspective. *Neuropsychoanalysis* **2003**, *5*, 5–21. [CrossRef]
24. Marlatt, G.A.; Donovan, D.M. *Relapse Prevention: Maintenance Strategies in the Treatment of Addictive Behaviors*; Guilford Press: New York, NY, USA, 2005.
25. Schimmenti, A.; Caretti, V. Psychic retreats or psychic pits? Unbearable states of mind and technological addiction. *Psychoanal. Psychol.* **2010**, *27*, 115–132. [CrossRef]
26. Gori, A.; Craparo, G.; Caretti, V.; Giannini, M.; Iraci-Sareri, G.; Bruschi, A.; Janiri, L.; Ponti, L.; Tani, F. Impulsivity, alexithymia and dissociation among pathological gamblers in different therapeutic settings: A multisaple comparison study. *Psychiatry Res.* **2016**, *246*, 789–795. [CrossRef]
27. Pace, U.; Zappulla, C.; Di Maggio, R.; Passanisi, A.; Craparo, G. Characteristics of regular gamblers in Italy: The role of control and emotion regulation. *Clin. Neuropsychiatry* **2015**, *12*, 111–117.
28. Rogier, G.; Velotti, P. Conceptualizing gambling disorder with the process model of emotion regulation. *J. Behav. Addict.* **2018**, *7*, 239–251. [CrossRef] [PubMed]
29. Gori, A.; Topino, E.; Craparo, G.; Bagnoli, I.; Caretti, V.; Schimmenti, A. A Comprehensive Model for Gambling Behaviors: Assessment of vulnerability and maintenance factors of Gambling Disorder. *J. Gambl. Stud.*. in press.
30. Sherrer, J.F.; Xian, H.; Kapp, J.M.; Waterman, B.; Shah, K.R.; Volberg, R.; Eisen, S.A. Association between exposure to childhood and lifetime traumatic events and lifetime pathological gambling in a twin cohort. *J. Nerv. Ment. Dis.* **2007**, *195*, 72–78. [CrossRef]
31. Laghi, F.; D'Alessio, M.; Pallini, S.; Baiocco, R. Attachment representations and time perspective in adolescence. *Soc. Indic. Res.* **2009**, *90*, 181–194. [CrossRef]
32. Cassidy, J.; Shaver, P.R. *Handbook of Attachment: Theory, Research, and Clinical Applications*, 2nd ed.; Guilford Press: New York, NY, USA, 2008.
33. Seiffge-Krenke, I.; Beyers, W. Coping trajectories from adolescence to young adulthood: Links to attachment state of mind. *J. Res. Adolesc.* **2005**, *15*, 561–582. [CrossRef]
34. Meeus, W.; Oosterwegel, A.; Vollebergh, W. Parental and peer attachment and identity development in adolescence. *J. Adolesc.* **2002**, *25*, 93–106. [CrossRef] [PubMed]
35. Musetti, A.; Schimmenti, A.; Corsano, P. Mass media influences on body image and body esteem in female adolescents: The mediating role of hopeful future expectations. *Atl. J. Commun.* **2020**. [CrossRef]
36. Sroufe, L.A. Attachment and development: A prospective, longitudinal study from birth to adulthood. *Attach. Hum. Dev.* **2005**, *7*, 349–367. [CrossRef]
37. Bowlby, J. *Attachment and Loss. Volume II. Separation: Anxiety and Anger*; Basic Books: New York, NY, USA, 1973.
38. Nickerson, A.B.; Nagle, R.J. The influence of parent and peer attachments on life satisfaction in middle childhood and early adolescence. *Soc. Indic. Res.* **2004**, *66*, 35–60. [CrossRef]
39. Welch-Ross, M. An integrative model of the development of autobiographical memory. *Dev. Rev.* **1995**, *15*, 338–365. [CrossRef]
40. Bowlby, J. *A Secure Base: Parent-Child Attachment and Healthy Human Development*; Basic Books: New York, NY, USA, 1988.
41. Muris, P.; Meesters, C.; van Melick, M.; Zwambag, L. Self-reported attachment style, attachment quality, and symptoms of anxiety and depression in young adolescents. *J. Personal. Individ. Differ.* **2001**, *30*, 809–818. [CrossRef]
42. Pavlidis, K.; McCauley, E. Autonomy and relatedness in family interactions with depressed adolescents. *J. Abnorm. Child Psychol.* **2001**, *29*, 11–21. [CrossRef] [PubMed]
43. Musetti, A.; Terrone, G.; Corsano, P.; Magnani, B.; Salvatore, S. Exploring the link among state of mind concerning childhood attachment, attachment in close relationships, parental bonding, and psychopathological symptoms in substance users. *Front. Psychol.* **2016**, *7*, 1193. [CrossRef]
44. Musetti, A.; Corsano, P. Multidimensional self-esteem and secrecy from friends during adolescence: The mediating role of loneliness. *Curr. Psychol.* **2019**. [CrossRef]
45. Rubin, K.; Fredstrom, B.; Bowker, J. Future directions in ... Friendship in childhood and early adolescence. *Soc. Dev.* **2008**, *17*, 1085. [CrossRef]

46. Allen, J.P. The attachment system in adolescence. In *Handbook of Attachment: Theory, Research, and Clinical Applications*, 2nd ed.; Guilford Press: New York, NY, USA, 2008; pp. 419–435.
47. Zimmermann, P. Attachment representation and characteristics of friendship relations during adolescence. *J. Exp. Child Psychol.* **2004**, *88*, 83–101. [CrossRef]
48. Dykas, M.J.; Cassidy, J. Attachment and the processing of social information across the life span: Theory and evidence. *Psychol. Bull.* **2011**, *137*, 19–46. [CrossRef]
49. Premack, D.; Woodruff, G. Does the chimpanzee have a theory of mind? *Behav. Brain Sci.* **1978**, *1*, 515–526. [CrossRef]
50. Calado, F.; Alexandre, J.; Griffiths, M.D. How coping styles, cognitive distortions, and attachment predict problem gambling among adolescents and young adults. *J. Behav. Addict.* **2017**, *6*, 648–657. [CrossRef] [PubMed]
51. McNally, A.M.; Palfai, T.P.; Levine, R.V.; Moore, B.M. Attachment dimensions and drinking-related problems among young adults. The mediational role of coping motives. *Addict. Behav.* **2003**, *28*, 1115–1127. [CrossRef]
52. Gupta, R.; Derevensky, J.; Marget, N. Coping strategies employed by adolescents with gambling problems. *Child Adolesc. Ment. Health* **2004**, *9*, 115–120. [CrossRef] [PubMed]
53. Nower, L.; Derevensky, J.; Gupta, R. The relationship of impulsivity, sensation seeking, coping and substance use in youth gamblers. *Psychol. Addict. Behav.* **2004**, *18*, 49–55. [CrossRef] [PubMed]
54. Jacobs, D.F. A general theory of addictions: A new theoretical model. *J. Gambl. Behav.* **1986**, *2*, 15–31. [CrossRef]
55. Jacobs, D.F. Juvenile gambling in North America: An analysis of long term trends and future prospects. *J. Gambl. Stud.* **2000**, *16*, 119–152. [CrossRef] [PubMed]
56. Gupta, R.; Derevensky, J.L. An empirical examination of Jacobs' general theory of addictions: Do adolescent gamblers fit the theory? *J. Gambl. Stud.* **1998**, *14*, 17–49. [CrossRef] [PubMed]
57. Winters, K.C.; Stinchfield, R.D.; Kim, L.G. Monitoring adolescent gambling in Minnesota. *J. Gambl. Stud.* **1995**, *11*, 165–183. [CrossRef]
58. Colasante, E.; Gori, M.; Bastiani, L.; Scalese, M.; Siciliano, V.; Molinaro, S. Italian adolescent gambling behaviour: Psychometric evaluation of the South Oaks Gambling Screen: Revised for Adolescents (SOGS-RA) among a sample of Italian students. *J. Gambl. Stud.* **2014**, *30*, 789–801. [CrossRef]
59. Kriss, A.; Steele, H.; Steele, M. Measuring attachment and reflective functioning in early adolescence: An introduction to the friends and family interview. *Res. Psychother. Psychopathol. Process. Outcome* **2013**, *15*, 87–95. [CrossRef]
60. Steele, H.; Steele, M. The construct of coherence as an indicator of attachment security in middle childhood: The Friends and Family Interview. In *Attachment in Middle Childhood*; Kerns, K., Richardson, R., Eds.; Guilford Press: New York, NY, USA, 2005; pp. 137–160.
61. Pace, C.S.; Muzi, S.; Steele, H. Adolescents' Attachment: Content and Discriminant Validity of the Friends and Family Interview. *J. Child Fam. Stud.* **2020**, *29*, 1173–1186. [CrossRef]
62. Hayes, A.F. *Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach*, 2nd ed.; Guilford Press: New York, NY, USA, 2018.
63. Griffiths, M.D. Adolescent gambling. In *Encyclopedia of Adolescence*; Bradford Brown, B., Prinstein, M.J., Eds.; Academic Press: San Diego, CA, USA, 2011; Volume 3, pp. 11–20. [CrossRef]
64. Bergevin, T.; Gupta, R.; Derevensky, J.; Kaufman, F. Adolescent gambling: Understanding the role of stress and coping. *J. Gambl. Stud.* **2006**, *22*, 195–208. [CrossRef]
65. Shead, N.W.; Derevensky, J.L.; Gupta, R. Risk and protective factors associated with youth problem gambling. *Int. J. Adolesc. Med. Health* **2010**, *22*, 39.
66. Kessler, R.C.; Hwang, I.; LaBrie, R.; Petukhova, M.; Sampson, N.A.; Winters, K.C.; Shaffer, H.J. DSM-IV pathological gambling in the National Comorbidity Survey Replication. *Psychol. Med.* **2008**, *38*, 1351–1360. [CrossRef] [PubMed]
67. Nower, L.; Martins, S.S.; Lin, K.H.; Blanco, C. Subtypes of disordered gamblers: Results from the N ational E pidemiologic S urvey on a lcohol and R elated C onditions. *Addiction* **2013**, *108*, 789–798. [CrossRef] [PubMed]
68. Blomgren, A.S.; Svahn, K.; Åström, E.; Rönnlund, M. Coping strategies in late adolescence: Relationships to parental attachment and time perspective. *J. Genet. Psychol.* **2016**, *177*, 85–96. [CrossRef]
69. Fonagy, P.; Target, M. Attachment and reflective function: Their role in self organization. *Dev. Psychopathol.* **1997**, *9*, 679–700. [CrossRef] [PubMed]
70. Laghi, F.; Pallini, S.; Baumgartner, E.; Guarino, A.; Baiocco, R. Parent and peer attachment relationships and time perspective in adolescence: Are they related to satisfaction with life? *Time Soc.* **2016**, *25*, 24–39. [CrossRef]
71. Luyten, P.; Fonagy, P. The Neurobiology of Attachment and Mentalizing: A Neurodevelopmental Perspective. In *Neurobiology of Personality Disorders*; Schmahl, C., Phan, K.L., Friedel, R.O., Eds.; Oxford University Press: Oxford, UK, 2018. [CrossRef]
72. Brüne, M.; Brüne-Cohrs, U. Theory of mind-evolution, ontogeny, brain mechanisms and psychopathology. *Neurosci. Biobehav. Rev.* **1988**, *30*, 437–455. [CrossRef]
73. Fonagy, P. Thinking about thinking: Some clinical and theoretical considerations in the treatment of a borderline patient. *Int. J. Psychoanal.* **1991**, *72*, 639–656.
74. Terrone, G.; Santona, A. The psychopathological risk in preadolescence. *G. Ital. Psicol.* **2012**, *39*, 697–706.
75. Boniwell, I.; Zimbardo, P.G. Balancing time perspective in pursuit of optimal functioning. *Posit. Psychol. Pract.* **2004**, *12*, 141–155. [CrossRef]

76. Keough, K.A.; Zimbardo, P.G.; Boyd, J.N. Who's smoking, drinking, and using drugs? Time perspective as a predictor of substance use. *Basic Appl. Soc. Psychol.* **1999**, *21*, 149–164. [CrossRef]
77. Apostolidis, T.; Fieulaine, N.; Soulé, F. Future time perspective as predictor of cannabis use: Exploring the role of substance perception among French adolescents. *Addict. Behav.* **2006**, *31*, 2339–2343. [CrossRef]
78. Wills, T.A.; Sandy, J.M.; Yaeger, A.M. Time perspective and early-onset substance use: A model based on stress-coping theory. *Psychol. Addict. Behav.* **2001**, *15*, 118. [CrossRef] [PubMed]
79. Årseth, A.K.; Kroger, J.; Martinussen, M.; Marcia, J.E. Meta-analytic studies of the relational issues of attachment and intimacy. *Identity Int. J. Theory Res.* **2009**, *9*, 1–32. [CrossRef]
80. Martinussen, M.; Kroger, J. Meta-analytic studies of identity status and personality: Introduction and overview. *Identity Int. J. Theory Res.* **2013**, *13*, 189–200. [CrossRef]
81. Lorains, F.K.; Cowlishaw, S.; Thomas, S.A. Prevalence of comorbid disorders in problem and pathological gambling: Systematic review and meta-analysis of population surveys. *Addiction* **2011**, *106*, 490–498. [CrossRef] [PubMed]
82. Main, M.; Goldwyn, R.; Hesse, E. *Adult Attachment Scoring and Classification System*; Department of Psychology, University of California at Berkeley: Berkeley, CA, USA, 2008.

Journal of *Personalized Medicine*

MDPI

Article

The Attentional Boost Effect in Young and Adult Euthymic Bipolar Patients and Healthy Controls

Giulia Bechi Gabrielli [1], Clelia Rossi-Arnaud [1], Pietro Spataro [2], Fabrizio Doricchi [1], Marco Costanzi [3], Alessandro Santirocchi [1], Gloria Angeletti [4], Gabriele Sani [5,6] and Vincenzo Cestari [1,*]

1 Department of Psychology, Faculty of Medicine and Psychology, Sapienza University, 00185 Rome, Italy; giulia.bechigabrielli@uniroma1.it (G.B.G.); clelia.rossi-arnaud@uniroma1.it (C.R.-A.); fabrizio.doricchi@uniroma1.it (F.D.); alessandro.santirocchi@uniroma1.it (A.S.)
2 Faculty of Economics, Universitas Mercatorum, 00100 Rome, Italy; pietro.spataro@unimercatorum.it
3 Department of Human Sciences, Lumsa University, 00100 Rome, Italy; m.costanzi@lumsa.it
4 Nesmos Department, Sapienza University, Sant'Andrea Hospital, 00100 Rome, Italy; gloria.angeletti@uniroma1.it
5 Institute of Psychiatry, Università Cattolica del Sacro Cuore, 00100 Rome, Italy; gabriele.sani@unicatt.it
6 Department of Psychiatry, Fondazione Policlinico Universitario "Agostino Gemelli" IRCCS, 00100 Rome, Italy
* Correspondence: Vincenzo.cestari@uniroma1.it

Abstract: In the Attentional Boost Effect (ABE), stimuli encoded with to-be-responded targets are later recognized more accurately than stimuli encoded with to-be-ignored distractors. While this effect is robust in young adults, evidence regarding healthy older adults and clinical populations is sparse. The present study investigated whether a significant ABE is present in bipolar patients (BP), who, even in the euthymic phase, suffer from attentional deficits, and whether the effect is modulated by age. Young and adult euthymic BP and healthy controls (HC) presented with a sequence of pictures paired with target or distractor squares were asked to pay attention to the pictures and press the spacebar when a target square appeared. After a 15-min interval, their memory of the pictures was tested in a recognition task. The performance in the detection task was lower in BP than in HC, in both age groups. More importantly, neither young nor adult BP exhibited a significant ABE; for HC, a robust ABE was only found in young participants. The results suggest that the increase in the attentional demands of the detection task in BP and in adult HC draws resources away from the encoding of target-associated stimuli, resulting in elimination of the ABE. Clinical implications are discussed.

Keywords: Attentional Boost Effect; bipolar disorder; euthymic patients; recognition memory

Citation: Bechi Gabrielli, G.; Rossi-Arnaud, C.; Spataro, P.; Doricchi, F.; Costanzi, M.; Santirocchi, A.; Angeletti, G.; Sani, G.; Cestari, V. The Attentional Boost Effect in Young and Adult Euthymic Bipolar Patients and Healthy Controls. *J. Pers. Med.* **2021**, *11*, 185. https://doi.org/10.3390/jpm11030185

Academic Editor: Toshiaki Onitsuka

Received: 27 November 2020
Accepted: 1 March 2021
Published: 6 March 2021

1. Introduction

The Attentional Boost Effect (ABE) represents a counterintuitive phenomenon in which the division of attention at encoding enhances later memory performance [1–3] (see Swallow and Jiang [4] for a review). In the latest version of the paradigm [5], participants were presented with a series of faces flanked by two target squares (e.g., orange), two distractor squares (e.g., blue), or no squares (the baseline condition). Participants were required to study the faces and simultaneously press the spacebar when the target squares appeared. When their memory of the faces was later probed in a yes/no recognition task, the performance was significantly better for the faces which were presented with target squares than for those presented with distractor squares or no square at all; no difference in performance was found between the latter two. This advantage of target-paired over distractor-paired images is referred to as the ABE. It is thought to be the result of a broad attentional enhancement linked to the detection of target squares [2,5]. More specifically, in their dual-task interaction model, Swallow and Jiang [4] proposed that, on the one hand, monitoring the squares interferes with image encoding, because it biases perceptual

resources away from the background stimuli and places additional cognitive demands to generate an appropriate response [6]. On the other hand, detecting the target squares and performing the associated motor response triggers temporal selective attention [7]. This mechanism facilitates perceptual processing of the background images by producing a transient increase in the release of norepinephrine from the locus coeruleus [8] (see Yebra et al. [9] for recent evidence). Under specific conditions, this processing enhancement exceeds the usual interference effects, leading to the ABE.

Despite being a recently described phenomenon, the ABE has been extensively investigated. First, it has been replicated with verbal materials in a variety of explicit memory tasks, including yes/no and forced-choice recognition [2,10,11], cued recall [12], and free recall [10]; the ABE has also been reported in perceptual implicit [3] and working memory tasks [13,14]. Second, studies have ruled out several potential explanations of the ABE, including accounts based on perceptual learning, target distinctiveness, attentional cueing, reinforcement learning, and perceptual grouping [2,15,16]. Third, studies that have included separate full-attention (FA) and divided-attention (DA) conditions have shown that the ABE represents a dynamic trade-off between attentional competition and attentional facilitation [2–4,10,17]. Here, the term 'attentional competition' refers to the finding that recognition of distractor-paired items is usually worse in the DA condition (in which participants have to encode the background stimuli and simultaneously detect the targets) than in the FA condition (in which the sole task is to encode the background stimuli), confirming the classical negative effects of DA on memory encoding. In contrast, the term 'attentional facilitation' refers to the finding that the recognition of target-paired items in the DA condition is boosted to the same level of the FA [2,10,17]. The ABE reflects a condition in which attentional facilitation exceeds attentional competition.

Most of the above-summarized evidence was gathered in studies of healthy young participants, typically university students. To date, only a handful of studies have examined the ABE in clinical populations [18–21]. Furthermore, except for a study by Rossi-Arnaud et al. [21], these studies used a modified version of the original ABE paradigm similar to the Rapid Serial Visual Presentation (RSVP) [13,14,22], in which participants encoded brief sequences of stimuli and the recognition task was administered immediately after the end of each sequence. Collectively, the results of these studies showed that patients with both amnestic mild cognitive impairment and post-traumatic stress disorder were severely impaired in terms of the recognition of scenes that were paired with targets. In contrast, these same patients performed like controls, or even displayed an enhanced performance, in terms of the recognition of scenes that were paired with distractors [19,20]. A different pattern was obtained in patients with Parkinson's disease before and after the administration of dopaminergic medications. At the baseline, drug-naive patients performed like controls. However, after receiving dopamine agonists for 12 weeks, patients outperformed controls for both target-paired and distractor-paired stimuli [18]. Only one previous study [21] has investigated the ABE in patients with schizophrenia, using a paradigm similar to that illustrated by Swallow and Jiang [2,5], in which patients and healthy controls encoded a long series of stimuli and the recognition test was separated from the encoding phase. The results showed that, unlike controls who displayed the typical ABE, patients with schizophrenia exhibited no memory advantage for target-paired pictures (Exp. 1) and words (Exp. 2).

The present study focused on bipolar disorder, which is characterized by mood alterations that include manic or hypomanic episodes (in which there is an elevation of tone mood), depressive episodes (in which there is a decline of tone mood), and mixed episodes, intermingled with intervals of euthymic remission [23]. We chose to examine this clinical population because there is growing evidence that individuals with bipolar disorder are affected by significant attentional deficits [24–28], even after controlling for mild residual symptomatology [29] and pharmacological treatment [30,31]. In particular, in the remission phase, bipolar patients (euthymic patients) show a decreased target sensitivity (omission errors) and slowed response latencies in detection tasks that require

sustained attention [29,32–38], in which participants have to monitor a continuous stream of stimuli to detect a pre-specified target.

Based on these results, our primary aim was to investigate the ABE in a sample of euthymic patients, using the most recent version of the paradigm [5]. Following the notion mentioned above, that the ABE represents a trade-off between attentional competition and attentional facilitation [2–4,10,17], we expected euthymic patients to exhibit a reduced or non-significant advantage for images encoded with target stimuli. Swallow and Jiang [2] (Exp. 5) showed that, when target detection requires additional attention resources, the negative effects of attentional competition exceeded the positive effects of attentional facilitation, thus resulting in elimination of the ABE. We reasoned that, if the maintenance of a fast and accurate performance in the target detection task requires more attention resources in euthymic patients than in healthy controls (as suggested by previous studies [29,32,33,35–37]), then the ABE should have been reduced or eliminated in the patient group.

In addition to testing the ABE in euthymic patients, a secondary aim of the present study was to investigate whether participants' age modulates this effect. Previous studies have typically reported a strong ABE in young adults between 18 and 35 years of age. In contrast, relatively few data have been reported for older participants and the overall findings are mixed. Bechi Gabrielli, Spataro, Pezzuti and Rossi-Arnaud [39] found that the ABE was abolished in older adults between 60 and 75 years when a 20-min interval separated the encoding and test phases. On the other hand, Prull [17], with a short study-test interval (2 min), reported that young-old adults (between 60 and 75 years) exhibited an ABE with a magnitude that did not differ from that of young adults, although a significant decrease was observable in older-old adults (from 75 to 94 years). Based on these results, Prull [17] speculated that the cognitive decline associated with healthy aging might produce vulnerable boosted memories that would be more easily disrupted over time, compared to the boosted memories of young adults; that is, due to processing resource limitations and/or associative deficits, long study-test delays would create a selective interference that impairs the boosted memories of older adults. The putative mechanisms underlying this impairment have been examined by a series of neuroimaging studies investigating the cerebral bases of the ABE. According to Swallow and Jiang [4], target detection in the ABE paradigm results in a transient increase in the release of norepinephrine from the locus coeruleus (LC), which in turn projects to a wide variety of cortical regions, including the hippocampus. More recent studies have showed that the memory enhancements following ABE manipulations are associated with widespread increases in functional connectivity between the LC and the parahippocampal gyrus [9] and between the visual cortex and the hippocampal formation [40]. Interestingly, for the present purposes, healthy aging is accompanied by (a) increased tau pathology and a reduced density of the LC, which are already evident from 20 years onwards [41], and (b) substantial shrinkage of the regional brain volume of the hippocampus, which accelerates with age (from 20 to 90 years) [42]. In addition to accounting for the creation of vulnerable boosted memories in older adults, the age-related changes occurring in these two structures suggest an additional inference: Namely, that significant reductions in the size of the ABE should be apparent in healthy adults well before the age of 60 years, at least when using long study-test intervals. To address this issue, in the present study, both the patient and healthy control samples were divided into two sub-groups: The young group included participants between 18 and 35 years, whereas the adult group included participants between 36 and 60 years. This allowed us to examine, for the first time, whether there is a significant reduction in the size of the ABE in the adult group.

2. Materials and Methods

Forty-two euthymic bipolar patients (BP) Type I, between 18 and 60 years, were recruited for the current study from the Psychiatric Ward of the Sant'Andrea Hospital in Rome. As stated above, they were divided into a young subgroup (n = 12; 5 females; age:

M = 27.4 years, range: 18–35 years; education: M = 13.7) and an adult subgroup (n = 30; 17 females; age: M = 50.3 years, range: 36–61 years; education: M = 12.7). The diagnosis of bipolar disorder was made according to the inclusion criteria specified by the DSM-5 [23]. All patients were under pharmacological treatment at the time of the study: Specifically, 33 patients (79%) were administered antipsychotics (e.g., seroquel, zyprexa, leponex, etc.); 36 (88%) were administered mood stabilizers (e.g., carbolithium, depakin, etc.); 14 (34%) were administered anxiolytics (e.g., diapezam); and 7 (16%) were administered antidepressants (e.g., anafranil, zoloft, etc.). To be included, patients had to be in the euthymic phase [23]. Forty-two healthy control subjects (HC), from 18 to 60 years old, were recruited as controls. They were likewise divided into a young subgroup (n = 15; 6 females; age: M = 26.7 years, range: 22–35 years; education: M = 12.7) and an adult subgroup (n = 27; 16 females; age: M = 51.1 years, range: 37–60 years; education: M = 13.5). Eight participants (3 from the BP group and 5 from the HC group) were additionally tested but excluded from statistical analyses because their accuracy in the detection task or the memory test fell two or more standard deviations below the overall mean. Four subtests of the WAIS-IV [43,44] were administered to all participants—the Digit Span subtest (forward and backward) to evaluate the working memory, and the Symbol Search and Digit Symbol-Coding subtests to evaluate the processing speed.

Potential differences in demographic characteristics and cognitive scores between bipolar patients and healthy controls were analyzed through a series of t-tests for independent samples. Separate analyses were conducted in the two age subgroups (see Table 1). In the young subgroup, significant differences were only found in the Symbol Search subtest of WAIS-IV, indicating lower scores in bipolar patients than in healthy controls: $t(25) = 2.13$, and $p = 0.042$. For the adult subgroup, significant differences were similarly obtained in the speed subtests of WAIS-IV, again indicating lower scores in bipolar patients than in healthy controls: $t(55) = 3.04$ and $p = 0.004$ for Symbol Search and $t(55) = 3.02$ and $p = 0.004$ for Digit Symbol-Coding. Bipolar patients and healthy controls were matched in terms of age and gender, as well as in the distribution of gender.

Table 1. Mean scores for the demographic and cognitive measures of euthymic bipolar patients (BP) and healthy control subjects (HC) in the two age subgroups (young-adults and adults). Standard errors are reported in parentheses. For the WAIS-IV subtests, weighted scores are reported.

Variables	Young-Adults		Adults	
	BP (n = 12)	**HC (n = 15)**	**BP (n = 30)**	**HC (n = 27)**
Age (years)	27.4 (1.8)	26.7 (1.6)	50.3 (1.1)	51.1 (1.2)
Education (years)	13.7 (1.0)	15.5 (0.9)	12.7 (0.6)	13.5 (0.7)
Gender (M/F)	7/5	9/6	13/17	11/16
Digit Span (forward)	8.7 (0.8)	9.5 (0.7)	8.7 (0.5)	9.7 (0.5)
Digit Span (backward)	8.5 (0.9)	10.1 (0.8)	8.1 (0.6)	9.7 (0.6)
Symbol Search	8.9 (0.7) [a]	10.9 (0.7) [b]	8.4 (0.5) [a]	10.5 (0.5) [b]
Digit Symbol-Coding	10.2 (0.7)	12.1 (0.6)	8.3 (0.4) [a]	10.2 (0.7) [b]

Note. The superscripts a and b indicate significant differences ($p < 0.05$) between couples of BP and HC means.

The study was carried out at the Sant'Andrea Hospital in compliance with ethical guidelines and written informed consent was obtained from each participant. Both healthy controls and bipolar patients participated in the study voluntarily.

A critical set of 45 neutral pictures were selected from the International Affective Picture System (IAPS) [45] valence: M = 5.28, on a 9-point Likert scale ranging from 1 = unhappy to 9 = very happy; arousal: M = 3.18, on a 9-point Likert scale ranging from 1 = relaxed to 9 = excited) (see Rossi-Arnaud, Spataro, Costanzi, Saraulli, and Cestari [1] for a study examining the ABE with emotional words and images). This initial set was further divided into three subgroups of 15 images. Each image could be associated with a red square (target condition); associated with a green square (distractor condition); or presented on its own, without squares (baseline condition). The use of the three subsets of images in

the different encoding conditions was counterbalanced across participants. An additional set of 124 non-critical neutral images were also selected from the IAPS, to be used as practice (5 images) and filler items (74 images) during the encoding phase, or as foils in the recognition task (45 images). Foils were as similar as possible to the critical images in terms of valence (M = 5.29) and arousal (M = 3.17). All images were pre-processed with Adobe Illustrator CS6 and presented on the 15" monitor of an HP Pavilion notebook using the software SuperLab 4.0 (Cedrus Corporation, San Pedro, CA, USA).

The experiment comprised an encoding phase, a 15-min interval, and a test phase (Bechi Gabrielli et al., 2018, Exp.1). In the encoding phase, participants were presented with a total of 124 images, at a rate of 500 ms/picture (no inter-stimulus interval). All of the stimuli were displayed on the 15″ display of an HP Pavilion notebook, with participants being sat at a distance of about 40 cm. For target and distractor trials, one image (1024 × 628 pixels) and one square (70 × 70 pixels; red or green, placed at the center of the image) appeared simultaneously on the screen for 100 ms, after which only the image remained visible for an additional 400 ms. For baseline trials, the images were presented for 500 ms, without squares. The entire presentation was divided into 16 continuous blocks of five images each (one practice block plus 15 critical blocks). Each block included 1 target image (presented with a red square), 1 distractor image (presented with a green square), 1 baseline image (presented without squares), and 2 filler images (presented with green squares). The target image was always located in the third position, whereas the distractor and baseline images were located in either the first or fifth position (the exact position was counterbalanced across blocks). In addition, from one to five filler images, always presented with green squares, were placed between adjacent blocks to reduce the regularity in the appearance of the target squares. Participants were told to pay attention to the images (incidental instructions, since they were not forewarned about the impending memory task) and simultaneously press the spacebar whenever they detected a red square. During the 15-min interval, both healthy controls and bipolar patients undertook the four WAIS-IV subtests. Finally, the recognition task involved the random presentation of 90 images, 45 old images (presented at encoding, including 15 target-paired, 15 distractor-paired, and 15 baseline images) and 45 new images (foils). For each image, the instructions were to press the key "v" (for "vecchio", old) or "n" (for "nuovo", new) if the participant judged it to be old or new, respectively.

3. Results

At encoding, the performance in the detection task was analyzed via a 2 × 2 ANOVA, considering group (healthy controls, HC vs. bipolar patients, BP) and age (young, Y vs. adult, A participants) as between-subject factors. The dependent variables were the mean percentages of targets correctly detected, the mean numbers of false alarms to distractor or baseline trials, and the mean detection times. The results showed that bipolar patients and healthy controls were equally accurate in the detection of target squares (M(HC) = 93.4% vs. M(BP) = 89.7%, F(1,80) = 2.90, $p = 0.09$, and $\eta 2 = 0.04$). Bipolar patients made more false alarms than healthy controls, although the overall percentages were very low (M(HC) = 0.23% vs. M(BP) = 1.00%, F(1,80) = 7.4, $p = 0.008$, and $\eta 2 = 0.09$). Finally, both groups were equally faster in target detection (M(HC) = 344.1 ms vs. M(BP) = 329.7 ms, F(1,80) = 1.95, $p = 0.17$, and $\eta 2 = 0.02$). When we analysed the main effects of age, we found that young and adult participants were equally accurate in the detection task (M(Y) = 92.5% vs. M(A) = 90.7%, F(1,80) = 0.69, $p = 0.41$, and $\eta 2 = 0.01$), and the two groups did not differ in the mean percentages of false alarms (M(Y) = 0.47% vs. M(A) = 0.75%, F(1,80) = 0.95, $p = 0.33$, and $\eta 2 = 0.01$). They were also equally faster in the detection of target squares (M(Y) = 337.1 ms vs. M(A) = 336.6 ms, F(1,80) = 0.002, $p = 0.96$, and $\eta 2 = 0.00$). We did not find significant interactions in any analysis.

For the recognition test, we first analysed the proportions of false alarms with a 2 (group: healthy controls vs. bipolar patients) × 2 (age: young vs. adult participants) ANOVA. We did not find a main effect of group: The mean proportions of false alarms were

comparable between bipolar patients and healthy controls (M(HC) = 0.15 vs. M(BP) = 0.18, F(1,80) = 1.23, p = 0.27, and η2 = 0.02). On the contrary, we found a significant main effect of age (F(1, 80) = 6.71, p = 0.01, and η2 = 0.08), indicating that adult participants (M = 0.20) committed more false alarms than young participants (M = 0.13). For this reason, all the subsequent statistical analyses were conducted on corrected recognition scores, computed as hits minus false alarms (this is a common procedure in studies examining the ABE in recognition tasks [1,21,39]). Note that the proportions of hits were adjusted by only considering those trials in which participants correctly performed the detection task [10]. These adjusted scores were submitted to a 2 (group: healthy controls vs. bipolar patients) × 2 (age: young vs. adult participants) × 3 (type of trial: target, distractor, and baseline images) mixed ANOVA. The results showed (a) a marginal main effect of trial type (F(2, 160) = 2.96, p = 0.054, and η2 = 0.04): The post-hoc comparisons demonstrated that the recognition of distractor-paired images (M = 0.26) was significantly worse than the recognition of baseline images (M = 0.32, p = 0.03); (b) a significant main effect of group (F(1,80) = 7.40, p = 0.008, and η2 = 0.09), indicating that healthy controls (M = 0.35) performed the recognition task significantly better than bipolar patients (M = 0.25); and (c) a significant two-way interaction between group and trial type (F(2, 160) = 4.59, p = 0.01, and η2 = 0.05), and a significant three-way interaction between group, age, and trial type (F(2, 160) = 4.92, p = 0.008, and η2 = 0.06). All other effects and interactions failed to reach the significance level (all Fs (1,80) < 2.01, p > 0.16).

A follow-up analysis of simple effects on the two-way interaction between group and trial type (see Figure 1) revealed that the effect of trial type was significant in healthy controls (F(2, 79) = 4.28, p = 0.017, and η2 = 0.10). For this group, the recognition of target-paired images (M = 0.42) was significantly more accurate than the recognition of distractor-paired images (M = 0.30, p = 0.013)—the Attentional Boost Effect. On the contrary, the recognition of baseline images (M = 0.34) did not differ from the recognition of target and distractor-paired images (p = 0.23 and p = 0.67, respectively). The effect of trial type was also significant in bipolar patients (F(2, 79) = 3.36, p = 0.040, and η2 = 0.08): For this group, the recognition of baseline images (M = 0.31) was significantly better than the recognition of distractor-paired images (M = 0.22, p = 0.048), but did not differ from the recognition of target-paired images (p = 0.18); no differences were found between the recognition of target-paired and distractor-paired images (p = 1.00).

When analysed in the opposite direction, this same interaction indicated that the effect of group was significant for target-paired images, with healthy controls (M = 0.42) outperforming bipolar patients (M = 0.22) (F(1, 80) = 11.31, p = 0.001, and η2 = 0.12). The two groups did not differ in the recognition of distractor-paired and baseline images (F(1, 80) = 2.87, MSE = 0.038, p = 0.094, and η2 = 0.04 and F(1, 80) = 0.54, p = 0.46, and η2 = 0.01, respectively).

A similar follow-up analysis of simple effects on the three-way interaction between group, age, and trial type (see Figure 2) revealed that the effect of trial type was significant for both young and adult healthy controls (F(2, 79) = 5.45, p = 0.006, and η2 = 0.12, and F(2, 79) = 3.19, p = 0.047, and η2 = 0.07, respectively). For young healthy controls, the recognition of target-paired images (M = 0.53) was significantly higher than the recognition of distractor-paired (M = 0.33, p = 0.01) and baseline images (M = 0.31, p = 0.008); no differences were found between these two conditions (p = 1.0). In contrast, adult healthy controls recognized baseline images (M = 0.37) better than distractor-paired images (M = 0.27, p = 0.039); the recognition of target-paired images (M = 0.31) did not differ from the recognition of distractor-paired and baseline images (p = 0.10 and p = 0.78, respectively). The effect of trial type was not significant in young and adult bipolar patients (F(2, 79) = 1.84, p = 0.17, and η2 = 0.04 and F(2, 79) = 1.97, p = 0.15, and η2 = 0.05, respectively), indicating no between-trial differences in these two subgroups. The same analysis showed that the effect of group was significant for young participants in the target condition (F(1, 80) = 10.25, MSE = 0.064, p = 0.002, and η2 = 0.11), and marginally significant for adult participants in the baseline condition (F(1, 80) = 3.72, p = 0.057, and η2 = 0.04). Therefore,

young healthy controls recognized target-paired images (M = 0.53) more accurately than young bipolar patients (M = 0.21); similarly, adult healthy controls (M = 0.37) recognized baseline images more accurately than adult patients (M = 0.27). The effect of group failed to reach the significance level in all other conditions (all Fs(1, 80) < 1.88, $p > 0.17$). Lastly, the follow-up analysis indicated that the effect of age was significant for healthy controls in the target condition (F(1, 80) = 7.07, $p = 0.009$, and $\eta 2 = 0.08$), indicating that young healthy controls recognized target-paired images (M = 0.53) more accurately than adult healthy controls (M = 0.31). The effect of age failed to reach the significance level in all other conditions (all Fs(1, 80) < 1.16, $p > 0.28$).

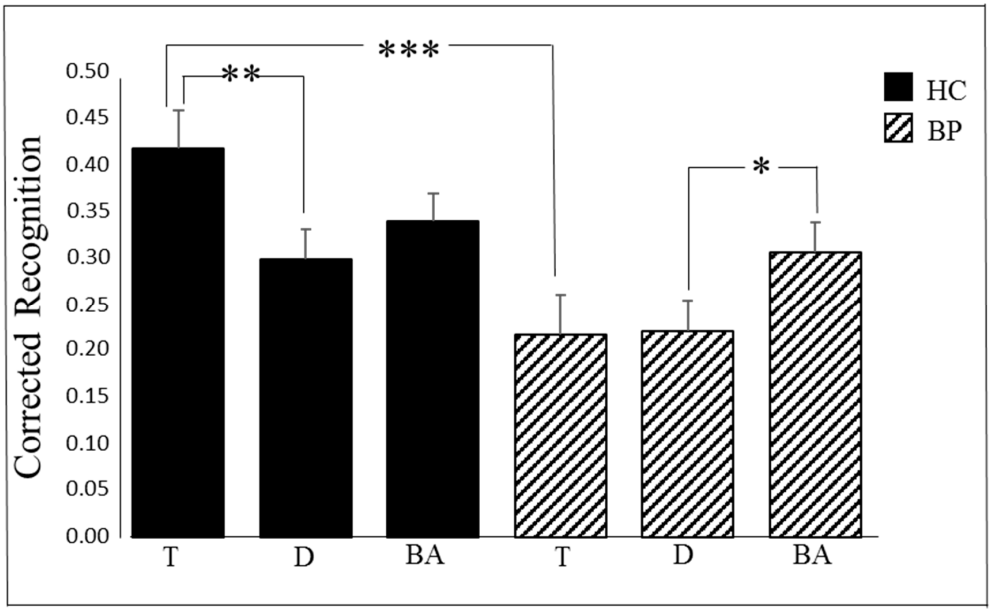

Figure 1. Mean proportions of corrected recognition (hits—false alarms) in bipolar patients (BP) and healthy control subjects (HC) as a function of trial type. Bars represent *SE*s. **Note**: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; T, target images; D, distractor images; BA, baseline images; HC, healthy controls; and BP, bipolar patients.

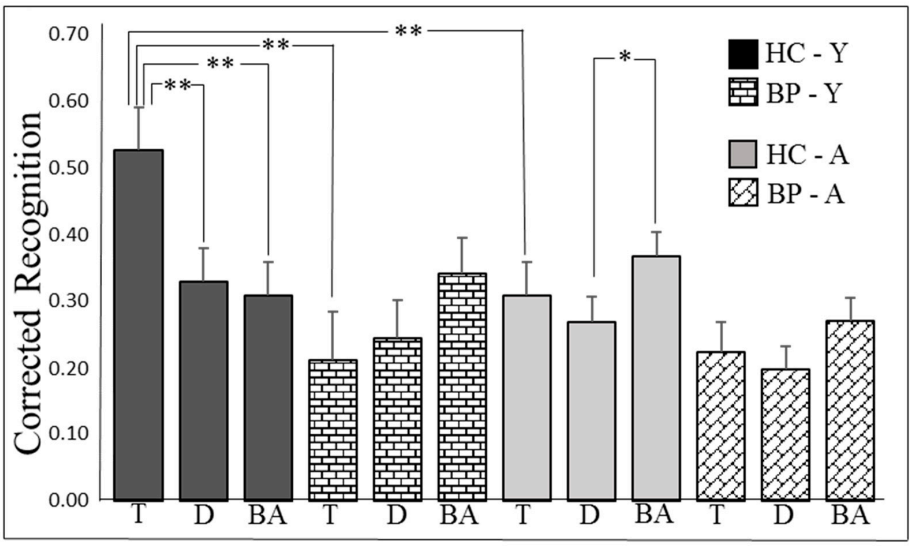

Figure 2. Mean proportions of corrected recognition (hits—false alarms) in bipolar patients (BP) and healthy control subjects (HC) as a function of trial type and age range (Y—young-adults, A—adult subjects). Bars represent *SE*s. **Note**: * $p < 0.05$; ** $p < 0.01$; T, target images; D, distractor images; and BA, baseline images.

4. Discussion

In the present study, using the most recent version of the paradigm by Swallow and Jiang [5], we examined the ABE in a sample of young (18–35 years) and adult (36–60 years) euthymic bipolar patients and in samples of matched healthy controls. The results showed that, during the encoding phase, bipolar patients were as accurate and fast as healthy controls in detecting the target squares, but produced significantly more false alarms. However, the overall incidence of false alarms was low in both groups. Turning to the recognition task, young healthy controls showed the typical ABE, with target-paired images being recognized better than distractor-paired images. In contrast, the ABE was abolished in adult healthy controls and bipolar patients, irrespective of age; in the latter group, the recognition of baseline images was significantly higher than the recognition of distractor images, suggesting enhanced attentional competition. Finally, healthy controls outperformed bipolar patients in the recognition of target images, whereas the two groups were equally accurate in the recognition of distractor and baseline images.

As mentioned in the introduction, the ABE represents a trade-off between attentional competition and attentional facilitation [2,4], such that any increase in the attentional requirements of the detection task should impair the encoding of target-associated stimuli, and thus reduce or even eliminate the memory facilitation produced. In agreement, Swallow and Jiang [2] (Exp. 5) showed that enhancing the difficulty of the detection task by asking participants to make different responses to target and distractor stimuli was sufficient to cancel the ABE. Several previous studies have already documented the attentional difficulties experienced by bipolar patients. They have shown that these deficits are not limited to the depressive and manic episodes, but extend to the euthymic phase [29,34,35,38]. Importantly, these patients show evident impairment in continuous performance tasks, in which they have to monitor a stream of stimuli to detect an infrequent pre-specified target [36,46]. Based on this literature, we expected that the maintenance of an accurate and fast performance in the detection task should recruit more attention resources in bipolar patients than in healthy controls and that the ensuing increase in the negative effect of attentional competition should eliminate the ABE.

Two results from the present study provide support for this prediction. First, bipolar patients exhibited a selective impairment in the recognition of target-paired images, together with an intact performance in the detection of target squares. Since, in our study, participants were not explicitly required to remember the background stimuli and were unaware of the following recognition test, it can be plausibly assumed that both bipolar patients and healthy controls emphasized and devoted more attention resources to the detection task than to the memory task (see Bechi Gabrielli et al. [39] for a discussion). Our data suggest that the maintenance of a fast and accurate performance in the detection task required more attention resources in bipolar patients than in healthy controls. As a consequence, bipolar patients had fewer resources available to encode the target-paired images into memory, resulting in a significant and selective deficit in the recognition of these images.

The second piece of evidence that supports this interpretation of the bipolar patients' performance comes from the significant two-way interaction between group and type of trial. This interaction highlighted that bipolar patients recognized baseline images significantly more accurately than distractor-paired images. Previous studies that have compared the FA and DA conditions have pointed out that the recognition of distractors is significantly lower in the DA than in the FA condition. These findings reflect the classical negative effects of divided attention [2,10,17]. In the paradigm used in the present study, the difference between the recognition of distractor and baseline images has been similarly proposed to reflect the attentional competition component of the ABE [5]. In line with this idea, a recent study using the Remember/Know procedure found that the proportions of 'remember' responses were significantly lower for distractor-paired than baseline words [47]. If this were the case, the present results might suggest that the negative effects of DA were stronger in bipolar patients than in healthy controls. Since the ABE emerges

from the interaction between attentional competition and attentional boost [2,4,10], the direct consequence of an increase in the interfering effects of attentional competition must necessarily be a reduction in the positive effects of the ABE. In sum, taken together with the selective impairment in the recognition of target-paired images, the finding mentioned above supports the idea that the maintenance of an adequate performance in the detection task is more attention-demanding in bipolar patients than in healthy controls and that the ensuing enhancement of the negative effects of DA was sufficient to eliminate the ABE.

In our experiment, we also investigated whether participants' age influenced the ABE. The large majority of previous studies have examined the ABE in young university students between 18 and 35 years [2,5,10,15]. To the best of our knowledge, only two studies were explicitly aimed at comparing the ABE of younger and older participants [17,39]. The results were mixed, likely because different study-test intervals (2 min vs. 20 min) were used in the two studies. On the basis of this evidence, Prull [17] proposed the so-called *vulnerable boost hypothesis*. Put simply, this hypothesis assumes that (a) maintaining boosted memories across a long study-test interval implies a substantial amount of interference, and (b) the negative effect of this interference would be larger in older than in younger adults, because of the reduced cognitive resources and/or the associative deficits commonly associated with aging. The results from two previous studies support this proposal. An advantage of target-paired images in older adult controls (age: M = 63.2 and M = 63.8 years) was found when using a short-term version of the ABE paradigm [18,20]. These results should be taken together with Prull's observation [17] of a significantly reduced size of the ABE in older-old adults, even when a very short 2-min study-test interval was used. Overall, these results suggest that the negative effects of interference increase linearly with age, such that (a) young adults show the ABE after both a short and long study-test interval; (b) young-old adults show the ABE after a short interval, but the effect is reduced or eliminated after a long interval; and (c) older-old adults already show a reduced ABE or no effect after a short interval.

To further clarify this issue, we recruited healthy controls and bipolar patients ranging from 18 to 60 years. We divided both samples into two age-subgroups: A 'young-adult' group, from 18 to 35 years of age, and an 'adult' group, ranging in age from 36 to 60 years. In line with our expectations, such a division had a strong impact on the ABE, as demonstrated by the significant three-way interaction between group, age, and type of trial. The follow-up analyses confirmed that the ABE was significant in young-adult healthy controls: Replicating previous results, the images encoded with targets were recognized significantly better than the images encoded with distractors or presented without squares [1–3,5,10,11,39,48]. In contrast, the ABE was abolished in adult healthy controls. Most importantly, we also found that the mechanisms accounting for the elimination of the ABE were similar to those discussed previously for bipolar patients: The follow-up analyses of the three-way interaction indicated that adult healthy controls recognized the baseline images significantly better than the distractor images and exhibited a significant and selective deficit in the recognition of target-paired images (compared to young-adult healthy controls). These results confirm an age-related impairment in the temporal selective attention processes at the basis of the ABE and further support the hypothesis that healthy ageing implicates an increase in the attentional resources required by the detection task, which in turn offsets the attentional facilitation enjoyed by target-paired stimuli [39]. Notably, our data indicate that this impairment is not limited to older participants between 60 and 75 years (as in Bechi Gabrielli et al. [39]); rather, when the study-test delay is sufficiently long, a sizable decrease in the magnitude of the ABE can already be observed in participants between 36 and 60 years.

A number of hypotheses can be put forward regarding the cerebral mechanisms that underlie the reduction of the positive effects of the ABE in healthy adult controls and bipolar patients. Currently, the neural underpinnings of the ABE are poorly understood [9]. An fMRI study by Swallow, Makovski and Jiang [49] reported that the regions that responded more strongly to target than distractor stimuli comprised those typically activated

in attentional selection tasks, including the anterior insula, the anterior cingulate, the intraparietal sulcus, the supramarginal gyrus, the precuneus, the basal ganglia, and the posterior brain stem in the vicinity of the locus coeruleus. Similarly, Bechi Gabrielli et al. [50] found that, compared to the processing of distractor-associated stimuli, the encoding of target-associated images produced a greater activation of regions within the ventral frontoparietal network, including the temporoparietal junction, the supramarginal area, the anterior cingulate cortex, and several subcortical regions. Interestingly, some of these areas were found to be dysfunctional in previous fMRI studies examining the performance of bipolar patients in sustained attention tasks. For example, Diwadkar et al. [51] showed that an increase in the attention demands of the detection task led to increased engagement of the frontal-striatal pathway in healthy controls, but disengagement in adolescents with a higher genetic risk for bipolar disorder. The already mentioned study by Sepede et al. [36] reported that, during errors in target detection, both patients and relatives showed a larger activation in the bilateral insula and the posterior part of the middle cingulate cortex. Finally, Brooks, Bearden, Hoblyn, Woodard, and Ketter [52] found that the omission errors of euthymic bipolar patients were strongly related to dorsolateral prefrontal hypometabolism and greater paralimbic, insula, and cingulate hypermetabolism. Although additional studies are needed to clarify the neural bases of the ABE and the differences between healthy and clinical populations, it seems reasonable to hypothesize that the reduction of the ABE in bipolar patients might be ascribed to a dysfunction of the ventral frontoparietal network.

In this respect, it should be highlighted that a significant deficit in the recognition of target-paired stimuli (coupled with an intact recognition of distractor-paired stimuli) has now been reported in a growing number of studies investigating the ABE in several psychiatric diseases, including amnestic mild cognitive impairment, post-traumatic stress disorder, schizophrenia, and bipolar disorder [18–21]. Most interestingly, from a clinical standpoint, two recent meta-analyses have pointed out that hypoactivation in brain regions regulating the ABE might signal vulnerability to develop different forms of psychopathology. For example, McTegue et al. [53] showed that, in tasks of cognitive control, hypoactivation in the right inferior prefrontal/insular cortex represented a transdiagnostic feature of schizophrenia, bipolar disorder, major depressive disorder, anxiety disorders, and substance use. Similarly, Janiri et al. [54] found that, in mood disorders, post-traumatic stress disorder, and anxiety disorders, the most consistent transdiagnostic abnormalities in task-related brain activity were identified in the inferior prefrontal cortex/insula, the inferior parietal lobule, and the putamen. Clearly, then, interventions aimed at improving the patients' performance in the ABE paradigm, targeting at least part of these shared brain phenotypes, might also improve clinical outcomes and reduce or prevent morbidity in the general population (see Kèri et al. [18] for an example).

From a clinical point of view, the present results may be relevant for translational neuroscience and psychiatry, especially with regards to the role of the hippocampus in the formation of bound representations linking the background stimuli with the central target items. A previous study by Szamosi and colleagues [20] reported that the hippocampal volume was positively associated with the recognition of target-paired images in the ABE paradigm, for both older controls and patients with amnestic mild cognitive impairment. Moreover, significant shrinkage of the hippocampal formation has been reported for older adults [42], as well as in several psychiatric populations, including individuals with bipolar disorder [55] and schizophrenic patients [56]. If, as suggested by the evidence described above, the ABE paradigm shows sensitivity to hippocampal pathology, then the recognition of target-paired images might be successfully used to detect the early stages of a wide range of clinical memory disorders [20].

The present study has some limitations that must be taken into account. First, all euthymic bipolar patients were under pharmacological treatment, usually with mood-stabilizing and antipsychotic treatments [57,58], and this might have influenced their neurocognitive performance. However, data from literature show that the attentional deficits of these patients endure after controlling for mild residual symptomatology [29]

and pharmacological treatment [30,31]. We are therefore inclined to believe that the significant impairment in the recognition of target-paired stimuli was genuine. Second, we used a relatively long interval between the encoding phase and the recognition task (20 min). Since Prull [17] found that healthy older adults exhibited an intact ABE when tested after 2 min from the encoding phase, investigating whether a significant ABE can be observed in bipolar patients after a short study-test interval represents an important avenue for future research.

5. Conclusions

In conclusion, our results are consistent with previous evidence showing attentional deficits in bipolar patients during the remission phase of the disease. In this clinical population, the absence of the ABE was mediated by a specific difficulty in the recognition of target-paired images, suggesting that temporal selective attention processes are defective in bipolar patients [4]. Based on the idea that the ABE represents a trade-off between attentional boost and attentional competition, we propose that the maintenance of a fast and accurate performance in the detection task is more attentionally demanding for patients than for healthy controls and that the increase in the negative effects of attentional competition is enough to eliminate the ABE. Our second important result is the absence of ABE in healthy adult controls. This confirms and extends the conclusions reported by Bechi Gabrielli et al. [39] and provides further evidence that the boosting mechanisms associated with target detection undergo an age-related decrease starting from about 35 years. Future studies should clarify the cerebral mechanisms leading to early attenuation of the ABE in healthy adults.

Author Contributions: Conceptualization, G.B.G., C.R.-A., P.S., F.D. and V.C.; data curation, G.B.G., P.S., M.C. and A.S.; investigation, G.B.G., A.S., G.A. and G.S.; methodology, G.B.G., C.R.-A., P.S., G.A., G.S. and V.C.; writing—original draft, G.B.G., C.R.-A., P.S., F.D. and V.C.; writing—review and editing, G.B.G., C.R.-A., P.S., F.D., M.C., A.S., G.A., G.S. and V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study adhered to the Principles of Human Rights, as adopted by the World Medical Association at the 18th WMA General Assembly, Helsinki, Finland, June 1964 and subsequently amended at the 64th WMA General Assembly, Fortaleza, Brazil, October 2013. All participants provided written informed consent to participate in the study after having received a complete explanation of study procedures and aims. Patients did not receive monetary compensation for this study.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data available on request. The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rossi-Arnaud, C.; Spataro, P.; Costanzi, M.; Saraulli, D.; Cestari, V. Divided attention enhances the recognition of emotional stimuli: Evidence from the attentional boost effect. *Memory* **2018**, *26*, 42–52. [CrossRef] [PubMed]
2. Swallow, K.M.; Jiang, Y.V. The attentional boost effect: Transient increases in attention to one task enhance performance in a second task. *Cognition* **2010**, *115*, 118–132. [CrossRef] [PubMed]
3. Spataro, P.; Mulligan, N.W.; Rossi-Arnaud, C. Divided attention can enhance memory encoding: The attentional boost effect in implicit memory. *J. Exp. Psychol. Learn. Mem. Cogn.* **2013**, *39*, 1223–1231. [CrossRef]
4. Swallow, K.M.; Jiang, Y.V. Attentional load and attentional boost: A review of data and theory. *Front. Psychol.* **2013**, *4*, 274. [CrossRef]
5. Swallow, K.M.; Jiang, Y.V. The attentional boost effect really is a boost: Evidence from a new baseline. *Atten. Percept. Psychophys.* **2014**, *76*, 1298–1307. [CrossRef] [PubMed]
6. Duncan, J. The locus of interference in the perception of simultaneous stimuli. *Psychol. Rev.* **1980**, *87*, 272. [CrossRef] [PubMed]
7. Olivers, C.N.; Meeter, M. A boost and bounce theory of temporal attention. *Psychol. Rev.* **2008**, *115*, 836. [CrossRef] [PubMed]

8. Aston-Jones, G.; Cohen, J.D. An integrative theory of locus coeruleus–norepinephrine function: Adaptive gain and optimal performance. *Annu. Rev. Neurosci.* **2005**, *28*, 403–450. [CrossRef] [PubMed]
9. Yebra, M.; Galarza-Vallejo, A.; Soto-Leon, V.; Gonzalez-Rosa, J.J.; de Berker, A.O.; Bestmann, S.; Oliviero, A.; Kroes, M.C.V.; Strange, B.A. Action boosts episodic memory encoding in humans via engagement of a noradrenergic system. *Nat. Commun.* **2019**, *10*, 1–12. [CrossRef] [PubMed]
10. Mulligan, N.W.; Spataro, P.; Picklesimer, M. The attentional boost effect with verbal materials. *J. Exp. Psychol. Learn. Mem. Cogn.* **2014**, *40*, 1049–1063. [CrossRef]
11. Spataro, P.; Mulligan, N.W.; Rossi-Arnaud, C. Limits to the attentional boost effect: The moderating influence of orthographic distinctiveness. *Psychon. Bull. Rev.* **2015**, *22*, 987–992. [CrossRef]
12. Spataro, P.; Mulligan, N.W.; Bechi Gabrielli, G.; Rossi-Arnaud, C. Divided attention enhances explicit but not implicit conceptual memory: An item-specific account of the attentional boost effect. *Memory* **2017**, *25*, 170–175. [CrossRef] [PubMed]
13. Lin, J.Y.; Pype, A.D.; Murray, S.O.; Boynton, G.M.; Fahle, M. Enhanced memory for scenes presented at behaviorally relevant points in time. *PLoS Biol.* **2010**, *8*, 1–6. [CrossRef] [PubMed]
14. Makovski, T.; Swallow, K.M.; Jiang, Y.V. Attending to unrelated targets boosts short-term memory for color arrays. *Neuropsychologia* **2011**, *49*, 1498–1505. [CrossRef]
15. Swallow, K.M.; Jiang, Y.V. The role of timing in the attentional boost effect. *Atten. Percept. Psychophys.* **2011**, *73*, 389–404. [CrossRef] [PubMed]
16. Swallow, K.M.; Jiang, Y.V. Goal-relevant events not to be rare to boost memory performance for concurrent images. *Atten. Percept. Psychophys.* **2012**, *74*, 70–82. [CrossRef]
17. Prull, M.W. The attentional boost effect for words in young and older adults. *Psychol. Aging* **2019**, *34*, 405–417. [CrossRef]
18. Kéri, S.; Nagy, H.; Levy-Gigi, E.; Kelemen, O. How attentional boost interacts with reward: The effect of dopaminergic medications in Parkinson's disease. *Eur. J. Neurosci.* **2013**, *38*, 3650–3658. [CrossRef]
19. Levy-Gigi, E.; Kéri, S. Falling out of time: Enhanced memory for scenes presented at behaviorally irrelevant points in time in posttraumatic stress disorder (PTSD). *PLoS ONE* **2012**, *7*, e42502. [CrossRef]
20. Szamosi, A.; Levy-Gigi, E.; Kelemen, O.; Kéri, S. The hippocampus plays a role in the recognition of visual scenes presented at behaviorally relevant points in time: Evidence from amnestic mild cognitive impairment (aMCI) and healthy controls. *Cortex* **2013**, *49*, 1892–1900. [CrossRef]
21. Rossi-Arnaud, C.; Spataro, P.; Saraulli, D.; Mulligan, N.W.; Sciarretta, A.; Marques, V.R.; Cestari, V. The attentional boost effect in schizophrenia. *J. Abnorm. Psychol.* **2014**, *123*, 588. [CrossRef] [PubMed]
22. Leclercq, V.; Le Dantec, C.C.; Seitz, A.R. Encoding of episodic information through fast task-irrelevant perceptual learning. *Vision Res.* **2014**, *99*, 5–11. [CrossRef]
23. American Psychiatric Association. *Manuale Diagnostico e Statistico dei Disturbi Mentali—Quinta Edizione. DSM-5*; Tr.it. Raffaello Cortina: Milano, Italy, 2014.
24. Cullen, B.; Ward, J.; Graham, N.A.; Deary, I.J.; Pell, J.P.; Smith, D.J.; Evans, J.J. Prevalence and correlates of cognitive impairment in euthymic adults with bipolar disorder: A systematic review. *J. Affect. Disord.* **2016**, *205*, 165–181. [CrossRef] [PubMed]
25. Lim, C.S.; Baldessarini, R.J.; Vieta, E.; Yucel, M.; Bora, E.; Sim, K. Longitudinal neuroimaging and neuropsychological changes in bipolar disorder patients: Review of the evidence. *Neurosci. Biobehav. Rev.* **2013**, *37*, 418–435. [CrossRef] [PubMed]
26. Palazzo, M.C.; Arici, C.; Cremaschi, L.; Cristoffanini, M.; Dobrea, C.; Dell'Osso, B.; Altamura, A. Cognitive Performance in Euthymic Patients with Bipolar Disorder vs Healthy Controls: A Neuropsychological Investigation. *Clin. Prac. Epidemiol. Ment. Health* **2017**, *13*, 71–81. [CrossRef] [PubMed]
27. Quraishi, S.; Frangou, S. Neuropsychology of bipolar disorder: A review. *J. Affect. Disord.* **2002**, *72*, 209–226. [CrossRef]
28. Robinson, L.J.; Thompson, J.M.; Gallagher, P.; Goswami, U.; Young, A.H.; Ferrier, I.N.; Moore, P.B. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *J. Affect. Disord.* **2006**, *93*, 105–115. [CrossRef]
29. Clark, L.; Iversen, S.D.; Goodwin, G.M. Sustained attention deficit in bipolar disorder. *Brit. J. Psychiatry* **2002**, *180*, 313–319. [CrossRef] [PubMed]
30. Thompson, J.M.; Gallagher, P.; Hughes, J.H.; Watson, S.; Gray, J.M.; Ferrier, I.N.; Young, A.H. Neurocognitive impairment in euthymic patients with bipolar affective disorder. *Brit. J. Psychiatry* **2005**, *186*, 32–40. [CrossRef] [PubMed]
31. Goswami, U.; Sharma, A.; Varma, A.; Gulrajani, C.; Ferrier, I.N.; Young, A.H.; Gallagher, P.; Thompson, J.M.; Moore, P.B. The neurocognitive performance of drug-free and medicated euthymic bipolar patients do not differ. *Acta Psychiatr. Scand.* **2009**, *120*, 456–463. [CrossRef] [PubMed]
32. Bora, E.; Vahip, S.; Gonul, A.S.; Akdeniz, F.; Alkan, M.; Ogut, M.; Eryavuz, A. Evidence for theory of mind deficits in euthymic patients with bipolar disorder. *Acta Psychiatr. Scand.* **2005**, *112*, 110–116. [CrossRef]
33. Clark, L.; Goodwin, G.M. State-and trait-related deficits in sustained attention in bipolar disorder. *Eur. Arch. Psychiatry Clin. Neurosci.* **2004**, *254*, 61–68. [CrossRef] [PubMed]
34. Harmer, C.J.; Clark, L.; Grayson, L.; Goodwin, G.M. Sustained attention deficit in bipolar disorder is not a working memory impairment in disguise. *Neuropsychologia* **2002**, *40*, 1586–1590. [CrossRef]
35. Liu, S.K.; Chiu, C.H.; Chang, C.J.; Hwang, T.J.; Hwu, H.G.; Chen, W.J. Deficits in sustained attention in schizophrenia and affective disorders: Stable versus state-dependent markers. *Am. J. Psychiatry* **2002**, *159*, 975–982. [CrossRef] [PubMed]

36. Sepede, G.; De Berardis, D.; Campanella, D.; Perrucci, M.G.; Ferretti, A.; Serroni, N.; Moschetta, F.S.; Del Gratta, C.; Salerno, R.M.; Ferro, F.M.; et al. Impaired sustained attention in euthymic bipolar disorder patients and non-affected relatives: An fMRI study. *Bipolar Disord.* **2012**, *14*, 764–779. [CrossRef] [PubMed]
37. Swann, A.C.; Pazzaglia, P.; Nicholls, A.; Dougherty, D.M.; Moeller, F.G. Impulsivity and phase of illness in bipolar disorder. *J. Affect. Disord.* **2003**, *73*, 105–111. [CrossRef]
38. Wilder-Willis, K.E.; Sax, K.W.; Rosenberg, H.L.; Fleck, D.E.; Shear, P.K.; Strakowski, S.M. Persistent attentional dysfunction in remitted bipolar disorder. *Bipolar Disord.* **2001**, *3*, 58–62. [CrossRef] [PubMed]
39. Bechi Gabrielli, G.; Spataro, P.; Pezzuti, L.; Rossi-Arnaud, C. When divided attention fails to enhance memory encoding: The attentional boost effect is eliminated in young-old adults. *Psychol. Aging* **2018**, *33*, 259. [CrossRef] [PubMed]
40. Moyal, R.; Turker, H.B.; Luh, W.M.; Swallow, K.M. Auditory Target Detection Enhances Visual Processing and Hippocampal Functional Connectivity. *bioRxiv* **2020**. [CrossRef]
41. Mather, M.; Harley, C.W. The locus coeruleus: Essential for maintaining cognitive function and the aging brain. *Trends Cogn. Sci.* **2016**, *20*, 214–226. [CrossRef] [PubMed]
42. Raz, N.; Lindenberger, U.; Rodrigue, K.M.; Kennedy, K.M.; Head, D.; Williamson, A.; Dahle, C.; Gerstorf, D.; Acker, J.D. Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. *Cereb. Cortex* **2005**, *15*, 1676–1689. [CrossRef]
43. Orsini, A.; Pezzuti, L. *WAIS-IV. Contributo alla Taratura Italiana*; Giunti OS: Firenze, Italy, 2013.
44. Wechsler, D. *WAIS-IV. Manuale di Somministrazione e Scoring*; Giunti OS: Firenze, Italy, 2013.
45. Lang, P.J.; Bradley, M.M.; Cuthbert, B.N. *International Affective Picture System (IAPS): Affective Ratings of Pictures and Instruction Manual*; Tech Rep A-8: University of Florida: Gainesville, FL, USA, 2008.
46. Clark, L.; Kempton, M.J.; Scarnà, A.; Grasby, P.M.; Goodwin, G.M. Sustained attention-deficit confirmed in euthymic bipolar disorder but not in first-degree relatives of bipolar patients or euthymic unipolar depression. *Biol. Psychiatry* **2005**, *57*, 183–187. [CrossRef]
47. Meng, Y.; Lin, G.; Lin, H. The role of distractor inhibition in the attentional boost effect: Evidence from the R/K paradigm. *Memory* **2019**, *27*, 750–757. [CrossRef] [PubMed]
48. Mulligan, N.W.; Spataro, P. Divided attention can enhance early-phase memory encoding: The attentional boost effect and study trial duration. *J. Exp. Psychol. Learn. Mem. Cogn.* **2015**, *41*, 1223–1228. [CrossRef]
49. Swallow, K.M.; Makovski, T.; Jiang, Y.V. Selection of events in time enhances activity throughout early visual cortex. *J. Neurophysiol.* **2012**, *108*, 3239–3252. [CrossRef]
50. Bechi Gabrielli, G.; Fagioli, S.; Spataro, P.; Macaluso, E.; Serra, L.; Bozzali, M.; Rossi-Arnaud, C. The attentional ventral network at the base of the Attentional Boost Effect. In Proceedings of the OHBM Annual Meeting—Organization of Human Brain Mapping, Rome, Italy, 9–13 June 2019.
51. Diwadkar, V.A.; Bakshi, N.; Gupta, G.; Pruitt, P.; White, R.; Eickhoff, S.B. Dysfunction and dysconnection in cortical–striatal networks during sustained attention: Genetic risk for schizophrenia or bipolar disorder and its impact on brain network function. *Front. Psychiatry* **2014**, *5*, 50. [CrossRef] [PubMed]
52. Brooks III, J.O.; Bearden, C.E.; Hoblyn, J.C.; Woodard, S.A.; Ketter, T.A. Prefrontal and paralimbic metabolic dysregulation related to sustained attention in euthymic older adults with bipolar disorder. *Bipolar Disord.* **2010**, *12*, 866–874. [CrossRef] [PubMed]
53. McTeague, L.M.; Huemer, J.; Carreon, D.M.; Jiang, Y.; Eickhoff, S.B.; Etkin, A. Identification of common neural circuit disruptions in cognitive control across psychiatric disorders. *Am. J. Psychiatry* **2017**, *174*, 676–685. [CrossRef] [PubMed]
54. Janiri, D.; Moser, D.A.; Doucet, G.E.; Luber, M.J.; Rasgon, A.; Lee, W.H.; Murrough, J.W.; Sani, G.; Eickhoff, S.B.; Frangou, S. Shared Neural Phenotypes for Mood and Anxiety Disorders: A Meta-analysis of 226 Task-Related Functional Imaging Studies. *JAMA Psychiatry* **2019**, *77*, 172–179. [CrossRef] [PubMed]
55. Otten, M.; Meeter, M. Hippocampal structure and function in individuals with bipolar disorder: A systematic review. *J. Affect. Disord.* **2005**, *174*, 113–125. [CrossRef] [PubMed]
56. Adriano, F.; Caltagirone, C.; Spalletta, G. Hippocampal volume reduction in first-episode and chronic schizophrenia: A review and meta-analysis. *Neuroscientist* **2012**, *18*, 180–200. [CrossRef] [PubMed]
57. Centorrino, F.; Fogarty, K.V.; Sani, G.; Salvatore, P.; Cimbolli, P.; Baldessarini, R.J. Antipsychotic drug use: McLean Hospital, 2002. *Hum. Psychopharmacol.* **2005**, *20*, 355–358. [CrossRef] [PubMed]
58. Sani, G.; Perugi, G.; Tondo, L. Treatment of bipolar disorder in a lifetime perspective: Is lithium still the best choice? *Clin. Drug Investig.* **2017**, *37*, 713–727. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Article

Finger Tapping as a Biomarker to Classify Cognitive Status in 80+-Year-Olds

Dieter F. Kutz [1,*], Stephanie Fröhlich [1], Julian Rudisch [1], Katrin Müller [2] and Claudia Voelcker-Rehage [1]

[1] Department of Neuromotor Behavior and Exercise, Institute of Sport and Exercise Sciences, University of Muenster, 48149 Münster, Germany; stephanie.froehlich@uni-muenster.de (S.F.); julian.rudisch@uni-muenster.de (J.R.); claudia.voelcker-rehage@uni-muenster.de (C.V.-R.)

[2] Institute of Human Movement Science and Health, Faculty of Behavioural and Social Sciences, Chemnitz University of Technology, 09126 Chemnitz, Germany; katrin.mueller@hsw.tu-chemnitz.de

* Correspondence: dieter.kutz@uni-muenster.de

Abstract: This study examined the association between finger tapping and cognitive function in a group of 225 elderly participants (116 males; age 79–92 years; M = 82.5; SD = 2.4). Finger tapping was assessed in two conditions: self-selected pace and fast pace. Based on cognitive assessments, including the MoCA and CERA-NP test battery, participants were classified as cognitively healthy individuals (CHI), participants with mild cognitive impairments (MCI), and those with possible MCI (pMCI). Results of the analyses show significant differences between groups, sex and the group × sex interaction in four parameters for the self-selected pace condition and eight parameters for the fast pace condition. These parameters were used for classification by means of linear discriminant analysis (LDA). The first LDA component showed significant differences between CHI and pMCI and between CHI and MCI. Furthermore, the second LDA component showed significant differences between CHI and pMCI as well as between pMCI and MCI. Nevertheless, the algorithm correctly classified only 50% of participants, regardless of group, suggesting that tapping parameters are only partially useful for classification in early stages of dementia. We discuss these findings in terms of the diadochokinetic nature of finger tapping as associated with the age-related degeneration of cortical and subcortical motor areas.

Keywords: aging; cerebellum; classification; cognitive decline; diadochokinesia; motor control; sensory motor performance; time perception

Citation: Kutz, D.F.; Fröhlich, S.; Rudisch, J.; Müller, K.; Voelcker-Rehage, C. Finger Tapping as a Biomarker to Classify Cognitive Status in 80+-Year-Olds. *J. Pers. Med.* **2022**, *12*, 286. https://doi.org/10.3390/jpm12020286

Academic Editor: Marco Costanzi

Received: 2 December 2021
Accepted: 11 February 2022
Published: 15 February 2022

1. Introduction

As societies age, more and more people become affected with dementia [1]. In Germany, according to the German Alzheimer Society e.V. [2], the number of people with dementia will have risen to three million by 2050. In addition to the personal cost, the disease causes substantial economic and social burdens [3]. However, these burdens can be alleviated by early diagnosis of dementia and its pre-stages, as such early detection can allow for more sustainable disease management and optimal health care for affected individuals [1]. It is therefore important to identify people with pre-dementia (e.g., persons with mild cognitive impairments, or MCI) early enough so they can start programs that will help them maintain their personal lifestyle and that will continuously assess the course of the dementia as it progresses.

In many therapeutic areas, diseases and treatments are evaluated using patient-reported outcome (PRO) measures (subjective measures), collected, for example, via questionnaires [4]. However, several barriers exist for using PRO measures in cognitive impairment. For example, disease-related disorders can impair memory and cause people to lose insight into how their disease is progressing [4,5]. In these cases, one must rely on the reports of clinicians or information from personal contacts, such as family members [4,6]. However, the accuracy of the information provided by family members may be

suboptimal, as biases may interfere or caregivers may lack knowledge regarding the disease symptoms [4,7]. Therefore, the validity of PRO measurements is limited. Furthermore, the sensitivity of current PRO measures for patients with mild cognitive impaired individuals (MCI) and Alzheimer's disease (AD) patients in the prodromal stage is limited, as they are not specifically designed for these milder conditions [4]. A combination of several neuropsychological tests (e.g., MoCA [8] and CERAD-NP [9]) may improve classification (e.g., [10,11]) and additionally identify a transitional stage between cognitively healthy individuals (CHI) and MCI, possible MCI (pMCI) as recently defined as individuals with some signs of cognitive impairment [1,10].

AD and cognitively healthy individuals have been shown to differ in performance of movement tasks (e.g., finger tapping [12]). Therefore, objective measurements, e.g., by technical systems that measure simple movements, are an alternative to PRO measurements because they are easy to use and inexpensive. For this purpose, researchers use computer-aided measuring systems that measure parameters of the movement by means of a keyboard [13], force sensors [12] or light beams [14]. With these types of devices, studies have shown differences in finger tapping tasks between age-matched healthy subjects and people with AD [12–14], MCI [13,14] and Parkinson's disease [14] in a mean age range of 71–82 years. These differences are mainly related to a slowing of the tapping rhythm and an increase in touch duration as well an increase in the variability of these parameters [12–14]. Such a study on the finger tapping behavior of group pMCI has not been previously conducted. In general, it should be noted that tapping is a diadochokinetic movement consisting of flexion followed by extension of the fingers. The timing of the change in movement is controlled by proprioceptive signals that are triggered when the force sensor is touched [15–17]. The tapping task therefore tests the ability to plan and execute rhythmically oppositely oriented movements. It is shown that the selected tap pace has an influence on the execution [13].

Therefore, in this study we aimed to use tapping parameters to distinguish between participants over 80 years old who were either cognitively healthy individuals (CHI), mild cognitive impaired individuals (MCI) or had possible MCI (pMCI), in two different conditions: as consistently as possible at a self-selected pace or as fast as possible without considering consistency (fast pace). We expected that in addition to reproducing known differences in tapping parameter between CHI and MCI groups [13,14], we would also find differences between pMCI subjects and the other groups. In addition, a recent study has shown for this study group that sex has an effect on force control [18]. It was therefore expected that sex differences in finger tapping parameters would be found. Based on these differences, we then developed a classifier to determine whether a subject belongs to a group.

2. Materials and Methods

This study is part of the SENDA study (Sensor-based systems for early detection of dementia, registered in the German Clinical Trials Register under DRKS00013167), which was conducted at Chemnitz University of Technology, Germany. The detailed study protocol was published earlier [1]. Only information relevant to the current research question is described here.

2.1. Participants

The SENDA study was advertised by local general practitioners and in newspapers. In total, 244 participants (123 males; age 79–93 years; M = 82.5; SD = 2.5) took part in the study and were recruited from January 2018 to March 2020. Study participation required walking ability, sufficient German language skills, residence in or around Chemnitz, Germany, and a self-organized means of travel to and from the laboratory. Volunteers were excluded before testing if any of the following criteria applied: (1) acute psychological disorder; (2) diagnosis of any neurocognitive or neurological disorder; (3) past traumatic head injury; (4) substance abuse; (5) participation in other clinical studies; (6) a

physician-directed ban from physical activities; (7) severe restrictions due to cardiovascular, pulmonary, or orthopedic diseases; or (8) failure to reach the minimum required score of 19 during screening with the Montreal Cognitive Assessment [8]. Each participant signed a written informed consent form, and all study proceedings were approved by the Ethics Committee of Chemnitz University of Technology, Germany, Faculty of Behavioral and Social Sciences (V232-17-KM-SENDA-07112017, approved on 19 December 2017). Each participant received 25 EUR compensation for his or her participation at three appointments.

The analysis for this article included 225 participants who took part at the baseline measurement (T1, see [1]). Exclusion from analysis was due to (1) dropout from the study before all needed testing was completed (n = 14) or (2) technical issues during the recording (n = 5). Due to the participants' old age, many followed a medication regimen (n = 211), which most often included medication for high blood pressure (n = 174), thrombosis prophylaxis, cholesterol reduction, stomach acid reduction or thyroid function. Demographic characteristics are reported in Table 1.

Table 1. Characteristics of the groups according to cognitive status.

	CHI	**pMCI**	**MCI**
n (in %)	79 (35)	80 (36)	66 (29)
m/f	35/44	43/37	38/28
Age in years	82.0 ± 0.3	82.5 ± 0.2	82.9 ± 0.3
Education in years	14.3 ± 0.4	13.9 ± 0.4	13.7 ± 0.4
MMSE (0–30)	28.3 ± 0.2	27.8 ± 0.2	27.3 ± 0.2
MoCA (0–30)	27.7 ± 0.1	25.8 ± 0.2	22.8 ± 0.2

Given are means ± SEM. CHI: cognitively healthy individuals; pMCI: possible MCI; MCI: mild cognitive impaired individuals. For details of the classification, see text. f: female, m: male; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

2.2. Neuropsychological Testing and MCI Classification

The neuropsychological testing and MCI classification are described in detail elsewhere [10]. Briefly, all participants went through an intensive neuropsychological test battery, which was carried out by trained testing staff at the university lab. The tests included the German version of the MoCA [8] and the German version of the Consortium to Establish a Registry for Alzheimer's Disease Neuropsychological Test Battery [9, CERAD-NP]. The MoCA was used to measure global cognitive functioning and to screen for MCI. The MoCA is the second-most-utilized geriatric cognitive screening tool after the Mini-Mental State Examination (MMSE) and has superior sensitivity to mild cognitive impairments [19]. The CERAD-NP examines the cognitive domains of memory, language, executive functions and visuo-construction. MCI classification was based on the recommendations of The National Institute on Aging and the Alzheimer's Association [20] and in accordance with the criteria proposed by [21]. Cognitive impairments were determined according to performance in MoCA (one sum score) and CERAD-NP (nine separate test scores). The following CERAD-NP scores were used: verbal fluency (number of animals named in 1 min), Boston naming test (number of objects correctly identified), phonematic fluency (number of words named with letter "S" in 1 min), constructional praxis (number of correctly copied characteristics), word list learning (number of words correctly remembered in third trial), word list recall (savings score), word list recognition (discriminability score), constructional praxis recall (savings score), and trail making test (quotient B/A). We followed a two-step procedure recommended for diagnosis of MCI in the general population, which states that, first, a screening should be used, and, second, in the case of abnormal findings, in-depth cognitive testing should follow [22]. A MoCA score below 26 points and at least one CERAD-NP test performance at least 1.5 standard deviations below the normative mean (taking into consideration age, sex and education level) resulted in the classification of participants with mild cognitive impairments (MCI). Correspondingly, participants were classified as being cognitively healthy individuals (CHI) if they scored 26 or more points on the MoCA and were also within the normative range

(no score below −1.5 SD) in all of the CERAD-NP tests [11]. Due to the application of the two-step process, an additional class was defined for participants who showed cognitive impairments only according to one of the two tests. They were categorized as possibly having MCI (pMCI). This group included either participants who had deficits in one specific domain of the CERAD-NP but their overall cognitive functioning was normal according to MoCA, or participants who had no strong impairment in any single domain but had small deficits in different domains adding up to a low MoCA score (<26). Although this group would be considered non-MCI according to [22], as these individuals neither showed abnormal scores in the screening (MoCA > 25) nor exhibited any cognitive impairments in in-depth clinical testing after abnormal testing, we opted to analyze this group separately to have high discriminatory power between CHI and MCI.

2.3. Tasks and Recording

Participants carried out three fine motor tasks [1], including (1) force modulation of a precision grip with the thumb and index finger [18]; (2) tapping with the index finger of the right hand, [based on, 12]; and (3) connecting dots on a touchscreen with a touch pen/tracing (as studied by [23]). Here we report the results of the second motor task, experiment (2).

For the finger tapping tasks, we used one force transducer with a diameter of 29.5 mm, a depth of 8 mm, and a measurement range of 0–22.5 kg (manufacturer: Measurement Specialties Inc., Hampton, VA, USA; Model: FX-1901-0001-50 L) [18]. Signals were pre-amplified (using a customized voltage amplifier), digitally converted and sampled at a frequency of 1000 Hz using a NI-DAQ USB-6002 (National Instruments, Austin, TA, USA). The force transducer was fixed in a self-built wooden board that was placed on the table in front of the participants to prevent any movement of the transducer during the task (see Figure 1a). Experimental procedures, i.e., data acquisition, were programmed using a customized LabView 2015 (National Instruments, Austin, TA, USA) script. The task involved tapping with one's dominant index finger on the force transducer, which participants carried out in two different conditions: as consistently as possible at a self-selected pace (cf., Figure 1b) or as fast as possible without considering consistency (fast pace). Each trial lasted 15 s. In order to reduce the influence of fatigue, the trials were carried out in blocks: the first three trials were in the self-selected pace condition and then two trials were performed for the fast pace condition. Participants received no visual feedback.

2.4. Data Processing, Parameter Extraction and Statistical Analyses

Data processing and parameter extraction were performed separately for each trial with a custom-made program in R ([24], ver. 3.6.3). The results were visually inspected and, when necessary, manually corrected. For determining the moment of finger contact with the force transducer, an individual threshold was calculated for each trial using a k-means algorithm with three means. The lowest mean value described the distribution of the noise of the non-contacted force transducer, the highest mean value described the distribution of the force peaks and the remaining mean value described the transition from the noise to the force peaks. The upper 95% confidence band of the first mean (the noise) was defined as the threshold. For the following analyses, the force curve was low-pass filtered using a second order Butterworth filter (cutoff frequency 100 Hz). Individual taps were identified using the filtered force curve. The tap start was defined as the moment when the force curve crossed the threshold after remaining below the threshold for the prior 100 ms. The tap end was the first moment after that when the force curve fell below the threshold (see Figure 1c). Based on the identified taps, the following parameters were extracted:

- tap duration: interval from tap start to tap end;
- tap cycle: interval from a tap start to the following tap start;
- offphase: interval from a tap end to the next tap start, namely the time when the finger is not in contact with the force transducer;
- force peak: force maximum of an individual tap; and

- time to peak: time from tap start to the moment of the force peak.

Visual inspection of the taps showed that an individual tap could be described by a trapeze (Figure 1c, right tap), having a force increase from tap start onwards (Figure 1c, flexion), reaching a plateau for several milliseconds (Figure 1c, plateau duration) and then followed by a decrease in force until tap end (Figure 1c, extension). To calculate these parameters, the tap was divided into two intervals at time to peak (first interval from tap start to time-to-peak, second from time-to-peak to tap end). For each interval, a two-linear spline model for the force curve over time was calculated [25]. From these calculations, the following parameters were extracted:

- flexion: first force slope in the first interval describing the flexion performance during tapping (Figure 1c, flexion);
- extension: second force slope of the second interval describing the extension performance during tapping (Figure 1c, extension);
- time to plateau: duration from tap start to the break point of the first interval (Figure 1c, right tap). This time describes the duration of the execution of flexion after contact of the finger with the force sensor; and
- plateau duration: duration from the first break point to the second break point (Figure 1c, right tap).

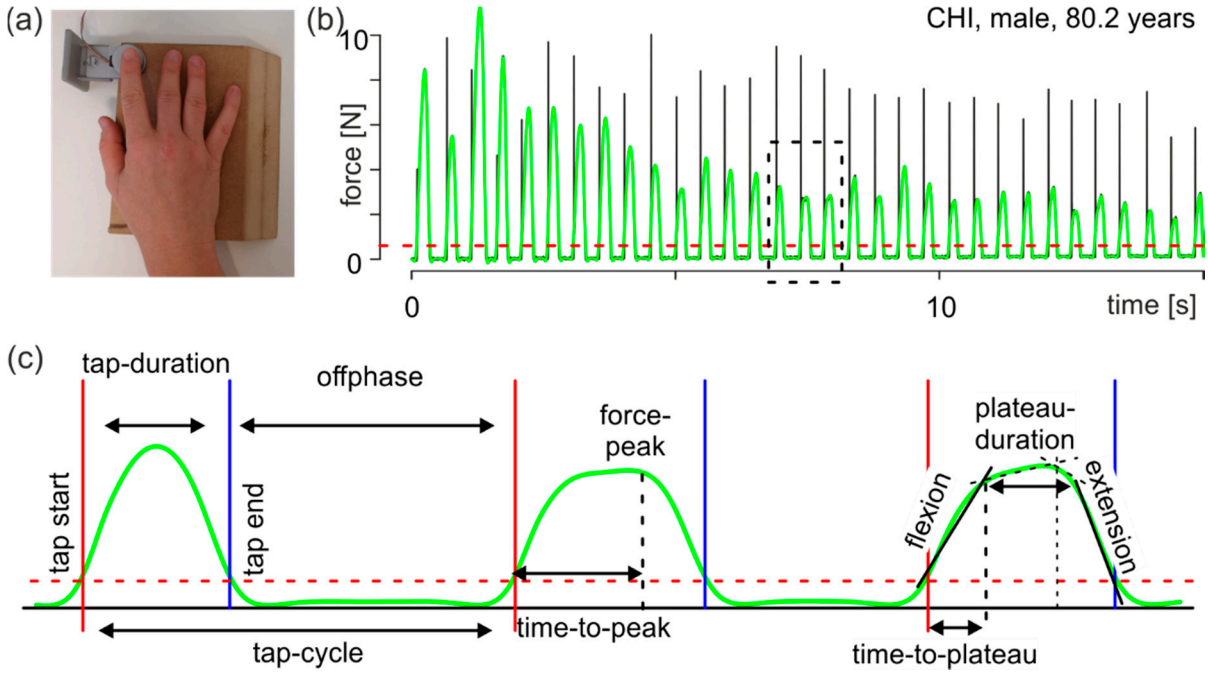

Figure 1. Methods. (**a**) Experimental setup. (**b**) Characteristic force curve of tapping. Black line: original recorded force curve; green line: low-pass filtered force curve; red dashed line: force threshold (see text); the dashed rectangle indicates the interval shown in c. (**c**) Example interval with three taps showing all time and force parameters (see text). Green line: filtered force curve; red vertical solid line: start point of an individual tap; blue vertical solid line: end point of an individual tap; red dashed line: force threshold; black vertical dashed line in the middle tap: time point of the force peak; black vertical dashed lines in the right tap: break points of the two-linear spline models (see text).

Of note, tapping is a diadochokinetic movement consisting of finger flexion followed by finger extension. The time to stop flexion and start extension is controlled by proprioceptive signals [15–17]. Therefore, the mean size of the time to plateau gives information on the planned movement (the shorter the time to plateau, the faster the movement), and its variability gives information on the participant's proprioceptive control at the spinal cord level (the smaller the better).

All time values were calculated in seconds, the force peak was calculated in Newtons and flexion and extension were calculated in Newtons/second. Since the distributions of a participant's parameters exhibited skewness and kurtosis that could not be fitted by a standard uniform procedure, each participant's individual finger tapping behavior was characterized by two values: first, the size of a parameter using the median of the values and, second, the variability of this parameter using the inter-quartile range (iqr). These values were calculated separately for all parameters in both conditions (self-selected pace and fast pace).

Since the group data were not normally distributed, they were logarithmically transformed for statistical analysis. A mixed ANOVA with a between-subjects factor with two levels (group and sex) and a within-subjects factor (condition) was performed. ANOVA was performed using the R package ez [26]. Effect size η^2_G is given to provide comparability [27]. Post-hoc comparisons were based on Fisher's least significant difference (FLSD) when appropriate. Linear discriminant analysis (LDA) was performed using the R package MASS (version 7.3–53) based on [28]. For LDA, the logarithmized parameters were z-transformed.

3. Results

This study was part of the SENDA study [1] and examined the finger tapping behavior of 225 participants over 80 years old who took part at baseline measurement T1 [1]. As described above, the study participants were classified into three groups according to their cognitive performance: cognitively healthy individuals (CHI, n = 79), participants with possible mild cognitive impairments (pMCI, n = 80), and participants with mild cognitive impairments (MCI, n = 66). Overall, 44,813 taps were recorded: 17,724 in the self-selected pace condition and 27,089 in the fast pace condition. In the self-selected pace condition, the groups did not behave differently: CHI performed 86.5 $\pm$ 4.1 taps on average (mean $\pm$ standard error of the mean (SEM)), pMCI 74.3 $\pm$ 3.4 taps, and MCI 74.9 $\pm$ 4.6 taps. In contrast, in the fast pace condition, MCI produced 114 $\pm$ 4.2 taps, significantly less than CHI (127 $\pm$ 3.2 taps, paired t-test with Bonferroni's correction, p = 0.019). pMCI (119 $\pm$ 2.9 taps) was not different compared with the other two groups.

3.1. Statistical Analyses

3.1.1. ANOVA

Group means and standard error of the mean (SEM) of the logarithmized parameter for the two conditions are given in Table 2. For convenience, all group mean values of the tapping parameters have been back-transformed into the respective physical dimensions and are listed in the supplement (Supplementary Table S1). In addition, a sex-specific breakdown of the values can be found in the supplement (Supplementary Table S2 and Supplementary Table S3). ANOVA shows that a total of four (out of 18) parameters in the self-selected pace condition (Table 3) and eight (out of 18) in the fast pace condition (Table 4) differed significantly ($p < 0.05$) in group, sex, or the group $\times$ sex interaction. For post-hoc comparison of significant effects, Fisher's least significant difference (FLSD) is given.

Mean values of the parameters (expressed as medians) were different between the groups in both conditions, i.e., the self-selected pace condition and the fast pace condition (Table 2). Post-hoc comparison of tap-cycle_median showed that inside each group, participants tapped faster in the fast pace condition than in the self-selected pace condition (FLSD (group + tapping condition) = 0.109). In contrast, group comparisons showed that only CHI differed from the other groups in the fast pace condition (FLSD (group) = 0.070, Table 4). For tap-duration_median, the post-hoc comparison showed that within each group, participants pressed the button for a shorter time during the fast pace condition than during the self-selected pace condition (FLSD (group + tapping condition) = 0.121). In contrast, in the group comparison, only MCI was significantly longer than the others in the fast pace condition (FLSD (group) = 0.070, Table 4).

Table 2. Logarithmized tapping parameters for both conditions for each group.

	Self-Selected Pace			Fast Pace		
	CHI (*n* = 79)	**pMCI (*n* = 80)**	**MCI (*n* = 66)**	**CHI (*n* = 79)**	**pMCI (*n* = 80)**	**MCI (*n* = 65) [1]**
tap-cycle_median	−0.593 ± 0.047	−0.433 ± 0.045	−0.440 ± 0.057	−1.442 ± 0.020	−1.380 ± 0.026	−1.365 ± 0.029
tap-cycle_iqr	−2.885 ± 0.081	−2.792 ± 0.080	−2.703 ± 0.099	−3.657 ± 0.066	−3.594 ± 0.050	−3.449 ± 0.080
tap-duration_median	−1.681 ± 0.050	−1.510 ± 0.054	−1.482 ± 0.066	−2.239 ± 0.020	−2.203 ± 0.026	−2.144 ± 0.029
tap-duration_iqr	−3.364 ± 0.074	−3.173 ± 0.081	−3.138 ± 0.089	−3.939 ± 0.046	−3.914 ± 0.037	−3.869 ± 0.053
offphase_median	−1.037 ± 0.051	−0.890 ± 0.049	−0.915 ± 0.060	−2.046 ± 0.025	−1.971 ± 0.032	−1.983 ± 0.035
offphase_iqr	−2.934 ± 0.067	−2.836 ± 0.070	−2.789 ± 0.083	−3.744 ± 0.059	−3.751 ± 0.053	−3.653 ± 0.075
force-peak_median	0.558 ± 0.109	0.527 ± 0.104	0.7901 ± 0.142	0.327 ± 0.089	0.403 ± 0.090	0.725 ± 0.112
force-peak_iqr	−0.449 ± 0.126	−0.594 ± 0.121	−0.328 ± 0.123	−0.454 ± 0.093	−0.420 ± 0.087	−0.189 ± 0.119
time-to-peak_median	−2.397 ± 0.054	−2.246 ± 0.057	−2.195 ± 0.072	−2.979 ± 0.019	−2.940 ± 0.028	−2.884 ± 0.030
time-to-peak_iqr	−3.934 ± 0.095	−3.710 ± 0.111	−3.619 ± 0.112	−4.677 ± 0.045	−4.631 ± 0.039	−4.644 ± 0.057
flexion_median	−3.743 ± 0.082	−3.852 ± 0.081	−3.676 ± 0.107	−3.551 ± 0.080	−3.508 ± 0.074	−3.228 ± 0.096
flexion_iqr	−4.853 ± 0.101	−4.935 ± 0.094	−4.764 ± 0.104	−4.497 ± 0.086	−4.497 ± 0.085	−4.306 ± 0.104
extension_median	−3.735 ± 0.089	−3.839 ± 0.086	−3.628 ± 0.114	−3.650 ± 0.078	−3.601 ± 0.074	−3.324 ± 0.094
extension_iqr	−4.766 ± 0.109	−4.896 ± 0.090	−4.763 ± 0.101	−4.624 ± 0.083	−4.604 ± 0.076	−4.443 ± 0.103
time-to-plateau_median	−2.722 ± 0.038	−2.682 ± 0.040	−2.617 ± 0.050	−3.053 ± 0.015	−3.030 ± 0.021	−2.985 ± 0.021
time-to-plateau_iqr	−4.446 ± 0.100	−4.448 ± 0.089	−4.235 ± 0.108	−4.871 ± 0.041	−4.855 ± 0.042	−4.888 ± 0.057
plateau-duration_median	−2.732 ± 0.076	−2.406 ± 0.084	−2.408 ± 0.097	−3.547 ± 0.025	−3.492 ± 0.032	-3.446 ± 0.040
plateau-duration_iqr	−3.778 ± 0.120	−3.457 ± 0.121	−3.434 ± 0.133	−5.347 ± 0.063	−5.278 ± 0.067	−5.207 ± 0.083

Given are means ± SEM. CHI: cognitively healthy individuals; MCI: participants with mild cognitive impairment; pMCI: participants with possible MCI. Note: units for time values are in log(s), those for force are in log(N) and those for flexion/extension are in log(N/s). Suffix _median specifies group medians and _iqr the inter-quartile range of the group. [1]: For technical reasons, the data for one participant from the fast pace condition are missing.

Table 3. ANOVA results for the self-selected pace condition. Only parameters with significant differences ($p < 0.05$) in at least one effect are shown. p values are false-discovery-rate-corrected for multiple comparisons.

Parameter	Effect	DFn	DFd	F	p	η^2_G
force-peak_median	group	2	219	1.22	0.30	0.01
	sex	1	219	6.32	0.03	0.03
	group × sex	2	219	3.85	0.06	0.03
	FLSD (sex)	0.263				
flexion_median	group	2	219	1.04	0.35	0.01
	sex	1	219	7.43	0.03	0.03
	group × sex	2	219	3.23	0.06	0.03
	FLSD (sex)	0.200				
extension_median	group	2	219	1.18	0.31	0.01
	sex	1	219	6.81	0.03	0.03
	group × sex	2	219	3.36	0.06	0.03
	FLSD (sex)	0.214				
plateau-duration_median	group	2	219	4.49	0.03	0.04
	sex	1	219	1.68	0.30	0.01
	group × sex	2	219	0.99	0.37	0.01
	FLSD (group)	0.238				

DFn: Degree of freedom (nominator); DFd: degree of freedom (denominator); η^2_G: generalized effect size; group: CHI, pMCI, MCI; sex: male, female; group × sex: interaction between group and sex; FLSD: Fisher's least significant difference.

Table 4. ANOVA results for the fast pace condition. Only parameters with significant differences ($p < 0.05$) in at least one effect are shown. p values are false-discovery-rate-corrected for multiple comparisons.

Parameter	Effect	DFn	DFd	F	p	η^2_G
tap-cycle_median	group	2	218	3.41	0.03	0.03
	sex	1	218	6.62	0.015	0.03
	group × sex	2	218	5.24	0.015	0.05
	FLSD (group)	0.070				
	FLSD (sex)	0.057				
	FLSD (group × sex)	0.097				
tap-cycle_iqr	group	2	218	3.24	0.06	0.03
	sex	1	218	7.11	0.03	0.03
	group × sex	2	218	2.11	0.12	0.02
	FLSD (sex)	0.147				
tap-duration_median	group	2	218	3.63	0.045	0.03
	sex	1	218	0.10	0.75	0.00
	group × sex	2	218	4.04	0.02	0.04
	FLSD (group)	0.070				
	FLSD (group × sex)	0.098				
offphase_median	group	2	218	2.69	0.07	0.02
	sex	1	218	13.4	<0.001	0.06
	group × sex	2	218	4.51	0.015	0.04
	FLSD (sex)	0.069				
	FLSD (group × sex)	0.117				
offphase_iqr	group	2	218	1.09	0.34	0.01
	sex	1	218	10.8	0.003	0.05
	group × sex	2	218	1.25	0.34	0.01
	FLSD (sex)	0.137				
force-peak_median	group	2	218	4.04	0.02	0.04
	sex	1	218	4.83	0.03	0.02
	group × sex	2	218	1.60	0.20	0.01
	FLSD (group)	0.267				
	FLSD (sex)	0.219				
flexion_median	group	2	218	3.72	0.045	0.03
	sex	1	218	6.34	0.03	0.03
	group × sex	2	218	1.06	0.35	0.01
	FLSD (group)	0.230				
	FLSD (sex)	0.188				
extension_median	group	2	218	3.78	0.03	0.03
	sex	1	218	8.10	0.015	0.04
	group × sex	2	218	1.03	0.36	0.01
	FLSD (group)	0.227				
	FLSD (sex)	0.185				

DFn: Degree of freedom (nominator); DFd: degree of freedom (denominator); η^2_G: generalized effect size; group: CHI, pMCI, MCI; sex: male, female; group × sex: interaction between group and sex; FLSD: Fisher's least significant difference.

In the fast pace condition, pMCI was significantly different from CHI for the parameter offphase_median and from MCI for the parameters force-peak_median, flexion_median, and extension_median. Therefore, for motor behavior, pMCI can be considered its own group between CHI and MCI. In addition, MCI differed significantly from CHI in the fast pace condition for the parameters tap-cycle_median, tap-cycle_iqr, tap-duration_median, force-peak_median, and flexion_median. In contrast, all significant variability measures showed sex differences (tap-cycle_iqr, offphase_iqr, Table 4).

Individual parameters clearly had small effect sizes, as shown by ηG^2. The highest significant value for group was 0.0393 (plateau-duration_median at self-selected pace; Table 3), for sex it was 0.0579 (offphase_median at fast pace; Table 4), and for group × sex it was 0.046 (tap-cycle_median at fast pace; Table 4). Hence, none of the parameters alone were suitable for assigning individual participants to one of the groups. Instead, classifying

participants required a combination of parameters with significant effects for group sex, or group × sex.

3.1.2. Linear Discriminant Analysis

Linear discriminant analysis (LDA) is a method of finding a linear combination of features that characterizes two or more classes of parameters. The resulting combination reduces the dimensionality and is used to classify the participants. Because a recent study for this study group showed that sex has an effect on force control [18], LDA was performed not only with parameters of the effect group but also with parameters of the effect of sex and the interaction group × sex. In total, four parameters of the self-selected pace condition and eight parameters of the fast pace showed significant differences ($p < 0.05$) for the effects of group, sex or the interaction group × sex. These parameters were analyzed using the R package MASS (see [28]). The LDA showed that the parameters can be combined into two linear combinations, LDA1 and LDA2. LDA1 explains 70% of the variance and LDA2 30%. The scales of the parameters are given in Table 5. Note that the suffix _self specifies the parameter of the self-selected pace condition and _fast specifies that of the fast pace condition.

Table 5. LDA scales.

Parameter	LDA1	LDA2
force-peak_median_self	0.38	0.93
flexion_median_self	−0.61	−0.97
extension_median_self	−0.69	0.21
plateau-duration_median_self	0.50	−0.69
tap-cycle_median_fast	−0.04	−4.50
tap-cycle_iqr_fast	1.01	0.31
tap-duration_median_fast	0.31	2.98
offphase_median_fast	0.17	2.45
offphase_iqr_fast	−1.02	0.09
force-peak_median_fast	−1.42	−3.17
flexion_median_fast	0.91	3.27
extension-median_fast	1.26	−0.15

The suffix _self specifies the parameter of the self-selected pace condition and _fast specifies that of the fast pace condition.

The distribution of the LDA scales of each group showed a significant difference of the medians among them for LDA-1 (Figure 2a). Post-hoc tests by means of a pairwise Wilcoxon rank-sum test confirmed the group difference for LDA1 between CHI and pMCI ($p < 0.001$, Bonferroni corrected) and between CHI and MCI ($p < 0.001$, Bonferroni corrected). For LDA-2 (Figure 2b), there was a difference in medians between CHI and pMCI as well as between MCI and pMCI. The post-hoc tests for LDA2 confirmed the significant difference between pMCI and MCI ($p < 0.05$, Bonferroni corrected) and between pMCI and CHI only at a trend level ($p < 0.1$, Bonferroni corrected). The same test for CHI vs. MCI revealed that for LDA2, the two groups were not significantly different at all ($p = 1$, Bonferroni corrected).

Astonishingly, reclassifying the participants based on the linear discriminant analysis only categorized 50% of participants into the right class, with the goodness of classification decreasing from CHI (49 of 79) to pMCI (41 of 80) to MCI (23 of 65). This is better than the theoretical probability of 1/3, but 50% were still misclassified (Table 6). The sensitivity of this classification for each group was CHI = 0.62, pMCI = 0.51, and MCI = 0.35; the specificity for each group was CHI = 0.70, pMCI = 0.69, and MCI = 0.86. Upon further inspecting the distribution of LDA1 and LDA2 over all correctly classified participants and misclassified participants, we found that for LDA1, correctly classified CHI participants were indeed different on this scale relative to correctly classified pMCI and MCI participants (Figure 3a). For LDA2, the difference between correctly classified pMCI participants and correctly classified participants of the other groups was clearly visible (Figure 3b). Importantly, the

LDA's inability to properly classify participants was related to the broad distribution of misclassified participants on both LDA1 and LDA2 scales (gray histograms in Figure 3a,b).

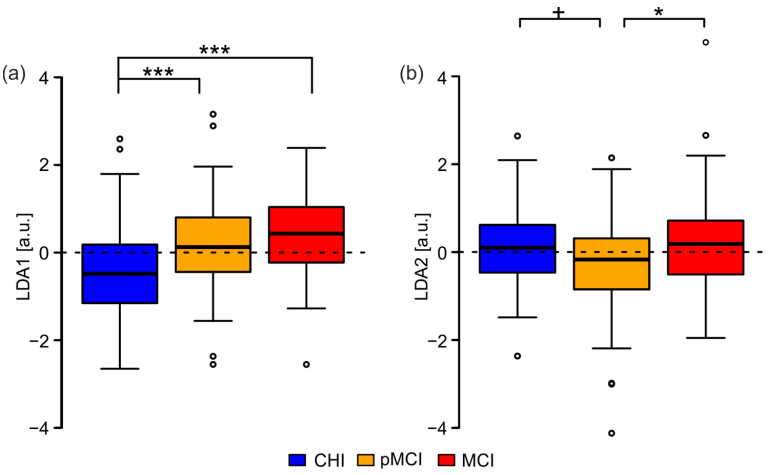

Figure 2. Boxplot of the LDA scales per group. (**a**) LDA1; (**b**) LDA2. CHI: cognitively healthy individuals; pMCI: participants with possible mild cognitive impairments; MCI: participants with mild cognitive impairments. $^{+}$ $p < 0.1$, * $p < 0.05$, *** $p < 0.001$.

Table 6. Confusion matrix of classification.

		Classification Based on Cognitive Assessments		
		CHI	**pMCI**	**MCI**
LDA classification	CHI	49	26	18
	pMCI	20	41	24
	MCI	10	13	23

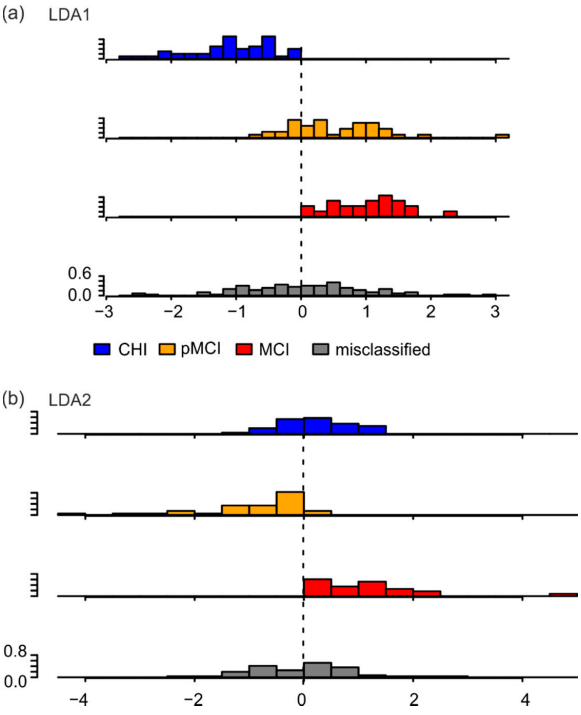

Figure 3. Histograms of probability densities of LDA values of correctly classified and misclassified participants. (**a**) LDA1; (**b**) LDA2. CHI: cognitively healthy individuals; pMCI: participants with possible mild cognitive impairments; MCI: participants with mild cognitive impairments.

The LDA with only parameters significant to the effect group reclassified only 47% of participants into the correct class. The distribution of LDA scales for each group showed a significant difference ($p < 0.05$) between medians only for LDA1 between CHI and pMCI and between CHI and MCI (data not shown). The histograms of the probability densities of the LDA values of correctly classified and misclassified participants mainly show a broadening of the distribution of misclassified participants (Supplement Figure S1). Overall, this indicates that finger tapping behavior was conditioned by cognitive status in only a subset of participants.

4. Discussion

The aim of the study was to develop a system that uses tapping parameters in a self-selected and fast tapping mode to distinguish cognitively healthy individuals (CHI) from people with possible MCI (pMCI) and people with mild cognitive impairments (MCI), specifically for individuals over 80 years old. For this purpose, the finger tapping behavior of 225 subjects over 80 years old was analyzed. ANOVA revealed differences between groups (CHI, pMCI, MCI), sexes (male, female) and their interaction (group × sex) for the self-selected pace condition (four parameters) and for the fast pace condition (eight parameters). These parameters were used for classification by means of a linear discriminant analysis (LDA). The first LDA component showed significant differences between CHI and pMCI, CHI and MCI, and pMCI and MCI. Furthermore, the second LDA component showed significant differences between CHI and pMCI and between pMCI and MCI. Nevertheless, when the algorithm was used to classify individual participants, it was correct in only 50% of cases. This shows that tapping parameters were only partially useful for classification.

Our results showed that pMCI, a group first described in the SENDA study [10], differed from both CHI and MCI. Previous studies on tapping behavior were mainly conducted with Alzheimer's patients (e.g., [12]) or MCI patients, (e.g., the CDR. 5 group in [13]). In this study, we additionally showed that in the self-selected pace condition, not only participants with MCI but also those with pMCI had a significantly slower tapping rhythm and prolonged touch duration compared to CHI (Table 2: tap-cyle_median, tap-duration_median, and plateau-duration_median).

However, the planned goal of classifying individual participants based on tapping parameters was only partially achieved. Thus, while 49 of 79 CHI participants were correctly classified on the basis of their motor performance, 30 of these participants were classified as pMCI or MCI. Furthermore, 42 of 65 MCI patients were apparently classified as CHI or pMCI. An explanation for the misclassification might be the simplicity of the task. Previous work has shown that no age effects exist in tasks with simple planned anticipatory grasp control, such as in tapping [29]; only tasks with higher complexity, such as activities of daily living, had recognizable differences [29]. In a recent study with a subset of the subjects described here, it was shown that all participants were comparably able to perform anticipatory grip strength control regardless of group membership [18]. It can therefore be assumed that the motor requirements of the tapping task were not sufficient to reliably separate between the groups.

In addition to the experimental condition, the neurological status of the participants must also be considered. All participants reported no neurological deficits (an exclusion criterion; see Methods). However, individuals may have had different degrees of age-related degeneration and in different relevant areas of the CNS (e.g., cortex, spinal cord, basal ganglia, cerebellum). Cortical activity can be measured via resting-state electroencephalography (EEG), usually performed with eyes closed and/or eyes open [30]. It is a measure of tonic brain activity [31] and this spontaneous EEG activity is thought to account for 80% of total brain activity [30,32]. Only a small additional percentage is accounted for by engagement in a task [32]. Thus, resting-state EEG studies describe the functional state of the cortex. A recent study [10] showed that in the subjects studied, cortical activity in

resting-state EEG did not differ between groups. Therefore, group differences in tapping parameters cannot be derived from cortical differences between the groups.

For Parkinson's disease, as an example disease of the basal ganglia, it is known that patients show a faster tapping rhythm than healthy subjects [14]. In contrast, our data show that in the self-selected pace condition pMCI and MCI tapped significantly slower than CHI, and in the fast pace condition there was a significant difference between CHI and MCI for this parameter (Table 2). This is consistent with behavior shown in MCI and Alzheimer's patients [12–14]. Therefore, it is reasonable to conclude that the group differences are not due to an influence of the basal ganglia.

The influence of spinal cord control can be derived from the parameter time-to-plateau (Figure 1). Tapping can be described as a diadochokinetic task. It consists of finger flexion followed by finger extension. The time to stop flexion and start extension is controlled by proprioceptive signals. The mean size of the parameter time-to-plateau gives information on the planned movement (the shorter the time to plateau, the faster the movement), and its variability gives information on the participant's proprioceptive control at the spinal cord level (the smaller the better). The parameter time-to-plateau was determined by the current speed of the movement (Figure 1, flexion) and the sensory feedback at touch, which could lead to deceleration of the movement and onset of the reverse movement (Figure 1, extension). If sensory feedback is insufficient, the stopping of the movement is delayed and much more variable. Thus, group differences can be inferred from the variability and mean magnitude of this parameter. For the self-selected pace condition, no group differences existed in either mean magnitude (Table 2 time-to-plateau_median) or variability (Table 2 time-to-plateau_iqr). In the fast pace condition, only one significant difference was found between CHI and pMCI or MCI for the parameter time-to-plateau_median. Therefore, in the fast pace condition, CHI performed a significantly faster motor program than the other groups. Because the variability of the time-to-plateau parameter was the same between groups in both pace conditions, it can be assumed that the degree of degeneration at the spinal cord level can be considered comparable between the groups.

The cerebellum is known to be generally important for coordinating motor performance, such as diadochokinesis, and it is additionally important for associating sensory information with movements as well as for adapting movements [33]. Some studies have highlighted the cerebellum's importance in the context of participants' associative learning of grip forces [34,35]. For example, in precision finger tasks such as the raspberry task [36,37], half of the young participants showed a conditioned change in force at just the second presentation of the conditioning stimulus [35] and personal observation of DFK. In contrast, cerebellar patients were significantly worse than control subjects at learning the necessary association [34]. For a successful association between the conditioned stimulus and the motor action, participants needed a well-planned and controlled execution of the task [35]; in cerebellar patients, this execution was impaired [25]. It is therefore possible that restrictions in tapping behavior can be explained not only by cognitive impairments, but also by age-related decline of the cerebellum. This is accompanied by a reduced ability to associate sensory information with the necessary timing of tapping. As they are spatially separated from the regions related to manual motor performance, parts of the cerebellum are also correlated with cognitive performance [38–41]. The anterior lobe and the top of the superior posterior lobe are correlated with motor skills, and the bottom parts of the posterior superior lobe and the inferior lobe are correlated with cognition [39]. Degeneration of cerebellar regions associated with the somatomotor network is more pronounced than that of regions associated with dorsal attention, ventral attention, or frontoparietal networks [38]. Furthermore, age-related degeneration of the motor cerebellum is comparable to the degeneration found in cerebellar diseases [38]. In contrast, Alzheimer's patients show degeneration of the cognitive part of the cerebellum without concomitant increased degeneration in the motor cerebellum [41]. Notably, the cerebellum is generally considered to be resistant to the neurotoxic effects of soluble amyloid-beta (Aβ), which is helpful in the early stages of AD [42]. However, assuming that a proportion of participants classified as

MCI are in a precursor phase to AD, it is still reasonable to hypothesize that the influence of the cerebellum on tapping behavior should be considered an age-related limitation rather than an effect of the developing disease.

In conclusion, the 30 misclassified participants in the CHI group may have had more degeneration of the motor cerebellum than those correctly classified into the CHI group (n = 49). Indicators for this difference are the values for the tap-cycle (correctly classified CHI: -0.697 ± 0.008; misclassified CHI: -0.422 ± 0.013; mean $\pm$ SEM in log [s]) and tap-duration (correctly classified CHI: -1.825 ± 0.007; misclassified CHI: -1.448 ± 0.016; mean $\pm$ SEM in log [s]) parameters in the self-selected condition. Thus, in this condition, the correctly classified CHI showed a significantly faster tapping rhythm with a shorter tapping duration ($p < 0.005$, Bonferroni corrected, both parameters). Similarly, it can be hypothesized that the 42 misclassified MCI participants had less degeneration of the cerebellum than the correctly classified MCI patients (n = 23). This can be seen from the values for the tap-cycle (correctly classified MCI: $-1{,}289 \pm 0.012$; misclassified MCI: -1.407 ± 0.005; mean $\pm$ SEM in log [s]) and tap-duration (correctly classified MCI: -2.017 ± 0.012; misclassified MCI: -2.213 ± 0.004; mean $\pm$ SEM in log [s]) parameters in the fast pace condition. In this condition, the correctly classified MCI showed a slower tap rhythm ($p < 0.1$, Bonferroni corrected) with a significantly longer tap duration ($p < 0.004$, Bonferroni corrected).

Overall, when investigating whether cognitive state can be assessed based on simple finger movements (such as tapping), one must also consider the possible degeneration of relevant motor systems (e.g., the cerebellum). To establish tapping as a good classifier, researchers need to perform additional motor tests to specifically determine the degeneration of the aforementioned areas and adequately assess their impact on tapping behavior.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jpm12020286/s1, Supplementary Table S1: Mean values of the tapping parameter for both conditions of each group (retransformed into physical dimensions). Supplementary Table S2: Logarithmized tapping parameter of female participants for both conditions and each group. Supplementary Table S3: Logarithmized tapping parameter of male participants for both conditions and each group. Supplementary Figure S1: Histograms of probability densities of LDA values of correctly classified and misclassified participants using only parameters that are significant for the effect group.

Author Contributions: S.F.: data collection; K.M.: project administration; J.R.: data revision, conceptualization of analysis; C.V.-R.: project conceptualization and administration, funding acquisition; S.F., K.M., J.R., C.V.-R. and D.F.K.: writing, review and editing; D.F.K., conceptualization, performance of the analysis, and writing of the first version of the manuscript. All authors contributed to manuscript revision and read and approved the submitted version. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the European Social Fund for Germany, the Sächsische Aufbaubank-Förderbank (SAB) of the Free State of Saxony (Grant No. 100310502), and Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under DFG Project-ID 416228727-SFB 1410). This funding source had no role in the study design, in the collection, analysis, and interpretation of data, or in the writing of the report. We acknowledge support from the Open Access Publication Fund of the University of Münster.

Institutional Review Board Statement: The studies involving human participants were reviewed and approved by Ethics Committee of the Chemnitz University of Technology, Faculty of Behavioral and Social Sciences (number V-232-17-KM-SENDA-07112017 approved on 19 December 2017).

Informed Consent Statement: The patients/participants provided written informed consent to participate in this study.

Data Availability Statement: The datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Acknowledgments: The authors would like to thank all the participants for their contribution. Further thanks go to Brennecka from the Scientific Editorial Service of the University of Münster for proofreading the manuscript. We acknowledge support from the Open Access Publication Fund of the University of Muenster.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Müller, K.; Fröhlich, S.; Germano, A.M.C.; Kondragunta, J.; Agoitia Hurtado, M.F.d.C.; Rudisch, J.; Schmidt, D.; Hirtz, G.; Stollmann, P.; Voelcker-Rehage, C. Sensor-based systems for early detection of dementia (SENDA): A study protocol for a prospective cohort sequential study. *BMC Neurol.* **2020**, *20*, 84. [CrossRef] [PubMed]
2. Deutsche Alzheimer Gesellschaft e.V. Informationsblatt 1 Die Häufigkeit von Demenzerkrankungen. 2018. Available online: https://www.deutsche-alzheimer.de/fileadmin/alz/pdf/factsheets/infoblatt1_haeufigkeit_demenzerkrankungen_dalzg.pdf (accessed on 25 October 2021).
3. Lin, P.-J.; Neumann, P.J. The economics of mild cognitive impairment. *Alzheimers Dement.* **2013**, *9*, 58–62. [CrossRef]
4. Frank, L.; Lenderking, W.R.; Howard, K.; Cantillon, M. Patient self-report for evaluating mild cognitive impairment and prodromal Alzheimer's disease. *Alzheimers Res. Ther.* **2011**, *3*, 12. [CrossRef] [PubMed]
5. Vogel, A.; Stokholm, J.; Gade, A.; Andersen, B.B.; Hejl, A.M.; Waldemar, G. Awareness of Deficits in Mild Cognitive Impairment and Alzheimer's Disease: Do MCI Patients Have Impaired Insight? *Dement. Geriatr. Cogn. Disord.* **2004**, *17*, 181–187. [CrossRef] [PubMed]
6. Farias, S.T.; Mungas, D.; Jagust, W. Degree of discrepancy between self and other-reported everyday functioning by cognitive status: Dementia, mild cognitive impairment, and healthy elders. *Int. J. Geriatr. Psychiatry* **2005**, *20*, 827–834. [CrossRef]
7. Argüelles, S.; Loewenstein, D.A.; Eisdorfer, C.; Argüelles, T. Caregivers' Judgments of the Functional Abilities of the Alzheimer's Disease Patient: Impact of Caregivers' Depression and Perceived Burden. *J. Geriatr. Psychiatry Neurol.* **2001**, *14*, 91–98. [CrossRef]
8. Nasreddine, Z.S.; Phillips, N.A.; Bédirian, V.; Charbonneau, S.; Whitehead, V.; Collin, I.; Cummings, J.L.; Chertkow, H. The Montreal Cognitive Assessment, MoCA: A Brief Screening Tool For Mild Cognitive Impairment. *J. Am. Geriatr. Soc.* **2005**, *53*, 695–699. [CrossRef]
9. Moms, J.C.; Heyman, A.; Mohs, R.C.; Hughes, J.P.; van Belle, G.; Fillenbaum, G.; Mellits, E.D.; Clark, C. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part I. Clinical and neuropsychological assesment of Alzheimer's disease. *Neurology* **1989**, *39*, 1159. [CrossRef]
10. Fröhlich, S.; Kutz, D.F.; Müller, K.; Voelcker-Rehage, C. Characteristics of Resting State EEG Power in 80+-Year-Olds of Different Cognitive Status. *Front. Aging Neurosci.* **2021**, *13*, 469. [CrossRef]
11. Fröhlich, S.; Müller, K.; Voelcker-Rehage, C. Normative Data for the CERAD-NP for Healthy High-Agers (80–84 years) and Effects of Age-Typical Visual Impairment and Hearing Loss. *J. Int. Neuropsychol. Soc.* **2021**, *in press*. [CrossRef]
12. Rabinowitz, I.; Lavner, Y. Association between Finger Tapping, Attention, Memory, and Cognitive Diagnosis in Elderly Patients. *Percept. Mot. Ski.* **2014**, *119*, 259–278. [CrossRef] [PubMed]
13. Bangert, A.S.; Balota, D.A. Keep Up the Pace: Declines in Simple Repetitive Timing Differentiate Healthy Aging from the Earliest Stages of Alzheimer's Disease. *J. Int. Neuropsychol. Soc.* **2012**, *18*, 1052–1063. [CrossRef] [PubMed]
14. Roalf, D.R.; Rupert, P.; Mechanic-Hamilton, D.; Brennan, L.; Duda, J.E.; Weintraub, D.; Trojanowski, J.Q.; Wolk, D.; Moberg, P.J. Quantitative assessment of finger tapping characteristics in mild cognitive impairment, Alzheimer's disease, and Parkinson's disease. *J. Neurol.* **2018**, *265*, 1365–1375. [CrossRef]
15. Cole, K.J.; Rotella, D.L.; Harper, J.G. Mechanisms for Age-Related Changes of Fingertip Forces during Precision Gripping and Lifting in Adults. *J. Neurosci.* **1999**, *19*, 3238–3247. [CrossRef] [PubMed]
16. Johansson, R.S.; Cole, K.J. Sensory-motor coordination during grasping and manipulative actions. *Curr. Opin. Neurobiol.* **1992**, *2*, 815–823. [CrossRef]
17. Johansson, R.S.; Cole, K.J. Grasp stability during manipulative actions. *Can. J. Physiol. Pharmacol.* **1994**, *72*, 511–524. [CrossRef] [PubMed]
18. Rudisch, J.; Müller, K.; Kutz, D.F.; Brich, L.; Sleimen-Malkoun, R.; Voelcker-Rehage, C. How Age, Cognitive Function and Gender Affect Bimanual Force Control. *Front. Physiol.* **2020**, *11*, 245. [CrossRef]
19. Breton, A.; Casey, D.; Arnaoutoglou, N.A. Cognitive tests for the detection of mild cognitive impairment (MCI), the prodromal stage of dementia: Meta-analysis of diagnostic accuracy studies. *Int. J. Geriatr. Psychiatry* **2019**, *34*, 233–242. [CrossRef]
20. Albert, M.S.; DeKosky, S.T.; Dickson, D.; Dubois, B.; Feldman, H.H.; Fox, N.C.; Gamst, A.; Holtzman, D.M.; Jagust, W.J.; Petersen, R.C.; et al. The diagnosis of mild cognitive impairment due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* **2011**, *7*, 270–279. [CrossRef]
21. Petersen, R.C.; Caracciolo, B.; Brayne, C.; Gauthier, S.; Jelic, V.; Fratiglioni, L. Mild cognitive impairment: A concept in evolution. *J. Intern. Med.* **2014**, *275*, 214–228. [CrossRef]

22. Petersen, R.C.; Lopez, O.; Armstrong, M.J.; Getchius, T.S.D.; Ganguli, M.; Gloss, D.; Gronseth, G.S.; Marson, D.; Pringsheim, T.; Day, G.S.; et al. Practice guideline update summary: Mild cognitive impairment. *Rep. Guidel. Dev. Dissem. Implement. Subcomm. Am. Acad. Neurol.* **2018**, *90*, 126–135. [CrossRef] [PubMed]
23. Yan, J.H. Effects of Aging on Linear and Curvilinear Aiming Arm Movements. *Exp. Aging Res.* **2000**, *26*, 393–407. [CrossRef] [PubMed]
24. R-CoreTeam. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
25. Meindl, T.; Schmid, B.C.; Timmann, D.; Kolb, F.P.; Kutz, D.F. Contribution of the Cerebellum to the Coupling of Grip Force and Pull Force During an Isometric Precision Grip Task. *Cerebellum* **2012**, *11*, 167–180. [CrossRef] [PubMed]
26. Lawrence, M.A. *ez: Easy Analysis and Visualization of Factorial Experiments*, R package version 4.4-0; R Core Team: Vienna, Austria, 2016.
27. Bakeman, R. Recommended effect size statistics for repeated measures designs. *Behav. Res. Methods* **2005**, *37*, 379–384. [CrossRef]
28. Venables, W.N.; Ripley, B.D. *Modern Applied Statistics with S*, 4th ed.; Springer: New York, BY, USA, 2002; p. 498.
29. Diermayr, G.; McIsaac, T.L.; Gordon, A.M. Finger Force Coordination Underlying Object Manipulation in the Elderly—A Mini-Review. *Gerontology* **2011**, *57*, 217–227. [CrossRef]
30. Cave, A.E.; Barry, R.J. Sex differences in resting EEG in healthy young adults. *Int. J. Psychophysiol.* **2021**, *161*, 35–43. [CrossRef]
31. Klimesch, W. EEG alpha and theta oscillations reflect cognitive and memory performance: A review and analysis. *Brain Res. Rev.* **1999**, *29*, 169–195. [CrossRef]
32. Shulman, R.G.; Rothman, D.L.; Behar, K.L.; Hyder, F. Energetic basis of brain activity: Implications for neuroimaging. *Trends Neurosci.* **2004**, *27*, 489–495. [CrossRef]
33. Manto, M.; Bower, J.M.; Conforto, A.B.; Delgado-Garcia, J.M.; da Guarda, S.N.; Gerwig, M.; Habas, C.; Hagura, N.; Ivry, R.B.; Marien, P.; et al. Consensus paper: Roles of the cerebellum in motor control–the diversity of ideas on cerebellar involvement in movement. *Cerebellum* **2012**, *11*, 457–487. [CrossRef]
34. Kutz, D.F.; Schmid, B.C.; Meindl, T.; Timmann, D.; Kolb, F.P. Contribution of the Cerebellum in Cue-Dependent Force Changes During an Isometric Precision Grip Task. *Cerebellum* **2016**, *15*, 439–450. [CrossRef]
35. Schmid, B.C.; Meindl, T.; Timmann, D.; Kolb, F.P.; Kutz, D.F. Motor learning of cue-dependent pull-force changes during an isometric precision grip task. *Hum. Mov. Sci.* **2015**, *39*, 138–153. [CrossRef] [PubMed]
36. Kutz, D.F.; Wölfel, A.; Meindl, T.; Timmann, D.; Kolb, F.P. Spatio-temporal human grip force analysis via sensor arrays. *Sensors* **2009**, *9*, 6330–6345. [CrossRef] [PubMed]
37. Kutz, D.F.; Wölfel, A.; Timmann, D.; Kolb, F.P. Dynamic torque during a precision grip task comparable to picking a raspberry. *J. Neurosci. Methods* **2009**, *177*, 80–86. [CrossRef] [PubMed]
38. Hulst, T.; van der Geest, J.N.; Thürling, M.; Goericke, S.; Frens, M.A.; Timmann, D.; Donchin, O. Ageing shows a pattern of cerebellar degeneration analogous, but not equal, to that in patients suffering from cerebellar degenerative disease. *NeuroImage* **2015**, *116*, 196–206. [CrossRef] [PubMed]
39. Koppelmans, V.; Hoogendam, Y.Y.; Hirsiger, S.; Mérillat, S.; Jäncke, L.; Seidler, R.D. Regional cerebellar volumetric correlates of manual motor and cognitive function. *Brain Struct. Funct.* **2017**, *222*, 1929–1944. [CrossRef] [PubMed]
40. Koziol, L.F.; Budding, D.; Andreasen, N.; D'Arrigo, S.; Bulgheroni, S.; Imamizu, H.; Ito, M.; Manto, M.; Marvel, C.; Parker, K.; et al. Consensus Paper: The Cerebellum's Role in Movement and Cognition. *Cerebellum* **2014**, *13*, 151–177. [CrossRef]
41. Gellersen, H.M.; Guell, X.; Sami, S. Differential vulnerability of the cerebellum in healthy ageing and Alzheimer's disease. *NeuroImage Clin.* **2021**, *30*, 102605. [CrossRef]
42. Liang, K.J.; Carlson, E.S. Resistance, vulnerability and resilience: A review of the cognitive cerebellum in aging and neurodegenerative diseases. *Neurobiol. Learn. Mem.* **2020**, *170*, 15. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

Development of a Nomogram for Predicting Depression in the Elderly Using Patient Health Questionnaire-9 among a Nationwide Sample of Korean Elderly

Haewon Byeon

Department of Medical Big Data, College of AI Convergence, Inje University, Gimhae-si 50834, Gyeonsangnam-do, Korea; bhwpuma@naver.com; Tel.: +82-10-7404-6969

Abstract: This cross-sectional study developed a nomogram that could allow medical professionals in the primary care setting to easily and visually confirm high-risk groups of depression. This study analyzed 4011 elderly people (≥60 years old) who completed a health survey, blood pressure, physical measurement, blood test, and a standardized depression screening test. A major depressive disorder was measured using the Korean version of the Patient Health Questionnaire (PHQ-9). This study built a model for predicting major depressive disorders using logistic regression analysis to understand the relationship of each variable with major depressive disorders. In the result, the prevalence of depression measured by PHQ-9 was 6.8%. The results of multiple logistic regression analysis revealed that the major depressive disorder of the elderly living alone was significantly ($p < 0.05$) related to monthly mean household income, the mean frequency of having breakfast per week for the past year, moderate-intensity physical activity, subjective level of stress awareness, and subjective health status. The results of this study implied that it would be necessary to continuously monitor these complex risk factors such as household income, skipping breakfast, moderate-intensity physical activity, subjective stress, and subjective health status to prevent depression among older adults living in the community.

Keywords: depression; nomogram; patient health questionnaire; multiple risk factors; epidemiological survey; high-risk group

Citation: Byeon, H. Development of a Nomogram for Predicting Depression in the Elderly Using Patient Health Questionnaire-9 among a Nationwide Sample of Korean Elderly. *J. Pers. Med.* **2021**, *11*, 645. https://doi.org/10.3390/jpm11070645

Academic Editor: Marco Costanzi

Received: 7 June 2021
Accepted: 6 July 2021
Published: 7 July 2021

1. Introduction

The prevalence of geriatric depression has been increasing due to the aging population [1]. The World Health Organization (2017) [2] forecasted that the prevalence of geriatric depression (60 years or older) worldwide has risen from 12% in 2015 to at least 22% in 2050, an almost two-fold increase. In particular, as the prevalence of depression in older adults has greatly increased while the global population is rapidly aging, depression has become a serious health problem for older adults [3]. The WHO [4] reported that the prevalence of depression among the global elderly population (≥60 years) was 12% in 2015. If this trend continues, it is predicted that one out of four older adults people (21.2%) would suffer from depression in 2050 [4]. In particular, it has been reported that the prevalence of major depressive disorders among adults in South Korea is lower than that in the United States and that in Europe, but the prevalence of the major depressive disorders among older adults in South Korea was relatively higher [5]. In a national survey of the elderly 65 years and older in South Korea [6,7], the prevalence of depressive disorders was 33.1–34.8%. Since South Korea is rapidly becoming an aging society, paying attention to the problems of older adults, who account for a majority of depressive disorder occurrences, is unavoidable.

Since the prevalence of depressive disorders is high and they cause various functional disorders, it is important to detect them early. Depressive disorders increase physical diseases and mortality because they worsen the performance of social functions and quality

of life and adversely affect physical and mental health [8]. In addition, since the onset of depressive symptoms is greatly affected by socio-cultural factors and health behaviors, it is necessary to prepare mental health policies optimized for each country. It is critical to conduct studies for predicting depressive disorders using reliable epidemiological data that can represent the population. Nevertheless, it is hard to differentiate depressive symptoms found in older adults from the symptoms due to aging such as a decrease in hormones, so it is not easy to detect high-risk groups of geriatric depression at an early stage and continuously manage them in the community.

Previous studies have reported that gender, age, low education level, social and economic status, undesirable lifestyle habits (e.g., smoking and excessive drinking), marital status, chronic diseases, and psychological stress affect geriatric depression [9–11]. However, the limitations of these previous studies are that (1) they were conducted in a single area or a small group of older adults [11] and (2) most were limited to exploring individual risk factors for depression in older adults [9,10]. Recent studies revealed that health risk behaviors tended to group together [12,13] and 17.6% of Brazilian men [14], 23% of UK men [15], and 15.2% of South Korean men [16] were exposed to at least three health risk behaviors, such as drinking, smoking, and obesity, at the same time. Therefore, the consideration of multiple health risk behaviors is required when developing a model for predicting major depressive disorders in order to present practical data that are necessary to detect high-risk groups at an early stage and prevent these disorders, based on evidence from older adults living in a community.

As far as we know, no study has developed a nomogram for predicting high-risk groups of geriatric depression while considering multiple health risk behaviors, using epidemiological data that can represent older adults living in local communities in South Korea. This study identified risk factors that could influence geriatric disorders among various aspects including physical activities and nutritional factors, preventative factors, sociodemographic factors and depression risk factors (e.g., health risk behaviors) confirmed in previous studies [17–19] by using the Patient Health Questionnaire (PHQ-9) [20,21], a standardized depression screening test widely used for epidemiological surveys globally. This study developed a nomogram that could allow medical professionals in the primary care setting to easily and visually confirm high-risk groups of depression.

2. Materials and Methods

2.1. Data Source

This cross-sectional study is a secondary data analysis study using raw data from the 7th National Health and Nutrition Examination Survey conducted from 2016 to 2018, supervised by the Korea Centers for Disease Control and Prevention under the Ministry of Health and Welfare. The National Health and Nutrition Examination Survey is a set of national statistics supervised by the Ministry of Health and Welfare and the Korea Centers for Disease Control and Prevention and is government-designated (approval number 1702) based on Article 17 of the Statistics Act. It was conducted after receiving written consent from participants and with the approval of the Institutional Bioethics Committee of the Korea Centers for Disease Control and Prevention (No.1041107-201806-HR-011-01). This study used the population living in South Korea and selected survey targets by using the stratified cluster sampling method and the systematic sampling method, based on the 2010 Population and Housing Census data (complete enumeration). The Seventh National Health and Nutrition Examination Survey investigated 24,269 people from 13,248 households in 576 surveyed districts, and the participation rate was 76.7% (n = 18,614). The National Health and Nutrition Examination Survey examines disease morbidity, activity restriction, quality of life, health behavior, and physical activity, and was conducted by interviews and a self-recording method during the survey period. A nutritional survey was performed by having a nutrition surveyor visit the home of the subject in person and conducting a food intake frequency survey using the interview method. This study analyzed 4011 older adults people ($\geq$60 years old) who completed

the health survey, blood pressure, physical measurement and blood tests, and a PHQ-9 (standardized depression screening test) [21].

2.2. Measurement and Definition of Variables

The dependent variable of this study was the prevalence of a major depressive disorder, measured using the Korean version of PHQ-9 [21]. PHQ-9 is a standardized depression screening test developed by Spitzer et al. (1999) [20] to diagnose mental health in primary health care centers. It is composed of nine items corresponding to the diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) for major depressive disorders. The PHQ-9 is a self-report test, and has excellent sensitivity and specificity [22]. Moreover, since it can simply check the severity of a major depressive disorder using only nine items, it has the advantage that it is highly likely to be applied to actual screening in epidemiological investigations as well as in the medical field [22]. The PHQ-9 asks a subject how often he or she has experienced anhedonia, depression, changes in sleep, fatigue, changes in appetite, guilt or worthlessness, decreased concentration, akathisia or feeling down, and suicidal thoughts in the past two weeks. It is evaluated on a four-point scale: "never", "for a few days", "more than one week", and "almost every day". The total score ranges from 0 to 27, and a higher score means more severe depression. The threshold of depression was defined as ten points (depression $\geq$10 points out of 27 points) based on the results of previous studies [23,24]. Choi (2017) [25] reported that the sensitivity and specificity of PHQ-9 were 81.1% and 89.9%, respectively. The reliability of the tool (Cronbach's α) was 0.89.

The explanatory variables included sociodemographic characteristics, physical characteristics, nutritional characteristics, health behaviors, and health status, referring to previous studies [9–11,17–19]. Sociodemographic characteristics were gender (male/female), age (60–64, 65–69, 70–74, 75–79, or over 80), living with a spouse (yes or no), education level ("elementary school graduation or below", "middle school graduation", "high school graduation", or "college graduation or above"), monthly mean household income (<KRW 1.5 million, $\geq$KRW 1.5 million and <KRW 2 million, $\geq$KRW 2 million and <KRW 3 million, or $\geq$KRW 3 million), and receiving national basic livelihood security (yes or no). Physical characteristics were waist circumference (cm), obesity by body mass index (BMI, kg/m^2) (underweight (<18.5 kg/m^2), normal ($\geq$18.5 kg/m^2 and < 23 kg/m^2), pre-obesity stage ($\geq$23 kg/m^2 and <25 kg/m^2), stage 1 obesity ($\geq$25 kg/m^2 and <30 kg/m^2), stage 2 obesity ($\geq$30 kg/m^2 and <35 kg/m^2), or stage 3 obesity ($\geq$35 kg/m^2)), and subjective body type perception (very thin, slightly skinny, average, slightly obese, or very obese). Nutritional characteristics were the mean frequency of having breakfast per week for the past year (rarely, "1–2 times per week", "3–4 times per week", or "5–7 times per week"), daily n-3 fatty acid intake (g/day), daily n-6 fatty acid intake (g/day), and daily vitamin c intake (mg/day). This study measured the n-3 fatty acid intake, n-6 fatty acid intake, and vitamin c intake by using the 24-h dietary recall method, and the survey data of food intake over one day were converted into a continuous variable and analyzed.

Health status variables were the usual level of stress awareness ("I hardly feel stressed", "I feel stressed a little", "I feel stressed a lot", or "I feel stressed very much"), subjective health status ("bad", "okay", or "good"), hypertension ("normal", "prehypertension", or "hypertension"), diabetes ("normal", "impaired fasting glucose", or "diabetes"), hypercholesterolemia (no or yes), and hypertriglyceridemia (no or yes). Hypertension was defined as systolic blood pressure $\geq$140 mmHg or diastolic blood pressure $\geq$90 mmHg based on the mean value of the second and third measurements among three measurements by medical personnel using a sphygmomanometer. Among those not diagnosed with hypertension, prehypertension was defined as 120 mmHg $\leq$ systolic blood pressure < 140 mmHg and 80 mmHg $\leq$ diastolic blood pressure < 90 mmHg. Hypercholesterolemia was defined as a person currently taking a cholesterol-lowering drug or a person who had a total cholesterol level of 240 mg/dL or more measured while fasting for more than 8 h. Diabetes was defined as a person receiving a hypoglycemic agent/insulin injection after being diagnosed

with diabetes by a doctor, or a person with a fasting blood sugar of 126 mg/dL or higher while fasting for 8 h or more. Impaired fasting glucose was defined as a person with a fasting blood sugar equal to or higher than 100 mg/dL and less than 126 mg/dL. Hypertriglyceridemia was defined as a person with a triglyceride content $\geq$ 200 mg/dL via a blood test while fasting for 12 h or more. Health behaviors were drinking experience (yes or no), intemperance frequency (non-drinkers, once a month or fewer, once a week or fewer, or almost every day), smoking experience (current smokers, ex-smoker or non-smokers), control weight over the past year ("never tried to control weight", "try to lose weight", "try to maintain weight", "try to gain weight"), moderate-intensity physical activity (yes or no), usual hours of sitting per day, usual minutes of sleep per day, and days of walking at least 30 min per week week ("never", "1 day", "2 days", "3 days", "4 days", "5 days" "6 days", or "7 days") using a questionnaire. Physical activity was measured with the Korean version of GPAQ, a standardized Korean version of the Global Physical Activity Questionnaire (GPAQ) developed by the WHO [26].

2.3. Development of Depression Prediction Model

This study built a model for predicting major depressive disorders using logistic regression analysis to understand the relationship (influence) of each variable with major depressive disorders. The variable selection was made using the backward selection method, and this study presented the OR and 95%CI of an unadjusted model that did not adjust confounding factors, and those of an adjusted model that adjusted confounding factors.

This study developed a nomogram based on the developed depression prediction model (final model) so that clinicians could easily interpret the prediction result (prediction probability). The nomogram developed in this study consisted of four elements (Figure 1). First, a point line was presented. The point line is a line placed at the top of the nomogram to indicate a score falling in a risk class. In the case of a logistic nomogram, it consists of 0–100 points.

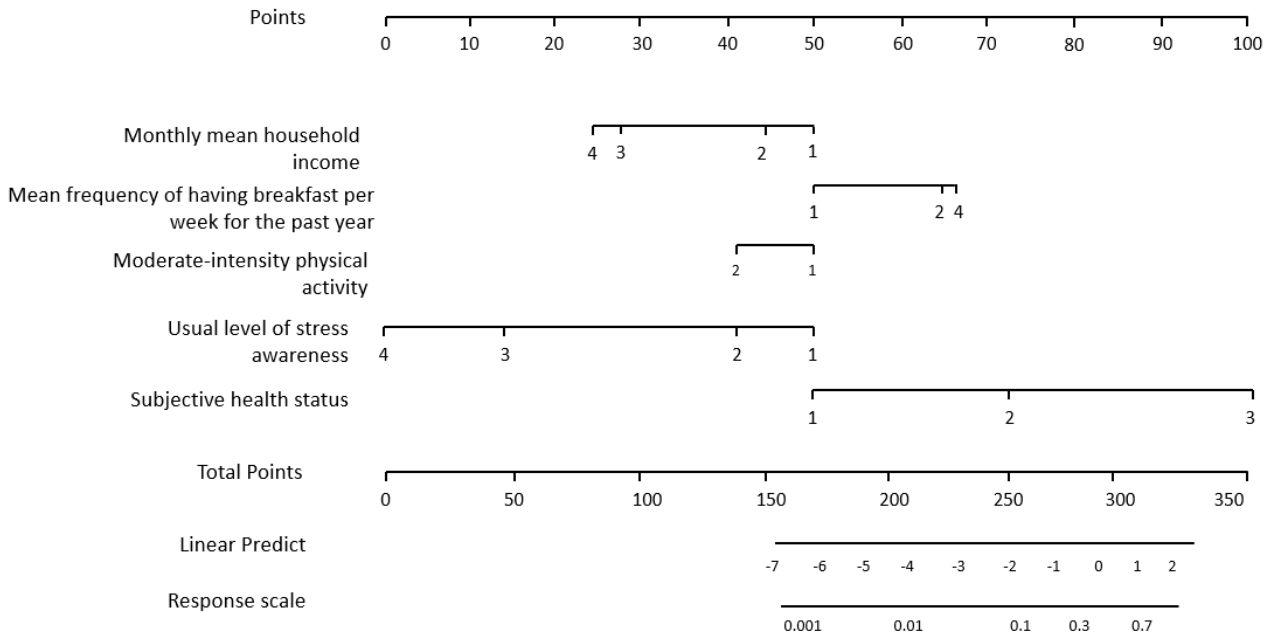

Figure 1. The nomogram for predicting depression in older adults in the community based on multiple risk factors.

Second, a risk factor line was presented. This line indicates the range of a risk factor that affects the occurrence of an event. The number of risk factor lines is equal to the number of risk factors. Third, this study presents a probability line. The probability line is the sum of finally calculated nomogram scores, and it is placed at the bottom of the

nomogram to derive the occurrence probability of a major depressive disorder. The fourth is the total point line, calculated and constructed based on a statistical model.

Monthly mean household income: 1 ≤ KRW 1.5 million, 2 ≥ KRW 1.5 million and <KRW 2 million, 3 ≥ KRW 2 million and < KRW 3 million, 4 ≥ KRW 3 million; Mean frequency of having breakfast per week for the past year: 1 = 5–7 times per week, 2 = 3–4 times per week, Rarely, 3 = 1–2 times per week, 4 = Rarely; Moderate-intensity physical activity: 1 = no, 2 = yes; Usual level of stress awareness: 1 = I feel stressed very much, 2 = I feel stressed a lot, 3 = I feel stressed a little,4 = I hardly feel stressed; Subjective health status:1 = good, 2 = normal, 3 = bad

2.4. Testing the Accuracy of a Nomogram for Predicting Geriatric Depression

Since the sample size of this model was not big enough (n = 4011) to validate the prediction model, this study used 10-fold cross-validation as a way to test the accuracy of the developed geriatric depression prediction nomogram to minimize the risk of overfitting, and presented the area under the curve (AUC), general accuracy, and calibration plot of each model. AUC is that of the receiver operating characteristic (ROC) curve. It is the most commonly used evaluation method in binary classification and is defined via diagnostic accuracy. A value closer to 1 means better diagnostic performance. The calibration plot is a figure for visually confirming the degree of agreement between the predicted probability in the nomogram and the observed probability.

3. Results

3.1. General Characteristics of Older Adults in the South Korean Community

The general characteristics of the 4011 subjects (56.4% were women and 43.6% were men) are presented in Table 1. Many subjects were non-drinkers (57.4%), non-smokers (61.3%), living with a spouse (71.5%), elementary school graduation or below (50.8%), a mean monthly household income less than KRW 1.5 million (42.8%), normal weight (46.3%), without moderate-intensity physical activity (96.4%), hypertension (58.8%), without diabetes (45.5%), without hypercholesterolemia (65.1%), and without hypertriglyceridemia (84.5%). The subjects usually sat 10.7 h per day on average (standard deviation 16.1), and slept 444.9 min per day on average (standard deviation 409.7). The prevalence of depression measured by PHQ-9 was 6.8%. Since only one person (0.1%) was in the stage 3 obesity class, it was merged with "stage 2 obesity" to make "stage 2 obesity or above" and the data were reanalyzed using chi-square and regression analyses.

Table 1. General characteristics of the subjects.

Characteristics	*n* (%)
Gender	
Male	1750 (43.6)
Female	2261 (56.4)
Binge(intemperance) frequency	
Non-drinkers	1265 (57.4)
Once a month or fewer	520 (23.6)
Once a week or fewer	265 (12.0)
Almost every day	154 (7.0)
Smoking experience	
Non-smokers	2445 (61.3)
Current smokers	424 (10.6)
Ex-smoker	1122 (28.1)
Living with a spouse	
Yes	2842 (71.5)
No	1132 (28.5)

Table 1. *Cont.*

Characteristics	*n* (%)
Education level	
Elementary school graduation or below	2036 (50.8)
Middle school graduation	698 (17.4)
High school graduation	793 (19.8)
College graduation or above	479 (12.0)
Monthly mean household income	
<KRW 1.5 million	1710 (42.8)
≥KRW 1.5 million and <KRW 2 million	376 (9.4)
≥KRW 2 million and <KRW 3 million	600 (15.0)
≥KRW 3 million	1305 (32.7)
Obesity by body mass index (BMI, kg/m^2)	
Underweight (<18.5 kg/m^2)	91 (2.3)
Normal (≥18.5 kg/m^2 and < 23 kg/m^2)	1839 (46.3)
Pre-obesity stage (≥23 kg/m^2 and <25 kg/m^2)	1272 (32.0)
Stage 1 obesity (≥25 kg/m^2 and <30 kg/m^2)	676 (17.0)
Stage 2 obesity (≥30 kg/m^2 and <35 kg/m^2)	92 (2.3)
Stage 3 obesity (≥35 kg/m^2)	1 (0.1)
Moderate-intensity physical activity	
Yes	146 (3.6)
No	3864 (96.4)
Hypertension	
Normal	777 (19.4)
Prehypertension	871 (21.8)
Hypertension	2352 (58.8)
Diabetes	
Normal	1675 (45.2)
Impaired fasting glucose	1126 (30.4)
Diabetes	901 (24.3)
Hypercholesterolemia	
No	2410 (65.1)
Yes	1292 (34.9)
Hypertriglyceridemia	
No	2720 (84.5)
Yes	498 (15.5)
Waist circumference (cm)	85.3 ± 8.9
Daily n-3 fatty acid intake (g/day)	1.7 ± 1.9
Daily n-6 fatty acid intake (g/day)	6.9 ± 6.0
Daily vitamin c intake (mg/day)	57.7 ± 61.8
Usual hours of sitting per day	10.7 ± 16.1
Usual minutes of sleep per day	444.9 ± 409.7
Major depressive disorder (PHQ-9)	
No	3738 (93.2)
Yes	273 (6.8)

3.2. Characteristics of Subjects According to the Prevalence of Depression

The characteristics of subjects according to the prevalence of depression are presented in Table 2. The results of the chi-square test revealed that the prevalence of a major depressive disorder was significantly ($p < 0.05$) affected by gender, current smoking status, marital status, education level, monthly mean household income, whether or not national basic livelihood security was received, subjective body type perception, the mean frequency of having breakfast per week for the past year, moderate-intensity physical activity, days of walking at least 30 min per week, the usual level of stress awareness, subjective health status, diabetes, hypertriglyceridemia, n-3 fatty acid intake (g), n-6 fatty acid intake (g), and vitamin C intake (mg).

Table 2. Characteristics of subjects according to the prevalence of depression, n (%).

Variable	Major Depressive Disorder		*p*
	No (*n* = 3738)	Yes (*n* = 237)	
Age			0.198
60–64	1016 (94.7)	57 (5.3)	
65–69	886 (92.8)	69 (7.2)	
70–74	766 (93.2)	56 (6.8)	
75–79	631 (92.3)	53 (7.7)	
80+	439 (92.0)	38 (8.0)	
Gender			<0.001
Male	1674 (95.7)	76 (4.3)	
Female	2064 (91.3)	197 (8.7)	
Binge(intemperance) frequency			0.540
Non-drinkers	1191 (94.2)	74 (5.8)	
Once a month or fewer	497 (95.6)	23 (4.4)	
Once a week or fewer	248 (93.6)	17 (6.4)	
Almost every day	147 (95.5)	7 (4.5)	
Smoking experience			0.001
Non-smokers	2271 (92.9)	174 (7.1)	
Current smokers	382 (90.1)	42 (9.9)	
Ex-smoker	1067 (95.1)	55 (4.9)	
Living with a spouse			<0.001
Yes	2702 (95.1)	140 (4.9)	
No	1007 (89.0)	125 (11.0)	
Education level			<0.001
Elementary school graduation or below	1846 (90.7)	190 (9.3)	
Middle school graduation	656 (94.0)	42 (6.0)	
High school graduation	762 (96.1)	31 (3.9)	
College graduation or above	470 (98.1)	9 (1.9)	
Monthly mean household income			<0.001
<KRW 1.5 million	1516 (88.7)	194 (11.3)	
≥KRW 1.5 million and <KRW 2 million	353 (93.9)	23 (6.1)	
≥KRW 2 million and <KRW 3 million	579 (96.5)	21 (3.5)	
≥KRW 3 million	1271 (94.7)	34 (2.6)	
Whether or not to receive national basic livelihood security			<0.001
No	3445 (94.1)	215 (5.9)	
Yes	292 (83.4)	58 (16.6)	
Obesity by body mass index (BMI, kg/m^2)			0.109
Underweight (<18.5 kg/m^2)	82 (90.1)	9 (9.9)	
Normal (≥18.5 kg/m^2 and < 23 kg/m^2)	1699 (92.4)	140 (7.6)	
Pre-obesity stage (≥23 kg/m^2 and <25 kg/m^2)	1184 (93.1)	88 (6.9)	
Stage 1 obesity (≥25 kg/m^2 and <30 kg/m^2)	644 (95.3)	32 (4.7)	
Stage 2 or 3 obesity (≥30 kg/m^2)	93 (95.7)	4 (4.3)	
Subjective body type perception			<0.001
Very thin	193 (85.0)	34 (15.0)	
Slightly skinny	465 (91.2)	45 (8.8)	
Average	1646 (95.3)	81 (4.7)	
Slightly obese	1155 (93.9)	75 (6.1)	
Very obese	264 (88.0)	36 (12.0)	
Mean frequency of having breakfast per week for the past year			<0.001
5–7 times per week	3091 (94.1)	193 (5.9)	
3–4 times per week	95 (86.4)	15 (13.6)	
1–2 times per week	62 (92.5)	5 (7.5)	
Rarely	99 (82.5)	21 (17.5)	
Control weight over the past year			<0.001
Try to lose weight	1087 (94.4)	64 (5.6)	
Try to maintain weight	691 (95.7)	31 (4.3)	
Try to gain weight	218 (87.2)	32 (12.8)	
Never tried to control weight	1729 (92.3)	144 (7.7)	
Moderate-intensity physical activity			<0.001
Yes	121 (82.9)	25 (17.1)	
No	3616 (93.6)	248 (6.4)	
Days of walking at least 30 min per week			<0.001
Never	904 (88.8)	114 (11.2)	
1 days	217 (93.1)	16 (6.9)	
2 days	325 (96.2)	13 (3.8)	
3 days	415 (92.8)	32 (7.2)	

Table 2. *Cont.*

Variable	Major Depressive Disorder		*p*
	No (*n* = 3738)	Yes (*n* = 237)	
4 days	256 (94.5)	15 (5.5)	
5 days	329 (95.6)	15 (4.4)	
6 days	170 (97.7)	4 (2.3)	
7 days	1107 (94.7)	62 (5.3)	
Usual level of stress awareness			<0.001
I feel stressed very much	111 (66.9)	55 (33.1)	
I feel stressed a lot	469 (81.1)	109 (18.9)	
I feel stressed a little	1970 (95.9)	85 (4.1)	
I hardly feel stressed	1170 (98.2)	22 (1.8)	
Subjective health status			<0.001
Good	899 (99.1)	8 (0.9)	
Okay	1885 (96.8)	63 (3.2)	
Bad	953 (82.5)	202 (17.5)	
Hypertension			0.502
Normal	731 (94.1)	46 (5.9)	
Prehypertension	812 (93.2)	59 (6.8)	
Hypertension	2184 (92.9)	168 (7.1)	
Diabetes			<0.001
Normal	1583 (94.5)	92 (5.5)	
Impaired fasting glucose	1066 (94.7)	60 (5.3)	
Diabetes	811 (90.0)	90 (10.0)	
Hypercholesterolemia			0.014
No	2270 (94.2)	140 (5.8)	
Yes	1190 (92.1)	102 (7.9)	
Hypertriglyceridemia			0.001
No	2558 (94.0)	162 (6.0)	
Yes	448 (90.0)	50 (10.0)	
Waist circumference (cm)	85.4 ± 8.9	84.6 ± 9.0	0.154
Daily n-3 fatty acid intake (g/day)	1.7 ± 1.8	1.3 ± 2.0	0.001
Daily n-6 fatty acid intake (g/day)	7.0 ± 5.9	5.6 ± 6.2	<0.001
Daily vitamin c intake (mg/day)	58.7 ± 62.6	43.2 ± 48.0	<0.001
Usual hours of sitting per day	10.5 ± 15.9	13.3 ± 18.0	0.007
Usual minutes of sleep per day	444.1 ± 393.1	455.9 ± 592.6	0.647

3.3. Development of a Model for Predicting Geriatric Depression in the Community

The final model for predicting geriatric depression in the community is presented in Table 3. The results of univariate logistic regression analysis (unadjusted model) revealed that the major depressive disorder of older adults living alone was significantly ($p < 0.05$) related with monthly mean household income, the mean frequency of having breakfast per week for the past year, moderate-intensity physical activity, the subjective level of stress awareness, and subjective health status. The analysis results of the adjusted model confirmed both risk factors and protective factors of major depressive disorders ($p < 0.05$). The monthly mean household income was a protective factor for depression. Older adults with KRW 2–2.99 million had 32% less risk of depression (OR = 0.68, 95% CI: 0.40–1.14) than older adults with less than KRW 1.5 million, and older adults with KRW 3 million or more had 72% less risk of depression (OR = 0.28, 95% CI: 0.18 to 0.43) than older adults with less than KRW 1.5 million ($p < 0.05$). It was also confirmed that independent risk factors for depression were rarely having breakfast per week for the past year (OR = 2.14, 95% CI: 1.16, 3.95), no moderate-intensity physical activity (OR = 2.05, 95% CI = 1.15, 3.64), very high subjective stress OR = 14.17, 95% CI = 7.71, 26.02), a lot of subjective stress (OR = 8.00, 95% CI = 4.75, 13.46), a little subjective stress (OR = 2.18, 95% CI = 1.30, 3.66), okay subjective health status (OR = 2.65, 95% CI = 1.18, 5.95), and bad subjective health status (OR = 11.21, 95% CI = 5.14, 24.40) ($p < 0.05$).

Table 3. The final model for predicting geriatric depression in the community: odds ratio (OR) and 95% confidence interval (CI).

Variables	Unadjusted Model	*p*	Adjusted Model [1]	*p*
Monthly mean household income				
<KRW 1.5 million (ref)	1.00		1.00	
≥KRW 1.5 million and <KRW 2 million	0.50 (0.32, 0.79)	0.003	0.68 (0.40, 1.14)	0.146
≥KRW 2 million and <KRW 3 million	0.28 (0.17, 0.44)	<0.001	0.30 (0.17, 0.53)	<0.001
≥KRW 3 million	0.20 (0.14, 0.30)	<0.001	0.28 (0.18, 0.43)	<0.001
Mean frequency of having breakfast per week for the past year				
5–7 times per week (ref)	1.00		1.00	
3–4 times per week	2.52 (1.43, 4.44)	0.001	1.87 (0.94, 3.71)	0.071
1–2 times per week	1.29 (0.51, 3.25)	0.587	0.92 (0.32, 2.63)	0.878
Rarely	3.39 (2.07, 5.56)	<0.001	2.14 (1.16, 3.95)	0.015
Moderate-intensity physical activity				
Yes (ref)	1.00		1.00	
No	3.01 (1.92, 4.72)	<0.001	2.05 (1.15, 3.64)	0.014
Usual level of stress awareness				
I hardly feel stressed (ref)	1.00		1.00	
I feel stressed very much	26.35 (15.48, 44.83)	<0.001	14.17 (7.71, 26.02)	<0.001
I feel stressed a lot	12.36 (7.72, 19.78)	<0.001	8.00 (4.75, 13.46)	<0.001
I feel stressed a little	2.29 (1.42, 3.68)	0.001	2.18 (1.30, 3.66)	0.003
Subjective health status				
Good (ref)	1.00		1.00	
Okay	3.75 (1.79, 7.87)	<0.001	2.65 (1.18, 5.95)	0.018
Bad	23.81 (11.68, 48.56)	<0.001	11.21 (5.14, 24.40)	<0.001

[1] Adjusted for monthly mean household income, mean frequency of having breakfast per week for the past year, moderate-intensity physical activity, usual level of stress awareness, and subjective health status.

3.4. Development and Validation of a Nomogram for Predicting Depression of Older Adults in the Community

The nomogram for predicting depression in older adults in the community based on multiple risk factors is presented in Figure 1. Subjective stress awareness had the greatest influence among the risk factors for depression for older adults in the community. The older adults who responded that they felt stressed very much had the highest risk of a major depressive disorder. For example, in this depression prediction nomogram (Figure 2), it was predicted that the depression risk probability of older adults who responded that their mean monthly household income was less than KRW 1.5 million, mean frequency of having breakfast per week for the past year was 5–7 times a week, did not do moderate-intensity physical activity, and hardly felt stressed, was 1.6%.

The developed nomogram for predicting depression of older adults was validated by using AUC, accuracy, and calibration plots. The AUC of the developed nomogram for predicting depression in older adults is presented in Figure 3. The results of 10-fold cross validation showed that the AUC and general accuracy of the nomogram were 0.91 and 0.96, respectively. This study compared predicted probability and observed probability using the calibration plot and the chi-square test for the group with depression and the group without depression (Figure 4) to find that there was no significant difference between predicted probability and observed probability ($p = 0.891$).

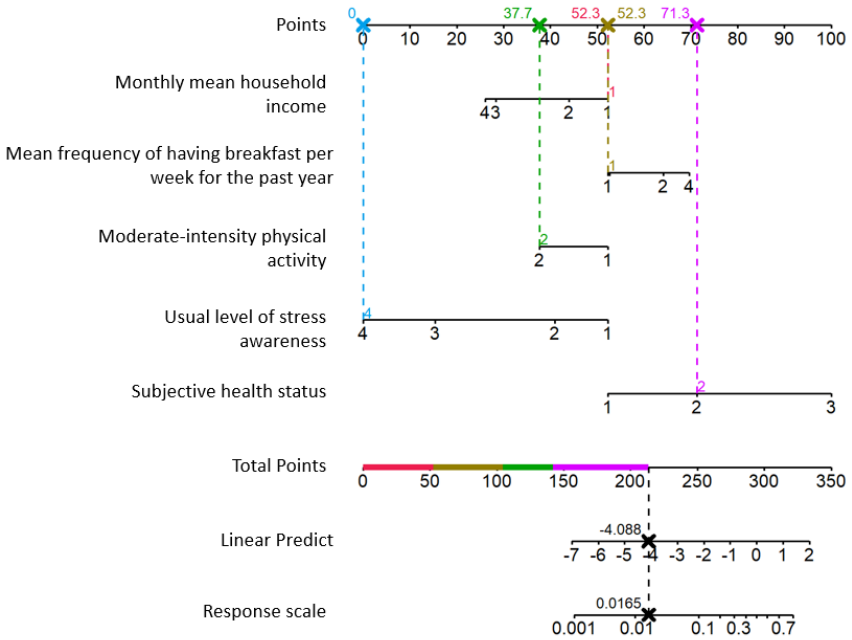

Figure 2. Application Example of Depression Prediction Nomogram for Older adults Living Alone: older adults who responded that mean monthly household income was less than KRW 1.5 million, mean frequency of having breakfast per week for the past year was 5–7 times a week, did not do moderate-intensity physical activity, and hardly felt stressed.

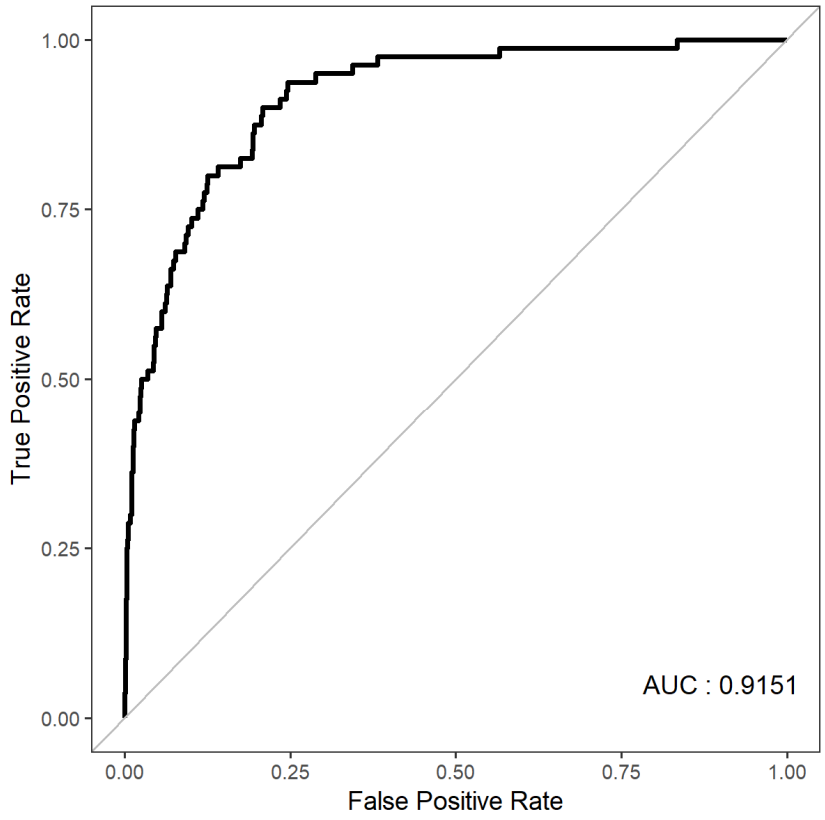

Figure 3. The AUC of the developed nomogram for predicting depression in older adults.AUC:the area under the curve.

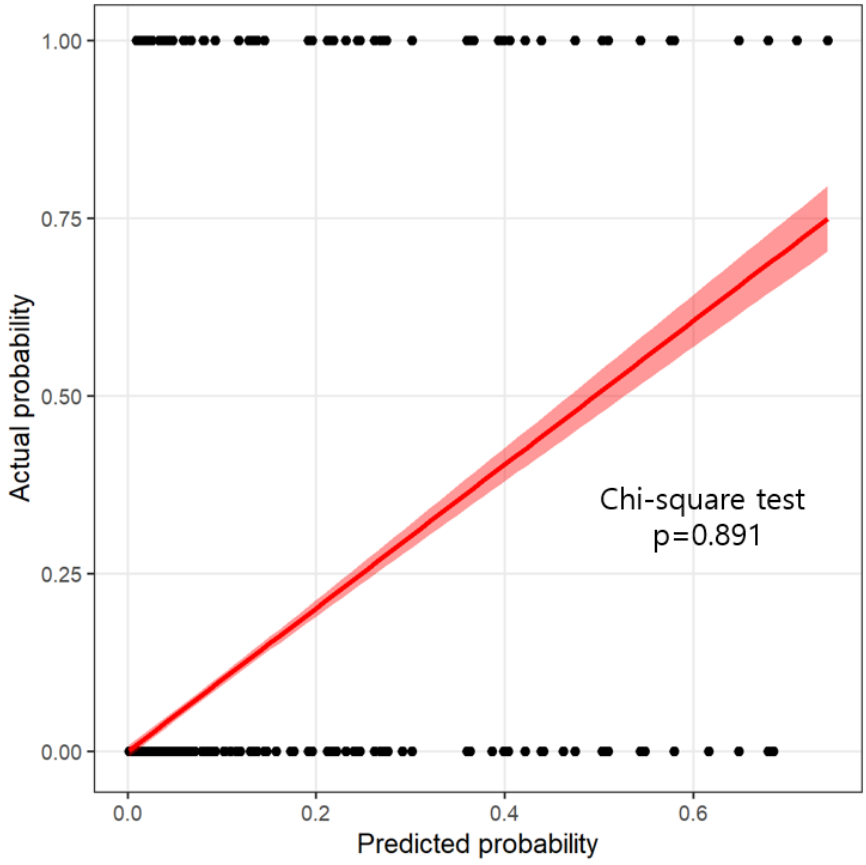

Figure 4. Calibration plot for test data: *X*-axis represents the predicted probability of major depressive disorder; *Y*-axis represents the actual major depressive disorder. An ideal (perfectly accurate) nomogram model would result in a plot in which the observed and predicted probabilities for given groups fall along the 45-degree line.

4. Discussion

This epidemiological study identified factors related to major depressive disorders in older adults in the community using PHQ-9. The results showed that a mean monthly household income of KRW 1.3 million won or more was an independent protective factor for depression. Skipping breakfast, absence of moderate-intensity physical activity, subjective stress, and subjective health status were independent risk factors for depression. It has been generally reported that a lower frequency of physical activity [27], lower socioeconomic status [28,29], higher stress [30], and poorer subjective health status [31] increase the prevalence of depression in the older adult population. Numerous previous studies [23,28,32] have suggested that regular physical activity was a major health promotion habit that can be critical in the prevention and treatment of depression. These studies have shown that physical activity can reduce the depression of individuals with diabetes, chronic stroke, or cancer as well as depression in healthy adults [28], that physically active older adults have less depression than physically inactive older adults [23], and that physical activity is effective in treating depression, preventing physiological side effects, and reducing the use of antidepressants [32].

Similar to the results of this study, Kim (2020) [33] evaluated 1447 older adults in South Korea and reported that the depression risk for older adults with a mean monthly household income of KRW 1.99 million or less was 5.4 times higher than that for older adults with a mean monthly income of KRW 4 million or more. The result agreed with the result of this study showing that a mean monthly household income of KRW 1.3 million or more was an independent protective factor against depression (older adults with KRW 1.3 million or more had a lower risk of depression than older adults with KRW 1.3 million

or less). Regarding the relationship between income level and depression, Kang et al. (2008) [34] explained that income level had an overall effect on health status, including depression, because a low income level affected access to health care services, as an index reflecting an individual's material status or resources to cope with a crisis.

Previous studies [23,28,32,33] that explored the risk factors of depression mainly identified individual risk factors for depression in the senile stage using regression analysis. Therefore, they were limited in understanding the multiple risk factors. This epidemiological study developed a nomogram to identify the multiple risk factors of depression in older adults living alone and predicted that older adults with an income level of KRW 1.3 million or less, skipping breakfast every day, no moderate-intensity physical activity, subjective perception of a lot of stress, and poor subjective health status had 85% depression risk, a very high risk. Therefore, detecting depression in this high risk group is required, among those who show these multiple risk factors at the same time, and continuous monitoring of this group is needed. Furthermore, the development of a bespoke prediction modeling is needed that can screen this high depression risk group early, including vulnerable groups such as older adults with low income, based on the results of this study.

Another important finding was that self-recognized stress and subjective health status were independent risk factors for depression in old age. As a person gets old, he or she experiences psychological pressure while going through difficult changes such as retirement from work, separation from children, the onset of various chronic diseases accompanied by physical weakness, and a sense of loss due to the death of close people (e.g., spouse, family, and friends) [35]. When the elderly eventually cannot stand the level of psychological pressure, they feel stressed. It has been reported that stress-related hormones reduce the number of neurotransmitters decreasing neurogenesis in the dentate nucleus of the hippocampus, which results in depression [36]. In particular, older adults tend to first complain of stress symptoms, physical symptoms, health anxiety, difficulty in concentration, and memory impairment, rather than directly complaining of depressive symptoms [37]. If these subjective symptoms complained of by older adults in the high depression risk group are neglected, their depressive symptoms may worsen and this can lead to suicide attempts in extreme cases [38]. Therefore, family members or neighbors of older adults need to continuously communicate with them and listen to the stress symptoms and physical symptoms that the older adults complain about in order to prevent depression. Moreover, when older adults complain of stress or health problems, it is necessary to bring them to a primary healthcare institution for screening.

The limitations of this study are as follows. First, it could not identify the detailed severity of depression or types of depression because it analyzed the prevalence of depression among older adults in the community based on the depression screening test mainly used in epidemiological investigations. Future studies are required to classify the types of depression into minor depressive disorder, subsyndromal depression, and various depressive symptoms using a medical diagnosis, and to explore risk factors according to depression type based on the results of this study. Second, since the food intake frequency survey used the 24-h dietary recall method, there was a possibility of a recall bias. Third, geriatric depression can be affected by social networks such as family and friends, but social networks were not considered. Therefore, future studies are needed to identify risk factors for depression, such as social networks and psychological factors. Fourth, Since the nomogram in this study was developed for the Korean elderly, there is a limit to its application to other cultures or countries. Fifth, since this study is a cross-sectional study, the results cannot be interpreted as a causal relationship, even if risk factors for depression are identified. Additional longitudinal studies are required to prove the causality of the risk factors for depression in older adults in the community found in this study.

5. Conclusions

The results of this study implied that it would be necessary to continuously monitor complex risk factors such as household income, skipping breakfast, moderate-intensity

physical activity, subjective stress, and subjective health status to prevent depression in older adults living in the community. Furthermore, the establishment of customized prevention policies is needed that can identify high-risk groups of geriatric depression early and continuously manage them.

Funding: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1D1A1B07041091, NRF-2021S1A5A8062526).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of National Biobank of Korea and Korean Centers for Disease Control and Prevention (protocol code KBN-2019-1327; KBN-2019-005 and date: 1 February 2019).

Informed Consent Statement: All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of the Korea Centers for Disease Control and Prevention (No.1041107-201806-HR-011-01).

Data Availability Statement: Restrictions apply to the availability of these data. Data was obtained from National Health and Nutrition Examination Survey and are available [from the National Health and Nutrition Examination Survey/https://data.go.kr/en/data/15076556/fileData.do (accessed on 1 July 2021) with the permission of Korea Centers for Disease Control and Prevention.

Acknowledgments: This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2018R1D1A1B07041091, NRF-2021S1A5A8062526).

Conflicts of Interest: The author declares no conflict of interest.

References

1. Park, J.H.; Lee, J.J.; Lee, S.B.; Huh, Y.; Choi, E.A.; Youn, J.C.; Jhoo, J.H.; Kim, J.S.; Woo, J.I.; Kim, K.W. Prevalence of major depressive disorder and minor depressive disorder in an elderly Korean population: Results from the Korean Longitudinal Study on Health and Aging (KLoSHA). *J. Affect. Disord.* **2010**, *125*, 234–240. [CrossRef]
2. World Health Organization. *Mental Health of Older Adults [Internet]*; World Health Organization: Geneva, Switzerland, 2017. Available online: https://www.who.int/news-room/fact-sheets/detail/mental-health-of-older-adults (accessed on 27 June 2021).
3. Sjöberg, L.; Karlsson, B.; Atti, A.R.; Skoog, I.; Fratiglioni, L.; Wang, H.X. Prevalence of depression: Comparisons of different depression definitions in population-based samples of older adults. *J. Affect. Disord.* **2017**, *221*, 123–131. [CrossRef]
4. World Health Organization. *Depression and Other Common Mental Disorders: Global Health Estimates [Internet]*; World Health Organization: Geneva, Switzerland, 2017. Available online: https://apps.who.int/iris/handle/10665/254610 (accessed on 27 June 2021).
5. Park, J.H.; Kim, K.W. A review of the epidemiology of depression in Korea. *J. Korean Med. Assoc.* **2011**, *54*, 362–369. [CrossRef]
6. Jeong, K.H.; Oh, Y.H.; Kang, E.N.; Kim, K.R.; Lee, Y.K.; Oh, M.A.; Hwang, N.H.; Kim, S.J.; Lee, S.H.; Lee, S.K.; et al. *The Survey on the Actual Conditions of Older Person in Korea*; Ministry of Health and Welfare: Sejong, Korea, 2017.
7. Kim, M.I.; Eo, Y.; Kim, S.E. A study of depression in the elderly by individual and community effects. *Health Soc. Welf. Rev.* **2019**, *39*, 192–221. [CrossRef]
8. Machado, M.O.; Veronese, N.; Sanches, M.; Stubbs, B.; Koyanagi, A.; Thompson, T.; Tzoulaki, I.; Solmi, M.; Vancampfort, D.; Schuch, F.B.; et al. The association of depression and all-cause and cause-specific mortality: An umbrella review of systematic reviews and meta-analyses. *BMC Med.* **2018**, *20*, 112. [CrossRef] [PubMed]
9. Joshi, S.; Mooney, S.J.; Kennedy, G.J.; Benjamin, E.O.; Ompad, D.; Rundle, A.G.; Bread, J.R.; Cerdá, M. Beyond METs: Types of physical activity and depression among older adults. *Age Ageing* **2016**, *45*, 103–109. [CrossRef] [PubMed]
10. Barnett, A.; Zhang, C.J.P.; Johnston, J.M.; Cerin, E. Relationships between the neighborhood environment and depression in older adults: A systematic review and meta-analysis. *Int. Psychogeriatr.* **2018**, *30*, 1153–1176. [CrossRef] [PubMed]
11. Nael, K.; Samer, R.; Kenneth, D.; Wasim, M. Gender, depression and physical impairment; an epidemiologic perspective from Aleppo, Syria. *Soc. Psychiatry Psychiatr. Epidemiol.* **2010**, *45*, 595–602. [CrossRef]
12. World Health Organization. *Global Status Report on Noncommunicable Diseases 2010*; World Health Organization: Geneva, Switzerland, 2019.
13. Chou, K.L. The prevalence and clustering of four major lifestyle risk factors in Hong Kong Chinese older adults. *J. Aging Health* **2008**, *20*, 788–803. [CrossRef]
14. Silva, D.A.; Peres, K.G.; Boing, A.F.; González-Chica, D.A.; Peres, M.A. Clustering of risk behaviors for chronic noncommunicable diseases: A population-based study in southern Brazil. *Prev. Med.* **2013**, *56*, 20–24. [CrossRef]

15. Zwolinsky, S.; Raine, G.; Robertson, S. Prevalence, co-occurrence and clustering of lifestyle risk factors among UK men. *J. Men Health* **2016**, *12*, 15–24.
16. Kang, K.W.; Sung, J.H.; Kim, C.Y. High risk groups in health behavior defined by clustering of smoking, alcohol, and exercise habits: National Heath and Nutrition Examination Survey. *J. Prev. Med. Public Health* **2010**, *43*, 73–83. [CrossRef]
17. Girgus, J.S.; Yang, K.; Ferri, C.V. The gender difference in depression: Are elderly women at greater risk for depression than elderly men? *Geriatrics* **2017**, *2*, 35. [CrossRef]
18. Fiske, A.; Wetherell, J.L.; Gatz, M. Depression in older adults. *Annu. Rev. Clin. Psychol.* **2009**, *5*, 363–389. [CrossRef] [PubMed]
19. Rodda, J.; Walker, Z.; Carter, J. Depression in older adults. *BMJ* **2011**, *343*, d5219. [CrossRef] [PubMed]
20. Spitzer, R.L.; Kroenke, K.; Wiliams, J.B.; Patient Health Questionnaire Primary Care Study Group. Validation and utility of a self-report version of PRIME-MD: The PHQ primary care study. *JAMA* **1999**, *282*, 1737–1744. [CrossRef] [PubMed]
21. Park, S.J.; Choi, H.R.; Choi, J.H.; Kim, K.W.; Hong, J.P. Reliability and validity of the Korean version of the Patient Health Questionnaire-9 (PHQ-9). *Anxiety Mood* **2010**, *6*, 119–124.
22. Han, C.S.; Ahnjo, S.M.; Kwak, J.H.; Pae, C.U.; Steffens, D.; Jo, I.H.; Park, M.H. Validation of the patient health questionnaire-9 Korean version in the elderly population: The Ansan geriatric study. *Compr Psychiatry* **2008**, *49*, 218–223. [CrossRef] [PubMed]
23. Lee, H.; Lee, J.A.; Brar, J.S.; Rush, E.B.; Jolley, C.J. Physical activity and depressive symptoms in older adults. *Geriatr Nurs.* **2014**, *35*, 37–41. [CrossRef]
24. Werneck, A.O.; Stubbs, B.; Fernandes, R.A.; Szwarcwald, C.L.; Silva, D.R. Independent relationships between different domains of physical activity and depressive symptoms among 60,202 Brazilian adults. *Gen. Hosp. Psychiaty* **2020**, *64*, 26–32. [CrossRef]
25. Choi, H.S.; Choi, J.H.; Park, K.H.; Joo, K.J.; Ga, H.; Koh, H.J.; Kim, S.R. Standardization of the Korean Version of Patient Health Questionnaire-9 as a screening instrument for major depressive disorder. *Korean J. Fam. Med.* **2007**, *28*, 114–119.
26. Lee, J.; Lee, C.; Min, J.; Kang, D.W.; Kim, J.Y.; Yang, H.; Park, J.; Lee, M.K.; Lee, M.; Park, I.; et al. Development of the Korean Global Physical Activity Questionnaire: Reliability and validity study. *Glob. Health Promot.* **2019**, *27*, 44–45. [CrossRef] [PubMed]
27. Zhang, S.; Xiang, K.; Li, S.; Pan, H.F. Physical activity and depression in older adults: The knowns and unknowns. *Psychiatry Res.* **2021**, *297*, 113738. [CrossRef] [PubMed]
28. Chi, S.H.; Wang, J.Y.; Tsai, A.C. Combined association of leisure-time physical activity and fruit and vegetable consumption with depressive symptoms in older Taiwanese: Results of a national cohort study. *Geriatr. Gerontol. Int.* **2016**, *16*, 244–251. [CrossRef] [PubMed]
29. Kim, J.; Kim, H. Demographic and environmental factors associated with mental health: A cross-sectional study. *Int. J. Environ. Res. Public Health* **2017**, *14*, 431. [CrossRef]
30. Tennant, C. Life events, stress and depression: A review of recent findings. *ANZJP* **2002**, *36*, 173–182. [CrossRef]
31. Pinquart, M. Correlates of subjective health in older adults: A meta-analysis. *Psychol. Aging* **2001**, *16*, 414–426. [CrossRef]
32. Mammen, G.; Faulkner, G. Physical activity and the prevention of depression: A systematic review of prospective studies. *Am. J. Prev. Med.* **2013**, *45*, 649–657. [CrossRef] [PubMed]
33. Kim, B. Factors influencing depressive symptoms in the elderly: Using the 7th Korea National Health and Nutrition Examination Survey (KNHANES VII-1). *J. Health Inform. Stat.* **2020**, *45*, 165–172. [CrossRef]
34. Kahng, S.K.; Kwon, T.Y. An exploratory study on the effects of socioeconomic status on depressive symptoms across lifespan. *Ment. Health Soc. Work* **2008**, *30*, 332–355.
35. Kim, S.Y.; Ko, S.G.; Kwon, J.H. The moderating effect of social support and coping on widowed elderly. *Korean Psychol. Assoc.* **2007**, *26*, 573–596.
36. McEwan, B.S. Mood disorders and allostatic load. *Biol. Psychiatry* **2003**, *54*, 200–207. [CrossRef]
37. Yoon, S.J.; Lee, Y.H.; Son, T.Y.; Oh, H.J.; Han, K.S.; Kim, K.H. Factors associated with dementia and depressive symptoms in older persons living in the community. *J. Korea Gerontol. Soc.* **2001**, *21*, 59–73.
38. Kok, R.M.; Reynolds, C.F. Management of depression in older adults: A review. *JAMA* **2017**, *317*, 2114–2122. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

Autism Spectrum Disorder and Childhood Apraxia of Speech: Early Language-Related Hallmarks across Structural MRI Study

Eugenia Conti [1], Alessandra Retico [2], Letizia Palumbo [2], Giovanna Spera [2], Paolo Bosco [1], Laura Biagi [1], Simona Fiori [1], Michela Tosetti [1], Paola Cipriani [1], Giovanni Cioni [1,3], Filippo Muratori [1,3], Anna Chilosi [1,†] and Sara Calderoni [1,3,*,†]

1 IRCCS Fondazione Stella Maris, 56128 Pisa, Italy; eugenia.conti@fsm.unipi.it (E.C.); p.bosco@fsm.unipi.it (P.B.); l.biagi@fsm.unipi.it (L.B.); s.fiori@fsm.unipi.it (S.F.); m.tosetti@fsm.unipi.it (M.T.); p.cipriani@fsm.unipi.it (P.C.); g.cioni@fsm.unipi.it (G.C.); f.muratori@fsm.unipi.it (F.M.); anna.chilosi@fsm.unipi.it (A.C.)

2 National Institute for Nuclear Physics (INFN), Pisa Division, 56127 Pisa, Italy; alessandra.retico@pi.infn.it (A.R.); letizia.palumbo@pi.infn.it (L.P.); giovanna.spera@pi.infn.it (G.S.)

3 Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy

* Correspondence: sara.calderoni@fsm.unipi.it

† These authors contributed equally to this work.

Received: 7 October 2020; Accepted: 9 December 2020; Published: 12 December 2020

Abstract: Autism Spectrum Disorder (ASD) and Childhood Apraxia of Speech (CAS) are developmental disorders with distinct diagnostic criteria and different epidemiology. However, a common genetic background as well as overlapping clinical features between ASD and CAS have been recently reported. To date, brain structural language-related abnormalities have been detected in both the conditions, but no study directly compared young children with ASD, CAS and typical development (TD). In the current work, we aim: (i) to test the hypothesis that ASD and CAS display neurostructural differences in comparison with TD through morphometric Magnetic Resonance Imaging (MRI)-based measures (ASD vs. TD and CAS vs. TD); (ii) to investigate early possible disease-specific brain structural patterns in the two clinical groups (ASD vs. CAS); (iii) to evaluate predictive power of machine-learning (ML) techniques in differentiating the three samples (ASD, CAS, TD). We retrospectively analyzed the T1-weighted brain MRI scans of 68 children (age range: 34–74 months) grouped into three cohorts: (1) 26 children with ASD (mean age ± standard deviation: 56 ± 11 months); (2) 24 children with CAS (57 ± 10 months); (3) 18 children with TD (55 ± 13 months). Furthermore, a ML analysis based on a linear-kernel Support Vector Machine (SVM) was performed. All but one brain structures displayed significant higher volumes in both ASD and CAS children than TD peers. Specifically, ASD alterations involved fronto-temporal regions together with basal ganglia and cerebellum, while CAS alterations are more focused and shifted to frontal regions, suggesting a possible speech-related anomalies distribution. Caudate, superior temporal and hippocampus volumes directly distinguished the two conditions in terms of greater values in ASD compared to CAS. The ML analysis identified significant differences in brain features between ASD and TD children, whereas only some trends in the ML classification capability were detected in CAS as compared to TD peers. Similarly, the MRI structural underpinnings of two clinical groups were not significantly different when evaluated with linear-kernel SVM. Our results may represent the first step towards understanding shared and specific neural substrate in ASD and CAS conditions, which subsequently may contribute to early differential diagnosis and tailoring specific early intervention.

Keywords: Autism Spectrum Disorders (ASD); childhood apraxia of speech; children; Magnetic Resonance Imaging (MRI); neuroanatomy; FreeSurfer

1. Introduction

Autism Spectrum Disorder (ASD) and Childhood Apraxia of Speech (CAS) are developmental disorders with distinct definitions and diagnostic criteria. Specifically, ASD includes a set of neurodevelopmental disorders characterized by social communication difficulties as well as restricted interests, repetitive activities and sensory abnormalities [1]. Recent estimates report an ASD prevalence of about one in 87 children aged 7–9 years [2] in Italy. ASD is a highly heterogeneous group of disorders, with multiple genetic backgrounds that may reflect multiple neuroanatomical underpinnings, which in turn are expressed with diverse behavioral manifestations [3,4].

CAS is a neurological childhood speech motor disorder in which the precision and consistency of movements underlying speech are impaired in the absence of neuromuscular deficits [5], and is included among Speech Sound Disorders (SSD) in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [1]. CAS core-deficit involves the planning and/or programming of the spatiotemporal parameters of movement sequences necessary for speech control [5] and is very frequently associated with an expressive language disorder [6].

The prevalence of CAS in the general population is low, 1–2 out of 1000 children [7], but it rises to 2.4% when considering children with SSD [8].

Despite the abovementioned differences in epidemiology and symptoms profile, an association between ASD and CAS has been suggested [9,10]. The prevalence of CAS is presumably higher in non-verbal or minimally verbal children with ASD, who represent about 25–30% of the ASD population without useful speech by age 5 [11].

ASD/CAS association is also supported by a possible shared genetic basis although only few syndromes or genes have been currently identified such as the 16p11.2 deletion syndrome [12] and the CNTNAP2 gene deletion on 7q35 position. The latter encodes a 'neurexin' protein that is associated with several neurodevelopmental disorders, including speech and language disorders [13] and autism [14, 15]. Moreover, studies on the function of FOXP2, which may be mutated in some CAS, underscored molecular intersections between networks involved in spoken language and pathways implicated in Intellectual Disability/ASD [16]. Furthermore, genes known to be regulated by FOXP2 have been implicated in disorders such as schizophrenia (e.g., DISC1) [17] and ASD (e.g., MET and MEF2C) [18,19].

From a clinical perspective, both ASD and CAS children experience a delayed expressive language acquisition that might contribute to difficulties in early differential diagnosis between these two conditions. This is of outmost importance as specific and early treatments are now available for these two conditions. A further difficulty relies on the lack of specific neurobiological markers, which would help distinguishing between ASD and CAS at an early age. The use of brain MRI has increased the potential for the application of advanced techniques for detecting brain abnormalities both in ASD and CAS [20,21]. In particular, morphometric and Diffusion-Weighted Imaging (DWI) MRI studies showed an early altered brain trajectory in ASD, involving mainly the fronto-temporal and basal ganglia circuits [22,23]. Morphometric and connectivity brain MRI abnormalities have been also reported in children with CAS and other speech sound disorders, with recurrent abnormalities involving the left supramarginal gyrus, fronto-temporal regions and basal ganglia among other regions [21,24–28].

In recent years, there has been a growing interest in the identification of shared brain abnormalities across psychiatric and neurodevelopmental disorders, especially among the disorders that frequently overlap in phenotypic presentation [29]. In particular, these studies enlarge our understanding of whether symptoms' overlap between some brain disorders could be at least partly explained by common altered neuroanatomy, or vice-versa, is subtended by disorder-specific brain underpinnings. In this framework, structural imaging studies have directly compared ASD with other neurodevelopmental disorders [30–32].

To our knowledge, no study has yet compared ASD and CAS through a structural morphometric MRI approach.

The main aim of this work has been to analyze structural MRI differences/similarities between children with ASD and CAS, which might share at least partly a genetic background, as well as

of clinical impairment in the language domain, though in different aspects. We decided to exclude comorbid cases (ASD plus CAS) since the presence of comorbidity could be a confounding factor in the identification of disorder-specific brain underpinnings.

In particular, we aimed:

(1) To test the hypothesis that the two clinical groups (ASD and CAS) display neurostructural differences in comparison with Typically Developing children (TD) through a morphometric MRI approach (ASD vs. TD; CAS vs. TD);
(2) To investigate possible disease-specific brain structural patterns in the two clinical groups (ASD vs. CAS);
(3) To evaluate the predictive power of machine-learning analysis in differentiating these three young populations (ASD, CAS, TD).

2. Participants and MRI Data Acquisition

We retrospectively selected MRI brain images of patients diagnosed with ASD and CAS after a comprehensive clinical evaluation at IRCCS Stella Maris Foundation (Pisa, IT), a tertiary care university hospital.

ASD group. Children were rigorously diagnosed with ASD according to the DSM-5 criteria [1] by a multidisciplinary team including a senior child psychiatrist, an experienced clinical child psychologist and a speech-language pathologist during three–five days of extensive evaluation. The diagnosis was confirmed by the Autism Diagnostic Observation Schedule (ADOS)-2 [33] administered by clinical psychologists who met standard requirements for research reliability. Inclusion criteria were: (a) age between 34 and 72 months, (b) Non-Verbal Intellectual Quotient (NVIQ) ≥70 and (c) spontaneous no-echolalic language of at least two-word associations, (d) absence of minimal signs potentially indicating comorbid CAS.

CAS group. CAS diagnosis was conducted by a multidisciplinary team on the basis of a comprehensive clinical, instrumental and neurological assessment as well as a video recorded speech–language evaluation. Following the international criteria for CAS diagnosis, speech and language performances were analysed by two independent expert observers according to a checklist including American Speech-Language-Hearing Association (ASHA) criteria [5] and Strand's features of CAS [6,10,34]. Inclusion criteria were: (a) age between 34 and 71 months, (b) NVIQ ≥ 70, (c) no ASD symptoms documented by neuropsychiatric and psychological observation.

Exclusion criteria for both clinical groups were: (a) structural anomalies detected by MRI; (b) presence of oro-facial structural abnormalities; (c) neurological or genetic diseases; (d) audiological deficits; (e) epilepsy; (f) any identified etiology of the two disorders based on DNA analysis or screening tests for inborn errors of metabolism (plasma and urine amino-acid analysis, urine organic acid measurement, urine muco-polysaccharides quantitation, plasma and urine creatine, and guanidinoacetate analysis).

A group of typically developing children (TD) who had undergone brain MRI for various reasons (including headache, seizures with fever, strabismus, cataract, paroxysmal vertigo, diplopia) was also recruited, as controls.

3. MRI Acquisition and Processing

MRI data were acquired using a GE 1.5 T Signa Neuroptimized System (General Electric Medical Systems) at IRCCS Stella Maris Foundation, fitted with 40 mT/m high-speed gradients. Within the MRI protocol for children, a whole-brain fast spoiled gradient recalled acquisition in the steady-state T1- weighted series (FSPGR) was collected in the axial plane, yielding to contiguous axial slices with voxel size of 1.1 × 1.1 × 1.1 mm. All children were sedated with a general anesthesia with a halogenated agent (Sevoflurane) while spontaneously breathing. For all MRI performed between 2012 and 2018 the same sequence of acquisition was used and the written informed consent from a parent

or guardian of children was obtained. This study was approved by the Pediatric Ethic Committee of Tuscany Region (Italy) through the ARIANNA Project (C52I16000020002) and RF 2016-022361560 Project, and was performed according to the Declaration of Helsinki and its later amendments or comparable ethical standards.

We used the ARIANNA platform [35] for data handling and processing.

4. FreeSurfer Processing and Feature Extraction

Structural MRI data were pre-processed according to the widely used FreeSurfer analysis pipeline (FreeSurfer v.6.0), to finally extract brain descriptive features, such as volume and thickness. FreeSurfer software is well documented and freely available for download at https://surfer.nmr.mgh.harvard.edu/. FreeSurfer is used as pre-processing workflow for structural MRI data to perform volumetric segmentation and cortical reconstruction through 31 processing steps. The technical details of these procedures are described in the publication by Fischl [36] and references therein. Briefly, this processing includes motion correction, removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures, intensity normalization, tessellation of the grey matter white matter boundary, automated topology correction, and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class. FreeSurfer is an extremely time-consuming procedure. It requires in order of a few hours (8 h on average) to segment a 3D volumetric image.

The FreeSurfer analysis pipeline computes for each structure several descriptive features. We considered in our analysis the following regional features: the volumes of subcortical structures and the volumes and thicknesses of the cortical structures, and some volume and thickness global measures. In detail, the cortex was parceled in 34 left and 34 right structures, whose volumes and thicknesses generated 138 cortical features (i.e., 68 volumes, 68 parcel thicknesses plus the mean left and mean right thickness values) contained in the *aparc* FreeSurfer output file. Thirty additional volume values were considered within those available in the *aseg* FreeSurfer output file, including the volumes of subcortical structures (thalamus, caudate, putamen, pallidum, hippocampus, amygdala, accumbens nuclei, corpus callosum and brainstem), and those of the ventricles, the subcortical cerebrospinal fluid, the optical chiasm and the cerebellum (white and gray matter). A complete list of the FreeSurfer features considered in this analysis is available in the Supplementary Materials.

5. Statistical Analysis

The entire set of brain features were statistically analysed in order to identify significant differences between patients with ASD, CAS and TD children. The ANOVA test was conducted for normally distributed features, whereas the Kruskal-Wallis test was used in case the features were not normally distributed. The Bonferroni method was used to correct the results for multiple comparisons. The effect sizes were evaluated in terms of Cohen's d. Furthermore, we performed machine learning (ML)-based multivariate analysis through Support Vector Machine (SVM) binary classifiers [37]. In particular, linear-kernel SVMs were implemented to evaluate the predictive power of neuroanatomical features in the binary classification of the ASD vs. TD, CAS vs. TD, ASD vs. CAS groups. We implemented a five-fold cross-validation scheme in this analysis to partition the available data in train and test sets and to evaluate the classifier performance. The classification performance was evaluated in terms of the mean and standard deviation of area of the ROC curve (AUC) obtained across 10 repetitions of the five-fold cross-validation.

The statistical analysis and the SVM classification were carried out with Matlab R2018a (The MathWorks, Inc., Natick, Massachusetts, U.S) through in-house developed scripts and functions. In particular, the *anova1*, *kruskalwallis* and *multcompare* matlab functions were used for statistical analysis and the *fitcsvm* function for the SVM classification.

6. Results

6.1. Participants

The initial cohort consisted of 98 patients who underwent brain structural MRI. Eighteen patients were excluded due to detection of minor brain anomalies (i.e., arachnoid cyst; periventricular leukomalacia; cortex anomalies such as heterotopia or dysplasia); seven patients were excluded due to low quality of MRI scans; five patients were excluded due to the presence of clinical comorbidities (other neurodevelopmental disorders).

The final cohort consisted of 68 children aged from 34 to 72 months, belonging to the three groups of ASD, CAS and TD, which were matched for gender and age. The age distributions are showed in Figure 1.

ASD group: 26 children; mean age ± standard deviation (SD) = 56 ± 11 months; CAS group: 24 children; mean age ± SD = 56 ± 10 months. TD group: 18 children; mean age ± SD = 55 ± 13 months.

Children's demographic characteristics are shown in Table 1 and Figure 1.

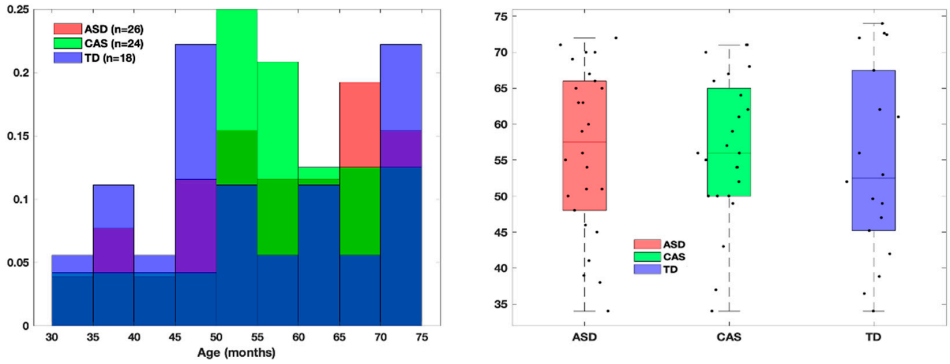

Figure 1. Age distribution of the subjects in the ASD, CAS and TD groups. The bar plot is shown on the left for the three groups, whereas the box plots with an overlay of the age value of the individual subjects, slightly scattered randomly along the x axis, are shown on the right. Abbreviations: ASD, Autism Spectrum Disorder; CAS, Childhood Apraxia of Speech; TD, Typical Development control.

Table 1. Demographic characteristics of the cohorts of subjects with ASD, with CAS and TD subjects. The number of subjects in each subgroup is reported (*n*) together with the male/female percentage with respect to the total number of subjects in each cohort.

Age in Months (Mean ± std [Range]) by Subjects' Category					
ASD (*n* = 26)		CAS (*n* = 24)		TD (*n* = 18)	
56 ± 11 (34–72)		57 ± 10 (34–71)		55 ± 13 (34–74)	
Males	Females	Males	Females	Males	Females
(*n* = 20, 77%)	(*n* = 6, 23%)	(*n* = 18, 75%)	(*n* = 6, 25%)	(*n* = 13, 72%)	(*n* = 5, 28%)
57 ± 11 [34–71]	54 ± 12 [39–72]	56 ± 10 [34–71]	57 ± 12 [37–68]	58 ± 12 [39–74]	47 ± 13 [34–67]

Abbreviations: ASD, Autism Spectrum Disorder; CAS, Childhood Apraxia of Speech; TD, Typical Development control.

6.2. Statistical Analysis

Statistical analysis of the complete set of FreeSurfer features was carried out. Significant differences in several brain structures across the three cohorts were detected through ANOVA statistical analysis and the Kruskall-Wallis test, as reported in Table 2 for cortical volumes, cortical thicknesses and subcortical volumes. The effect sizes in terms of Cohen's *d* are also reported in the table. A visual representation of all altered brain regions, as segmented by FreeSurfer, is shown in Figure 2, where their overlay on the anatomical image of a representative subject is reported. In particular, brain regions whose features showed statistically significant differences in the comparison among the three groups of children are highlighted. We summarize in the text below the results of the between-group comparisons ASD vs. TD, CAS vs. TD and ASD vs. CAS, reported in Table 2 column (a), Table 2 columns (b) and (c), and Table 2 column (d), respectively.

Table 2. Cortical volumes showing a statistically significant difference in the comparison among children with ASD, children with CAS and TD children. The test statistics (either ANOVA or Kruskal-Wallis), the p-values, the specification of which group comparisons showed statistically significant differences and the related effect size in terms of Cohen's *d* are reported.

Comparison among ASD, CAS and TD Groups		Statistical Test §		Cohen's *d* in the Between-Group Comparisons			
				(a)	(b)	(c)	(d)
		F/X^2	*p* Value	ASD > TD	CAS > TD	CAS < TD	ASD > CAS
Cortical volumes	Left Paracentral volume	4.1	0.02	0.83	0.79	/	/
	Left Posterior Cingulate volume	4.0	0.02	0.73	/	/	/
	Left Supra Marginal volume §	7.7	0.02	0.58	0.48	/	/
	Right Caudal Middle Frontal volume §	5.9	0.05	0.77	/	/	/
	Right Pars Triangularis volume §	8.3	0.01	/	0.53	/	/
	Right Superior Temporal volume	5.9	0.004	0.95	/	/	/
Cortical Thickness	Right Superior Temporal thickness	4.1	0.02	/	/	/	0.79
	Right Frontal Pole thickness	4.1	0.02	/	/	0.97	/
Subcortical structures, cerebellum and global measures	Left Caudate volume	5.8	0.005	1.04	/	/	0.68
	Left Cerebellum Cortex volume	4.1	0.02	0.97	/	/	/
	Left Hippocampus volume §	12	0.002	1.15	/	/	0.57
	Left Nucleus Accumbens §	11	0.004	0.92	0.97	/	/
	Left Putamen volume §	7.7	0.02	0.89	/	/	/
	Right Caudate volume §	8.0	0.02	0.89	/	/	/
	Right Cerebellum Cortex volume	4.5	0.01	1.02	/	/	/
	Right Hippocampus volume §	12	0.002	1.19	/	/	0.56
	Right Putamen volume §	9.3	0.01	0.88	/	/	/
	SubCortical Gray matter volume	5.3	0.008	0.97	/	/	/
	Total Gray matter volume	3.1	0.05	0.71	/	/	/

Abbreviations. ASD, Autism Spectrum Disorder; CAS, Childhood Apraxia of Speech; TD, Typical Development control. § Features with not-normal distribution undergone Kruskal-Wallis test instead of ANOVA.

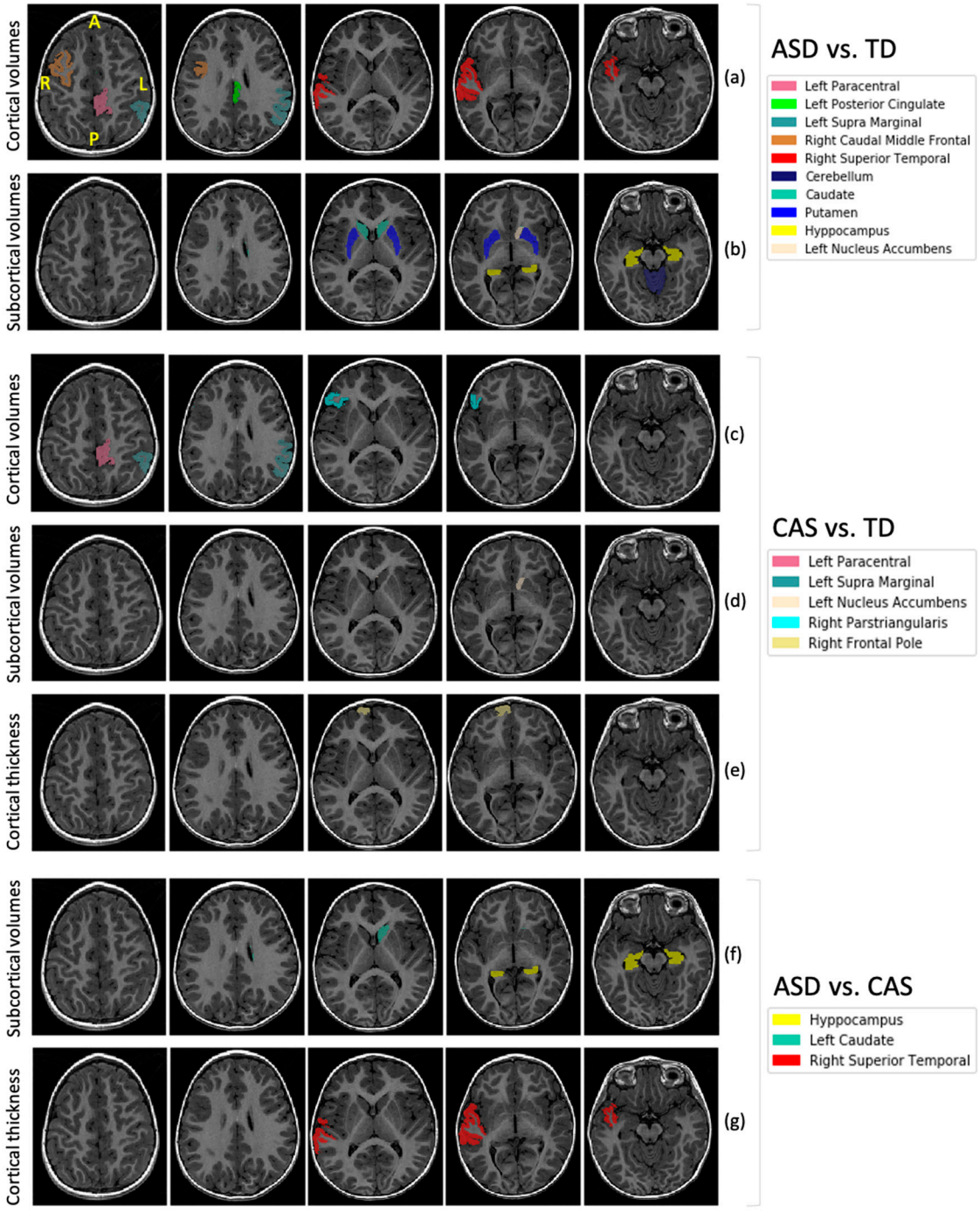

Figure 2. Visualization of brain regions whose features showed statistically significant differences among the three groups of subjects (see Table 2 for the complete list of features, including also global volumes). The overlay of FreeSurfer segmented regions onto the anatomical image of a representative subject is shown for specific alterations in the ASD vs. TD (**a**,**b**), CAS vs. TD (**c**–**e**) and ASD vs. CAS (**f**,**g**) comparisons. The involved set of features, i.e., the cortical and subcortical volumes, and the cortical thicknesses, are indicated in each figure row.

6.3. Comparison between ASD and TD

The significant neuroanatomical differences between ASD and TD are reported in Table 2 (a), visualized in Figure 2a,b and discussed below.

Cortical volumes. Overall, ASD presented increased volumes in comparison with TD. No contra-comparison results were found. The Cohen's d effect sizes vary from medium effect size to large effect size. ASD presented increased cortical volumes within the fronto-temporal lobe: left paracentral volume (d = 0.83), left posterior cingulate volume (d = 0.73), left supramarginal volume (d = 0.58), right caudal middle frontal volume (d = 0.77) and right superior temporal volume (d = 0.95).

Subcortical volumes. Overall, ASD presented increased subcortical volumes in comparison with TD; no contra comparison results were found. ASD presented increased volumes in the following regions: caudate (left d = 1 and right d = 0.89), putamen (left d = 0.89 and right d = 0.88), hippocampus (left d = 1.15 and right d = 1.19) and left nucleus accumbens (d = 0.92).

Cortical thickness. No statistically significant results were detected.

Global measures and cerebellum. An increase in cortex volumes of the cerebellum (left d = 0.97 and right d = 1) was detected in children with ASD with respect to TD. Children with ASD also presented an increased global subcortical grey matter volume (d = 0.97) and total grey matter volume (d = 0.71) with respect to TD.

6.4. Comparison between CAS and TD

The significant neuroanatomical differences between CAS and TD are reported in Table 2 (b) and (c), visualized in Figure 2c–e, and discussed below.

Cortical volumes. Overall, children with CAS presented higher values of cortical volume within the frontal lobe, in particular in left paracentral (d = 0.80), right pars triangularis (d = 0.52) and in left supramarginal (d = 0.50).

Subcortical volumes. CAS showed an increased volume in left nucleus accumbens (d = 0.97) with respect to TDs.

Cortical thickness. CAS presented reduced values of cortical thickness in the frontal lobe, in particular in the right frontal pole (d = 0.97).

Global measures and cerebellum. No statistically significant results were detected.

6.5. Comparison between ASD and CAS

The significant neuroanatomical differences between ASD and CAS are reported in Table 2 (d), visualized in Figure 2f,g, and discussed below.

Cortical volumes. No statistically significant differences between ASD and CAS were directly detected in cortical volumes.

Subcortical volumes. Statistically significant greater volumes of the left caudate (d = 0.68) and of the hippocampi (left d = 0.57 and right d = 0.56) have been detected in ASD with respect to CAS.

Cortical thickness. ASD showed statistically significant higher cortical thickness in the right superior temporal (d = 0.89) in comparison to CAS.

Global measures and cerebellum. No statistically significant results were detected.

6.6. Machine Learning Analysis

The performances obtained with the binary linear-kernel SVM in the ASD vs. TD, CAS vs. TD, ASD vs. CAS classification are reported in Table 3. The classification performance achieved by different groups of features (e.g., cortical volumes/thickness, subcortical volumes, and their combinations) are reported in the table in terms of the ROC curve (AUC) values obtained according to a five-fold cross-validation scheme. The error assigned to each AUC value is computed as the standard deviation over 10 repetitions of the five-fold cross-validation.

Table 3. Classification performance in the binary classification between the ASD vs. TD, CAS vs. TD and ASD vs. CAS groups using structural features. The performances are expressed in terms of the area under the ROC curve (AUC) achieved in the classification of different groups of features (e.g., cortical volumes/thickness, subcortical volumes, global volumes and their combinations). The cross-validation scheme implemented consisted in 10 repetitions of a five-fold cross validation.

Features		AUC (Mean ± SD)		
		ASD vs. TD (n = 44)	CAS vs. TD (n = 42)	ASD vs. CAS (n = 50)
Subcortical volumes + cerebellum	m = 34	0.75 ± 0.16	0.48 ± 0.17	0.42 ± 0.14
Subcortical volumes and global measures	m = 38	0.76 ± 0.14	0.54 ± 0.18	0.45 ± 0.12
Cortical volumes	m = 68	0.53 ± 0.17	0.52 ± 0.18	0.45 ± 0.17
Cortical thicknesses	m = 70	0.52 ± 0.19	0.62 ± 0.21	0.64 ± 0.17
All cortical features (volumes and thicknesses)	m = 138	0.63 ± 0.18	0.59 ± 0.17	0.50 ± 0.15
All structural features and global measures	m = 176	0.73 ± 0.19	0.61 ± 0.17	0.45 ± 0.16

Abbreviations: AUC, area under the ROC curve; SD, standard deviation; m, number of features in each selected group of features (each table row); n, number of subjects used in each classification problem (each table column); ASD, Autism Spectrum Disorder; CAS, Childhood Apraxia of Speech; TD, Typical Development control.

It can be noticed from Table 3 that the most informative groups of features driving the discrimination performance between children with ASD and TD are the subcortical volumes, either including or not the global measures. In those cases, AUC values of 0.76 ± 0.14 and of 0.75 ± 0.16 have been obtained. The classification performance remains high when the combination of all features is considered, leading to an AUC of 0.73 ± 0.19.

By contrast, the group of children with CAS does not appear to be distinguishable from the group of TD children by means of a linear-kernel SVM classification. Despite that the classification performance obtained is not above the chance level, the set of cortical thickness features showed an average AUC value of 0.62, and the combination of all features an AUC of 0.61.

It turns out also that the groups of children with ASD and with CAS are indistinguishable from each other by means of a linear-kernel SVM classification, as reported in the rightmost column of Table 3 (all chance-level AUC values). In this case, only the set of cortical thickness features displayed an average AUC value of about 0.64.

7. Discussion

To our knowledge, this is the first structural morphometric MRI study comparing ASD, CAS and TD. This may represent the first step towards understanding the neural substrate that characterizes the two conditions, possibly being of utmost importance for future early tailored intervention strategies. Furthermore, it could be helpful to understand as early as possible whether children with non-verbal or minimally verbal ASD have a CAS on the basis of neuroanatomical brain configuration, given the difficulty of directly testing these children.

7.1. Are ASD and CAS Brain Different from TD Brain?

7.1.1. ASD Versus TD

ASD children displayed an overall increase of total grey matter volume in comparison with TD, specifically distributed in the fronto-temporal regions.

Grey matter volume increase is one of the most consistent structural findings in ASD, and it is particularly striking in younger children [20], thus supporting the early brain overgrowth of ASD related to abnormal cortical development and expansion [38,39]. It is of interest that the cortical volumes' increase found in our ASD children versus TD is mainly distributed in fronto-temporal lobes, known to be crucial for socio-communicative skills development [40]. The importance of these two

brain lobes in the pathophysiology of ASD was further corroborated by two recent studies [41,42], which identified both frontal and temporal lobe volumes as the most discriminative features in the ASD-control classification. Critically, the left supramarginal gyrus, which belongs to the inferior parietal lobule, was also increased in volume in ASD children. This region is massively connected with both Broca's and Wernicke's areas, and it is altered during processing of some language aspects, including pragmatics [43], and sentence comprehension [44] in children with ASD.

Besides the well-replicated alterations in the fronto-temporal cortical regions, an increased volume in some subcortical structures was detected in the current work. Specifically, both right and left hippocampi were significantly increased in ASD group when compared with TD. The hippocampus is considered relevant for the ASD pathophysiology, since it is connected to the amygdala within the limbic system and implicated in crucial functions of the "social brain" [45]. Our result of increased hippocampi volumes is consistent with findings from a large study of 98 patients with ASD (age-range: 7.5–18 years) compared to 31 controls [46], and it is also concordant with a longitudinal investigation in children between the ages of 8 and 12 years [47]. However, findings on hippocampal volume in autism are quite controversial, as some authors did not identify differences compared with controls [48], while significantly decreased volumes were found by other authors [49,50].

In addition, we identified a significant enlargement of the striatum (the part of the basal ganglia composed of three subnuclei: caudate, putamen, and nucleus accumbens) in patients with ASD in comparison with TD. Structural alterations of the basal ganglia, and in particular of the caudate nucleus and the putamen, have been frequently detected in ASD, both in children [51] and adults [52], and associated with the severity of restricted and repetitive behaviors [53]. Instead, the nucleus accumbens is generally reported as a critical node within the brain's reward circuitry, but, more broadly, it is also involved in action selection, integration of cognitive and affective information, and suppression of inappropriate actions [54].

Interestingly, decreased structural and functional connectivity between the ventral tegmental area of the midbrain and the nucleus accumbens, namely two brain areas crucial for processing social reward, was found in ASD children compared with TD children [55]. Such brain alterations appear to be related to the level of social interaction impairments in patients with ASD, providing support for the link between abnormalities in reward processing and widespread deficits in social engagement and communication in autism (i.e., the social motivation theory [56]).

The increased cerebellum volume we found is in agreement with some [49,57], but not all [20,58] literature findings. The cerebellum has been traditionally considered to be primarily involved in motor control and coordination, but its function comprises other domains typically impaired in ASD children, such as language, social cognition, and affective regulation [59]. In keeping with these data, a consensus paper recently highlighted a crucial role of the neuroanatomical cerebellar alterations in ASD [60].

We also detected an increased volume in the right superior temporal gyrus (STG), a region implicated in the processing of semantic [61] and prosodic cues [62], thus being two language aspects frequently impaired in children with ASD [63]. In this context, two previous fMRI investigations observed increased brain activation in the right STG of ASD children during processing of prosodic cues, such as anger and irony [64,65], that may reflect a more effortful processing required for the interpretation of prosodic information. The increased grey matter volume in right STG detected in the current study is consistent with three previous sMRI investigations focused respectively on toddlers (mean age: 30 months) [66], preschoolers (mean age: 53 months) [67], and children/adolescents (age range: 8.8–18.3 years) [68] with ASD, supporting the view that volumetric alterations in this region are present across the developmental age.

Cortical thickness analysis reported, in the current sample, no significant differences between ASD patients and TD peers. Some previous studies have shown cortical thickness differences between participants with ASD and controls across the whole brain, and in particular, a greater cortical thickness in fronto-temporal regions implicated in the processing of language and social information [69–71].

Nevertheless, other investigations have failed to detect between-groups differences in cortical thickness [72]. Additionally, cortical thinning in the pars opercularis of the inferior frontal gyrus was reported both in preschoolers [67] and in adults [73] with ASD. These discrepancies could be partly explained by study differences in sample size, age, patients' characteristics and clinical symptoms' severity, MRI acquisition and processing protocols. Longitudinal studies identified that differences in cortical thickness between patients with ASD and TD controls change over time [74,75], suggesting that an altered cortical thickness trajectory may constitute a more reliable neuroanatomical marker in ASD.

7.1.2. CAS Versus TD

Apraxia of speech (AOS) is the main symptom in adults after infarcts to the left hemisphere involving the inferior frontal region, in particular the posterior part of Broca's area [76] and the insular cortex [77] or adjacent white matter [78]. Instead, Childhood Apraxia of Speech (CAS) is a developmental disorder whose brain correlates remain largely unknown and little evidence is available to date [21,24,28].

Our results show structural brain differences in children with CAS in comparison with TD. In particular, altered cortical volumes in areas crucial for speech and language were found, with increased volumes distributed within the parietal lobe (supramarginal gyrus), the frontal lobe (para-central, pars triangularis), and decreased volume in the nucleus accumbens.

The involvement of the left supramarginal gyrus in CAS has been previously described by Kadis [24], who found an increased thickness of this region in children with CAS aged as in our sample. Anatomo-clinical correlation studies in brain-damaged patients with a selective impairment of the auditory-verbal memory span indicate that the inferior parietal lobule (supramarginal gyrus) of the left hemisphere, at the temporo-parietal junction, represents the main neural correlate of the 'store' component of phonological short-term memory [79]. The left supramarginal gyrus is reported to play an important role in speech production, its damage thus being associated with deficits in phonemic discrimination and speech planning [80]. Furthermore, the supramarginal gyrus is part of a neural dorsal pathway receiving inputs from the auditory cortex and has reciprocal connections with the opercular part of the inferior frontal gyrus and the ventral premotor area [81,82], both involved in articulatory planning [83]. Indeed, as recently suggested by Nakamichi et al. [84] in a functional Near Infrared Spectroscopy (fNIRS) study, the supramarginal gyrus seems to be involved in phonemic processing and articulatory learning through an "articulatory loop" in which phonemic and oral somatosensory information are mapped onto motor representations for articulation. Partially overlapping with our findings, morphological abnormalities of supramarginal gyrus and, bilaterally, of temporal planum and Heschl's gyrus have been described in children with a subtype of speech sound disorder characterized by persistent speech sound errors [27].

Another resulting altered region in terms of increased values in CAS vs. TD in the current work was the pars triangularis within the Broca's area [85–87]. This region has been associated with linguistic processes, including syntax and semantics [86,88,89], but its precise functional role still remains controversial. Recently, Elmer and colleagues [90] suggested that pars triangularis can be considered as a "hub" region of the language-control network and would have a role in supporting verbal working memory functions during simultaneous language translation.

A further area within the frontal lobe founded as volumetrically increased in CAS vs. TD was the left paracentral region, a sensory-motor area involved in motor control in adults, whose eventual role in the acquisition of speech motor control during development is still unknown.

However it is of note that the paracentral region is contiguous to the precentral region reported as altered in the diffusion study on children with CAS by Fiori et al. [21].

At subcortical level, the nucleus accumbens, which is part of the ventral striatum and belongs to a broad language learning network [91], showed a decreased volume in CAS compared to controls.

As far as cortical thickness is concerned, the right frontal pole showed reduced values in CAS compared to TD. It is of note that the frontal pole (Broadmann Area 10) has been described as the

most evolved region in humans [92] and it is essential for attention control, manipulation of stored knowledge and modulation of complex actions, cognition emotion, and behaviour [93]. However, the interpretation of this finding in children with CAS is still unclear.

7.2. Which Regions Directly Differentiate ASD vs. CAS?

Comparison between ASD and CAS showed that the caudate and the hippocampus volumes, together with the superior temporal thickness, were shown to be increased in ASD vs. CAS.

The basal ganglia (BG), which consist of the striatum, caudate nucleus and putamen, are involved in motor function [94] as well as in learning and memory processes [95] and have been widely related to the repetitive and stereotyped behaviors characteristic of autism spectrum [96,97]. Bilaterally reduced grey matter density in the caudate nuclei was described in patients with CAS related to FOXP2 mutations. In particular, MRI studies of the Ke family [98] and a recent report of an unrelated male child with a FOXP2 intragenic deletion [99] confirmed reductions of the caudate nucleus bilaterally, as well as of the globus pallidus and hippocampus.

As above mentioned in the ASD vs. TD section, the superior temporal gyrus has been widely described as having a crucial role in the social brain development, and not surprisingly has been found to be increased in our ASD population. Incidentally, the superior temporal gyrus is also involved in language comprehension that is often more severely impaired than language production in children with ASD [100], whereas an inverse profile characterizes children with CAS.

Data concerning hippocampus increased volumes in ASD are consistent with the findings of other studies, whereas there is only a case report by Liéogeois et al. (2016) [99] describing bilateral hippocampal and basal ganglia volume reduction (thalamus, globus pallidus, and caudate nucleus) in a 8 years old child with FOXP2 related CAS

Since the abovementioned regions (caudate, superior temporal gyrus and hippocampus) significantly differentiated not only ASD from CAS, but also ASD from TD, it may be hypothesized that these results are more ascribable to ASD higher values than to CAS lower values in the direct comparison.

7.3. Is Machine Learning Informative about Diagnosis Prediction?

Non-invasive brain imaging techniques coupled with advanced image analysis methodologies based on machine learning (ML) have been recently used to provide an automated classification of diseases, including ASD [101], whereas no application in the CAS field is yet reported. We have included, then, a ML analysis in the current work to estimate the predictive capabilities of neuroanatomical descriptive features in a binary comparison between two out of the three groups of children.

In the cases of CAS vs. TD and of ASD vs. CAS comparisons, the performances achieved did not score above the chance level. This means that the two groups are quite indistinguishable at least with linear-kernel SVM. It has to be noted that the high standard deviation assigned to the AUC values is mainly due to the limited sample size, which did not allow avoidance of the overfitting problem. Increasing the dataset population could sensibly reduce the standard deviation on the AUC values and the classification trends that are barely visible (AUC~0.6) may become apparent. It is also worth mentioning that any subtle relationship between neuroanatomical features that may characterize the two clinical groups of this study could be very hard to catch by a linear classifier. Non-linear approaches could be implemented. However, even in this case, the limited data samples (less than 30 subjects per group) with respect to the large amount of image features (sets from ~30 to more than 150 features have been considered) would not avoid the occurrence of overfitting, which causes a reduction in the generalization ability of the classifier.

In conclusion our ML analysis highlights that the group of children with ASD shows a distinct brain pattern with respect to the control group, focused especially on subcortical brain regions. The comparison between the children with CAS and the control group showed a trend towards the possibility of identifying a relevant pattern focused on cortical thickness, that represents the only relevant trend in the direct comparison between the two clinical groups. Indeed, while ASD present

more widespread brain alterations, confirming previous data and opening the possibility to predictive power of ML algorithms based on MRI data, a predictive role of brain patterns in CAS is still not supported by the current sample size.

7.4. Final Considerations

In summary our work reports a general consistency with the previous literature in the brain structural comparison between ASD versus TD and CAS versus TD, with resulting effect size ranging from medium to large. It is of interest that all but one structure showed higher volumes in both ASD and CAS versus TD in this young population, in accordance with previous literature, especially for the ASD group. The only opposite trend regards cortical thickness (CAS < TD in frontal pole cortical thickness), but the significance of this result is not clear, cortical thickness not being univocally interpreted in the literature, and being age-dependent [102]

The more widespread abnormalities have been observed comparing ASD vs. TD, while more focused alterations have been found by comparing CAS vs. TD. Considering all the structures (cortical and subcortical volumes and cortical thickness), ASD alterations involve fronto-temporal regions together with basal ganglia and cerebellum, while CAS alterations seem to be more focused on and shifted to frontal regions, thus suggesting a possibly more specific speech-language related distribution of anomalies. We can speculate that autistic children's brain atypicalities are more widespread in the superior temporal gyrus, historically considered the site of sound processing and auditory association cortex, but also linked to social cognition and implicated in visual analysis of social information. Moreover, the superior temporal gyrus has been involved in the perception of the emotional facial stimuli and more generally in social interactions. Conversely, atypicalities in CAS are more shifted in the frontal regions, where sensory motor circuitries are represented. Furthermore, it can be considered that overlapping structural regions between the two conditions can assume a different role at a functional level.

7.5. Strenghts and Weaknesses of the Study

To the best of our knowledge this is the first study comparing young children presenting with ASD, CAS and TD at a neurostructural level. Though our results suggest the possibility of detecting brain correlates potentially able to disentangle the two conditions from typical development from an early age (when clinical specific phenotypization is not easy), ML analysis in our sample has partially reached an optimal predictive power. Indeed, while our ML analyses confirm the possibility of detecting brain patterns with reliable predictive power in the ASD vs. TD comparison, this is not the same for CAS vs. TD and ASD vs. CAS. This can be ascribable to limited sample size, which does not allow a complete representation of the different distribution of possible alterations in the two conditions and also prevents the possibility of implementing more complex non-linear classifier models.

Certainly, the study has strengths and weakness. First, this is a whole brain analysis not driven by an a priori hypothesis, thus minimizing possible interpretation biases. Second, clinical groups, with a short age range, have been evaluated from expert clinicians (multidisciplinary team) in the field of ASD and CAS. Thirdly, the single-site recruitment of study participants limited the noise associated with the collection of data from different MRI scanners and different sequences. Our results should be interpreted in the light of some methodological limitations among which the small sample size is the most crucial. Indeed, our sample size of about 20 children per group is quite small, thus limiting the generalizability of the results and the strength of the conclusions [103]. Hence, it will be critical in the future to recruit larger samples in order to replicate our findings, and to provide a more robust characterization of the clinical and neuroanatomical profiles of children with ASD and CAS. This study's limits are mitigated by the fact that patients have been carefully selected so as to be included in a limited age-range, in order to minimize age dependent structural brain alterations. Notably, the sample has been reduced from the initial cohort with the aim of obtaining a well-selected and homogeneous pool of MRI data, coupled with an extensive multidisciplinary clinical characterization of patients.

Furthermore, the retrospective nature of this study has not allowed us to apply appropriate clinical evaluations, such as ADOS-2 to the CAS population and standardized CAS protocols to the ASD population.

Future studies should collect a further group of patients with ASD and comorbid CAS in order to investigate the extent to which these cases differ from the "pure" disorders.

Though remaining cautious about interpretation, our results may represent the first step towards understanding the neural substrate that characterizes ASD and CAS conditions and therefore, in the future, for identifying neurobiological markers that may support early diagnosis of non-verbal or minimally verbal children with ASD.

Furthermore, the identification of a specific motor speech disorder associated to ASD is crucial for tailoring an appropriate early intervention.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4426/10/4/275/s1, Table S1: FreeSurfer features considered in the analysis.

Author Contributions: E.C., A.C., and S.C. conceived the study. E.C. and L.B. collected MRI and clinical data. E.C., A.C., and S.C. were major contributors in writing the manuscript. A.R., S.F., M.T. and P.C. contribute to interpretation of results and to the manuscript finalization. A.R., L.P., G.S., and P.B. performed the analysis. F.M. and G.C. contributed to review and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work has been partially funded by the Tuscany Government (Bando FAS Salute by Sviluppo Toscana, ARIANNA Project) and by the Italian Ministry of Health RF 2016 022361560. E.C. and S.C. were partially funded by AIMS-2-Trials European Project.

Conflicts of Interest: The authors have no conflict of interest to declare.

References

1. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*; American Psychiatric Publication: Washington, DC, USA, 2013.
2. Narzisi, A.; Posada, M.; Barbieri, F.; Chericoni, N.; Ciuffolini, D.; Pinzino, M.; Romano, R.; Scattoni, M.L.; Tancredi, R.; Calderoni, S.; et al. Prevalence of Autism Spectrum Disorder in a large Italian catchment area: A school-based population study within the ASDEU project. *Epidemiol. Psychiatr. Sci.* **2018**, *29*. [CrossRef] [PubMed]
3. Loth, E.; Murphy, D.G.; Spooren, W. Defining precision medicine approaches to autism spectrum disorders: Concepts and challenges. *Front. Psychiatry* **2016**, *7*, 188. [CrossRef] [PubMed]
4. Lombardo, M.V.; Lai, M.C.; Baron-Cohen, S. Big data approaches to decomposing heterogeneity across the autism spectrum. *Mol. Psychiatry* **2019**, *24*, 1435–1450. [CrossRef] [PubMed]
5. American Speech-Language-Hearing Association. Childhood Apraxia of Speech [Technical Report]. 2007. Available online: www.asha.org/policy/ (accessed on 11 December 2020).
6. Chilosi, A.M.; Lorenzini, I.; Fiori, S.; Graziosi, V.; Rossi, G.; Pasquariello, R.; Cipriani, P.; Cioni, G. Behavioral and neurobiological correlates of childhood apraxia of speech in Italian children. *Brain Lang.* **2015**, *150*, 177–185. [CrossRef] [PubMed]
7. Shriberg, L.D.; Aram, D.M.; Kwiatkowski, J. Developmental apraxia of speech: II. Toward a diagnostic marker. *J. Speech Lang. Hear. Res.* **1997**, *40*, 286–312. [CrossRef] [PubMed]
8. Shriberg, L.D.; Strand, E.A.; Jakielski, K.J.; Mabie, H.L. Estimates of the prevalence of speech and motor speech disorders in persons with complex neurodevelopmental disorders. *Clin. Linguist. Phon.* **2019**, *33*, 707–736. [CrossRef] [PubMed]
9. Tierney, C.D.; Kurtz, M.; Souders, H. Clear as mud: Another look at autism, childhood apraxia of speech and auditory processing. *Curr. Opin. Pediatr.* **2012**, *24*, 394–399. [CrossRef]
10. Shriberg, L.D.; Paul, R.; Black, L.M.; Van Santen, J.P. The hypothesis of apraxia of speech in children with autism spectrum disorder. *J. Autism Dev. Disord.* **2011**, *41*, 405–426. [CrossRef]
11. Tager-Flusberg, H.; Kasari, C. Minimally verbal school-aged children with autism spectrum disorder: The neglected end of the spectrum. *Autism Res.* **2013**, *6*, 468–478. [CrossRef]

12. Weiss, L.A.; Shen, Y.; Korn, J.M.; Arking, D.E.; Miller, D.T.; Fossdal, R.; Saemundsen, E.; Stefansson, H.; Ferreira, M.A.R.; Green, T.; et al. Association between Microdeletion and Microduplication at 16p11.2 and Autism. *N. Engl. J. Med.* **2008**, *358*, 667–675. [CrossRef]
13. Rice, M.L. Toward epigenetic and gene regulation models of specific language impairment: Looking for links among growth, genes, and impairments. *J. Neurodev. Disord.* **2012**, *4*, 1–14. [CrossRef] [PubMed]
14. Gregor, A.; Albrecht, B.; Bader, I.; Bijlsma, E.K.; Ekici, A.B.; Engels, H.; Hackmann, K.; Horn, D.; Hoyer, J.; Klapecki, J.; et al. Expanding the clinical spectrum associated with defects in CNTNAP2 and NRXN1. *BMC Med. Genet.* **2011**, *12*. [CrossRef] [PubMed]
15. Wang, J.; Gong, J.; Li, L.; Chen, Y.; Liu, L.; Gu, H.T.; Luo, X.; Hou, F.; Zhang, J.; Song, R. Neurexin gene family variants as risk factors for autism spectrum disorder. *Autism Res.* **2018**, *11*, 37–43. [CrossRef] [PubMed]
16. Fisher, S.E.; Scharff, C. FOXP2 as a molecular window into speech and language. *Trends Genet.* **2009**, *25*, 166–177. [CrossRef] [PubMed]
17. Walker, R.M.; Hill, A.E.; Newman, A.C.; Hamilton, G.; Torrance, H.S.; Anderson, S.M.; Ogawa, F.; Derizioti, P.; Nicod, J.; Vernes, S.C.; et al. The DISC1 promoter: Characterization and regulation by FOXP2. *Hum. Mol. Genet.* **2012**, *21*, 2862–2872. [CrossRef] [PubMed]
18. Chen, Y.C.; Kuo, H.Y.; Bornschein, U.; Takahashi, H.; Chen, S.Y.; Lu, K.M.; Yang, H.Y.; Chen, G.M.; Lin, J.R.; Lee, Y.H.; et al. Foxp2 controls synaptic wiring of corticostriatal circuits and vocal communication by opposing Mef2c. *Nat. Neurosci.* **2016**, *19*, 1513–1522. [CrossRef]
19. Mukame, Z.; Konopka, G.; Wexler, E.; Osborn, G.E.; Dong, H.; Bergman, M.Y.; Levitt, P.; Geschwind, D.H. Regulation of MET by FOXP2, genes implicated in higher cognitive dysfunction and autism risk. *J. Neurosci.* **2011**, *31*, 11437–11442. [CrossRef]
20. Pagnozzi, A.M.; Conti, E.; Calderoni, S.; Fripp, J.; Rose, S.E. A systematic review of structural MRI biomarkers in autism spectrum disorder: A machine learning perspective. *Int. J. Dev. Neurosci.* **2018**, *71*, 68–82. [CrossRef]
21. Fiori, S.; Guzzetta, A.; Mitra, J.; Pannek, K.; Pasquariello, R.; Cipriani, P.; Tosetti, M.; Cioni, G.; Rose, S.E.; Chilosi, A. Neuroanatomical correlates of childhood apraxia of speech: A connectomic approach. *NeuroImage Clin.* **2016**, *12*, 894–901. [CrossRef]
22. Conti, E.; Calderoni, S.; Marchi, V.; Muratori, F.; Cioni, G.; Guzzetta, A. The first 1000 days of the autistic brain: A systematic review of diffusion imaging studies. *Front. Hum. Neurosci.* **2015**, *9*. [CrossRef]
23. Conti, E.; Mitra, J.; Calderoni, S.; Pannek, K.; Shen, K.K.; Pagnozzi, A.; Rose, S.; Mazzotti, S.; Scelfo, D.; Tosetti, M.; et al. Network over-connectivity differentiates autism spectrum disorder from other developmental disorders in toddlers: A diffusion MRI study. *Hum. Brain Mapp.* **2017**, *38*, 2333–2344. [CrossRef] [PubMed]
24. Kadis, D.S.; Goshulak, D.; Namasivayam, A.; Pukonen, M.; Kroll, R.; De Nil, L.F.; Pang, E.W.; Lerch, J.P. Cortical thickness in children receiving intensive therapy for idiopathic apraxia of speech. *Brain Topogr.* **2014**, *27*, 240–247. [CrossRef] [PubMed]
25. Morgan, A.; Bonthrone, A.; Liegeois, F.J. Brain basis of childhood speech and language disorders: Are we closer to clinically meaningful MRI markers? *Curr. Opin. Pediatr.* **2016**, *28*, 725–730. [CrossRef] [PubMed]
26. Alexander-Bloch, A.; Raznahan, A.; Bullmore, E.; Giedd, J. The convergence of maturational change and structural covariance in human cortical networks. *J. Neurosci.* **2013**, *33*, 2889–2899. [CrossRef]
27. Preston, J.L.; Molfese, P.J.; Mencl, W.E.; Frost, S.J.; Hoeft, F.; Fulbright, R.K.; Landi, N.; Grigorenko, E.L.; Seki, A.; Felsenfeld, S.; et al. Structural brain differences in school-age children with residual speech sound errors. *Brain Lang.* **2014**, *128*, 25–33. [CrossRef]
28. Morgan, A.T.; Su, M.; Reilly, S.; Conti-Ramsden, G.; Connelly, A.; Liégeois, F.J. A Brain Marker for Developmental Speech Disorders. *J. Pediatr.* **2018**, *198*, 234–239.e1. [CrossRef]
29. Boedhoe, P.S.W.; van Rooij, D.; Hoogman, M.; Twisk, J.W.R.; Schmaal, L.; Abe, Y.; Alonso, P.; Ameis, S.H.; Anikin, A.; Anticevic, A.; et al. Subcortical brain volume, regional cortical thickness, and cortical surface area across disorders: Findings from the ENIGMA ADHD, ASD, and OCD working groups. *Am. J. Psychiatry* **2020**, *177*, 834–843. [CrossRef]
30. Park, M.T.M.; Raznahan, A.; Shaw, P.; Gogtay, N.; Lerch, J.P.; Mallar Chakravarty, M. Neuroanatomical phenotypes in mental illness: Identifying convergent and divergent cortical phenotypes across autism, ADHD and schizophrenia. *J. Psychiatry Neurosci.* **2018**, *43*, 201–212. [CrossRef]

31. Swanson, M.R.; Shen, M.D.; Wolff, J.J.; Elison, J.T.; Emerson, R.W.; Styner, M.A.; Hazlett, H.C.; Truong, K.; Watson, L.R.; Paterson, S.; et al. Subcortical Brain and Behavior Phenotypes Differentiate Infants With Autism Versus Language Delay. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* **2017**, *2*, 664–672. [CrossRef]
32. Carlisi, C.O.; Norman, L.J.; Lukito, S.S.; Radua, J.; Mataix-Cols, D.; Rubia, K. Comparative Multimodal Meta-analysis of Structural and Functional Brain Abnormalities in Autism Spectrum Disorder and Obsessive-Compulsive Disorder. *Biol. Psychiatry* **2017**, *82*, 83–102. [CrossRef]
33. Lord, C.; Rutter, M.; DiLavore, P.C.; Risi, S.; Gotham, K.; Bishop, S. *ADOS-2 Autism Diagnostic Observation Schedule*, 2nd ed.; Western Psychological Services: Torrance, CA, USA, 2012.
34. Murray, E.; Mc Cabe, P.; Heard, R.; Ballard, K.J. Differential diagnosis of children with suspected childhood apraxia of speech. *J. Speech Lang. Hear. Res.* **2015**, *58*, 43–60. [CrossRef] [PubMed]
35. Retico, A.; Arezzini, S.; Bosco, P.; Calderoni, S.; Ciampa, A.; Coscetti, S.; Cuomo, S.; De Santis, L.; Fabiani, D.; Fantacci, M.E.; et al. ARIANNA: A research environment for neuroimaging studies in autism spectrum disorders. *Comput. Biol. Med.* **2017**, *87*, 1–7. [CrossRef] [PubMed]
36. Fischl, B.; Salat, D.H.; Busa, E.; Albert, M.; Dieterich, M.; Haselgrove, C.; Van Der Kouwe, A.; Killiany, R.; Kennedy, D.; Klaveness, S.; et al. Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron* **2002**, *33*, 341–355. [CrossRef]
37. Cortes, C.; Vapnik, V. Support-Vector Networks. *Mach. Learn.* **1995**, *20*, 273–297. [CrossRef]
38. Casanova, M.F.; Buxhoeveden, D.P.; Switala, A.E.; Roy, E. Minicolumnar pathology in autism. *Neurology* **2002**, *58*, 428–432. [CrossRef] [PubMed]
39. Piven, J.; Elison, J.T.; Zylka, M.J. Toward a conceptual framework for early brain and behavior development in Autism. *Mol. Psychiatry* **2017**, *22*, 1–10. [CrossRef]
40. Carper, R.A.; Moses, P.; Tigue, Z.D.; Courchesne, E. Cerebral lobes in autism: Early hyperplasia and abnormal age effects. *Neuroimage* **2002**, *16*, 1038–1051. [CrossRef]
41. Gori, I.; Giuliano, A.; Muratori, F.; Saviozzi, I.; Oliva, P.; Tancredi, R.; Cosenza, A.; Tosetti, M.; Calderoni, S.; Retico, A. Gray Matter Alterations in Young Children with Autism Spectrum Disorders: Comparing Morphometry at the Voxel and Regional Level. *J. Neuroimaging* **2015**, *25*, 866–874. [CrossRef]
42. Katuwal, G.J.; Cahill, N.D.; Baum, S.A.; Michael, A.M. The Predictive Power of Structural MRI in Autism Diagnosis. In Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS, Milan, Italy, 25–29 August 2015; pp. 4270–4273.
43. Hesling, I.; Dilharreguy, B.; Peppé, S.; Amirault, M.; Bouvard, M.; Allard, M. The integration of prosodic speech in high functioning Autism: A preliminary fMRI study. *PLoS ONE* **2010**, *5*, e11571. [CrossRef]
44. Eigsti, I.M.; Stevens, M.C.; Schultz, R.T.; Barton, M.; Kelley, E.; Naigles, L.; Orinstein, A.; Troyb, E.; Fein, D.A. Language comprehension and brain function in individuals with an optimal outcome from autism. *NeuroImage Clin.* **2016**, *10*, 182–191. [CrossRef]
45. Groen, W.; Teluij, M.; Buitelaar, J.; Tendolkar, I. Amygdala and Hippocampus Enlargement During Adolescence in Autism. *J. Am. Acad. Child Adolesc. Psychiatry* **2010**, *49*, 552–560. [CrossRef] [PubMed]
46. Schumann, C.M.; Hamstra, J.; Goodlin-Jones, B.L.; Lotspeich, L.J.; Kwon, H.; Buonocore, M.H.; Lammers, C.R.; Reiss, A.L.; Amaral, D.G. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J. Neurosci.* **2004**, *24*, 6392–6401. [CrossRef] [PubMed]
47. Barnea-Goraly, N.; Frazier, T.W.; Piacenza, L.; Minshew, N.J.; Keshavan, M.S.; Reiss, A.L.; Hardan, A.Y. A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2014**, *48*, 124–128. [CrossRef] [PubMed]
48. Aylward, E.H.; Minshew, N.J.; Goldstein, G.; Honeycutt, N.A.; Augustine, A.M.; Yates, K.O.; Barta, P.E.; Pearlson, G.D. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology* **1999**, *53*, 2145–2150. [CrossRef]
49. Sparks, B.F.; Friedman, S.D.; Shaw, D.W.; Aylward, E.H.; Echelard, D.; Artru, A.A.; Maravilla, K.R.; Giedd, J.N.; Munson, J.; Dawson, G.; et al. Brain structural abnormalities in young children with autism spectrum disorder. *Neurology* **2002**, *59*, 184–192. [CrossRef]
50. Nicolson, R.; DeVito, T.J.; Vidal, C.N.; Sui, Y.; Hayashi, K.M.; Drost, D.J.; Williamson, P.C.; Rajakumar, N.; Toga, A.W.; Thompson, P.M. Detection and mapping of hippocampal abnormalities in autism. *Psychiatry Res. Neuroimaging* **2006**, *148*, 11–21. [CrossRef]
51. Langen, M.; Durston, S.; Staal, W.G.; Palmen, S.J.M.C.; van Engeland, H. Caudate Nucleus Is Enlarged in High-Functioning Medication-Naive Subjects with Autism. *Biol. Psychiatry* **2007**, *62*, 262–266. [CrossRef]

52. Hollander, E.; Anagnostou, E.; Chaplin, W.; Esposito, K.; Haznedar, M.M.; Licalzi, E.; Wasserman, S.; Soorya, L.; Buchsbaum, M. Striatal volume on magnetic resonance imaging and repetitive behaviors in autism. *Biol. Psychiatry* **2005**, *58*, 226–232. [CrossRef]
53. Calderoni, S.; Bellani, M.; Hardan, A.Y.; Muratori, F.; Brambilla, P. Basal ganglia and restricted and repetitive behaviours in Autism Spectrum Disorders: Current status and future perspectives. *Epidemiol. Psychiatr. Sci.* **2014**, *23*, 235–238. [CrossRef]
54. Floresco, S.B. The nucleus accumbens: An interface between cognition, emotion, and action. *Annu. Rev. Psychol.* **2015**, *66*, 25–32. [CrossRef]
55. Supekar, K.; Kochalka, J.; Schaer, M.; Wakeman, H.; Qin, S.; Padmanabhan, A.; Menon, V. Deficits in mesolimbic reward pathway underlie social interaction impairments in children with autism. *Brain* **2018**, *141*, 2795–2805. [CrossRef] [PubMed]
56. Kohls, G.; Chevallier, C.; Troiani, V.; Schultz, R.T. Social "wanting" dysfunction in autism: Neurobiological underpinnings and treatment implications. *J. Neurodev. Disord.* **2012**, *4*, 1–20. [CrossRef] [PubMed]
57. Cauda, F.; Geda, E.; Sacco, K.; D'Agata, F.; Duca, S.; Geminiani, G.; Keller, R. Grey matter abnormality in autism spectrum disorder: An activation likelihood estimation meta-analysis study. *J. Neurol. Neurosurg. Psychiatry* **2011**, *82*, 1304–1313. [CrossRef] [PubMed]
58. Foster, N.E.V.; Doyle-Thomas, K.A.R.; Tryfon, A.; Ouimet, T.; Anagnostou, E.; Evans, A.C.; Zwaigenbaum, L.; Lerch, J.P.; Lewis, J.D.; Hyde, K.L. Structural Gray Matter Differences during Childhood Development in Autism Spectrum Disorder: A Multimetric Approach. *Pediatr. Neurol.* **2015**, *53*, 350–359. [CrossRef]
59. Stoodley, C.J.; Schmahmann, J.D. Functional topography in the human cerebellum: A meta-analysis of neuroimaging studies. *Neuroimage* **2009**, *44*, 489–501. [CrossRef]
60. Fatemi, S.H.; Aldinger, K.A.; Ashwood, P.; Bauman, M.L.; Blaha, C.D.; Blatt, G.J.; Chauhan, A.; Chauhan, V.; Dager, S.R.; Dickson, P.E.; et al. Consensus paper: Pathological role of the cerebellum in Autism. *Cerebellum* **2012**, *11*, 777–807. [CrossRef]
61. St George, M.; Kutas, M.; Martinez, A.; Sereno, M.I. Semantic integration in reading: Engagement of the right hemisphere during discourse processing. *Brain* **1999**, *122*, 1317–1325. [CrossRef]
62. Buchanan, C.P. A Neuropsychological Investigation of the "Weak Central Coherence" Anomaly in Autism. Ph.D. Thesis, Rosalind Franklin University, North Chicago, IL, USA, 2002.
63. Condouris, K.; Meyer, E.; Tager-Flusberg, H. The relationship between standardized measures of language and measures of spontaneous speech in children with autism. *Am. J. Speech-Lang. Pathol.* **2003**, *12*, 349–358. [CrossRef]
64. Wang, A.T.; Lee, S.S.; Sigman, M.; Dapretto, M. Neural basis of irony comprehension in children with autism: The role of prosody and context. *Brain* **2006**, *129*, 932–943. [CrossRef]
65. Eigsti, I.M.; Schuh, J.; Mencl, E.; Schultz, R.T.; Paul, R. The neural underpinnings of prosody in autism. *Child Neuropsychol.* **2012**, *18*, 600–617. [CrossRef] [PubMed]
66. Xiao, Z.; Qiu, T.; Ke, X.; Xiao, X.; Xiao, T.; Liang, F.; Zou, B.; Huang, H.; Fang, H.; Chu, K.; et al. Autism spectrum disorder as early neurodevelopmental disorder: Evidence from the brain imaging abnormalities in 2–3 years old toddlers. *J. Autism Dev. Disord.* **2014**, *44*, 1633–1640. [CrossRef] [PubMed]
67. Retico, A.; Gori, I.; Giuliano, A.; Muratori, F.; Calderoni, S. One-class support vector machines identify the language and default mode regions as common patterns of structural alterations in young children with autism spectrum disorders. *Front. Neurosci.* **2016**, *10*. [CrossRef]
68. Jou, R.J.; Minshew, N.J.; Keshavan, M.S.; Vitale, M.P.; Hardan, A.Y. Enlarged right superior temporal gyrus in children and adolescents with autism. *Brain Res.* **2010**, *1360*, 205–212. [CrossRef] [PubMed]
69. Hardan, A.Y.; Muddasani, S.; Vemulapalli, M.; Keshavan, M.S.; Minshew, N.J. An MRI study of increased cortical thickness in autism. *Am. J. Psychiatry* **2006**, *163*, 1290–1292. [CrossRef]
70. Hyde, K.L.; Samson, F.; Evans, A.C.; Mottron, L. Neuroanatomical differences in brain areas implicated in perceptual and other core features of autism revealed by cortical thickness analysis and voxel-based morphometry. *Hum. Brain Mapp.* **2010**, *31*, 556–566. [CrossRef] [PubMed]
71. Raznahan, A.; Lenroot, R.; Thurm, A.; Gozzi, M.; Hanley, A.; Spence, S.J.; Swedo, S.E.; Giedd, J.N. Mapping cortical anatomy in preschool aged children with autism using surface-based morphometry. *NeuroImage Clin.* **2013**, *2*, 111–119. [CrossRef]

72. Hazlett, H.C.; Poe, M.D.; Gerig, G.; Styner, M.; Chappell, C.; Smith, R.G.; Vachet, C.; Piven, J. Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch. Gen. Psychiatry* **2011**, *68*, 467–476. [CrossRef]
73. Hadjikhani, N.; Joseph, R.M.; Snyder, J.; Tager-Flusberg, H. Anatomical differences in the mirror neuron system and social cognition network in autism. *Cereb. Cortex* **2006**, *16*, 1276–1282. [CrossRef]
74. Zielinski, B.A.; Prigge, M.B.D.; Nielsen, J.A.; Froehlich, A.L.; Abildskov, T.J.; Anderson, J.S.; Fletcher, P.T.; Zygmunt, K.M.; Travers, B.G.; Lange, N.; et al. Longitudinal changes in cortical thickness in autism and typical development. *Brain* **2014**, *137*, 1799–1812. [CrossRef]
75. Smith, E.; Thurm, A.; Greenstein, D.; Farmer, C.; Swedo, S.; Giedd, J.; Raznahan, A. Cortical thickness change in autism during early childhood. *Hum. Brain Mapp.* **2016**, *37*, 2616–2629. [CrossRef] [PubMed]
76. Hillis, A.E.; Work, M.; Barker, P.B.; Jacobs, M.A.; Breese, E.L.; Maurer, K. Re-examining the brain regions crucial for orchestrating speech articulation. *Brain* **2004**, *127*, 1479–1487. [CrossRef] [PubMed]
77. Dronkers, N.F. A new brain region for coordinating speech articulation. *Nature* **1996**, *384*, 159–161. [CrossRef] [PubMed]
78. Ackermann, H.; Riecker, A. The contribution(s) of the insula to speech production: A review of the clinical and functional imaging literature. *Brain Struct. Funct.* **2010**, *214*, 419–433. [CrossRef] [PubMed]
79. Vallar, G.; Papagno, C.; Rusconi, M.L.; Bisiach, E. Vestibular Stimulation, Spatial Hemineglect and Dysphasia. Selective Effects? *Cortex* **1995**, *31*, 589–593. [CrossRef]
80. Gow, D.W.; Caplan, D.N. New levels of language processing complexity and organization revealed by granger causation. *Front. Psychol.* **2012**, *3*. [CrossRef]
81. Budisavljevic, S.; Dell'Acqua, F.; Rijsdijk, F.V.; Kane, F.; Picchioni, M.; McGuire, P.; Toulopoulou, T.; Georgiades, A.; Kalidindi, S.; Kravariti, E.; et al. Age-related differences and heritability of the perisylvian language networks. *J. Neurosci.* **2015**, *35*, 12625–12634. [CrossRef]
82. Rushworth, M.F.S.; Taylor, P.C.J. TMS in the parietal cortex: Updating representations for attention and action. *Neuropsychologia* **2006**, *44*, 2700–2716. [CrossRef]
83. Price, C.J. The anatomy of language: A review of 100 fMRI studies published in 2009. *Ann. N. Y. Acad. Sci.* **2010**, *1191*, 62–88. [CrossRef]
84. Nakamichi, N.; Takamoto, K.; Nishimaru, H.; Fujiwara, K.; Takamura, Y.; Matsumoto, J.; Noguchi, M.; Nishijo, H. Cerebral hemodynamics in speech-related cortical areas: Articulation learning involves the inferior frontal gyrus, ventral sensory-motor cortex, and parietal-temporal sylvian area. *Front. Neurol.* **2018**, *9*. [CrossRef]
85. Price, C.J. A review and synthesis of the first 20years of PET and fMRI studies of heard speech, spoken language and reading. *Neuroimage* **2012**, *62*, 816–847. [CrossRef] [PubMed]
86. Rüschemeyer, S.A.; Fiebach, C.J.; Kempe, V.; Friederici, A.D. Processing lexical semantic and syntactic information in first and second language: FMRI evidence from German and Russian. *Hum. Brain Mapp.* **2005**, *25*, 266–286. [CrossRef] [PubMed]
87. Friederici, A.D. The Neural Basis of Language Development and Its Impairment. *Neuron* **2006**, *52*, 941–952. [CrossRef] [PubMed]
88. Bornkessel-Schlesewsky, I.; Schlesewsky, M. Reconciling time, space and function: A new dorsal-ventral stream model of sentence comprehension. *Brain Lang.* **2013**, *125*, 60–76. [CrossRef] [PubMed]
89. Friederici, A.D.; Gierhan, S.M.E. The language network. *Curr. Opin. Neurobiol.* **2013**, *23*, 250–254. [CrossRef] [PubMed]
90. Elmer, S. Broca pars triangularis constitutes a "hub" of the language-control network during simultaneous language translation. *Front. Hum. Neurosci.* **2016**, *10*. [CrossRef] [PubMed]
91. Krishnan, S.; Watkins, K.E.; Bishop, D.V.M. Neurobiological Basis of Language Learning Difficulties. *Trends Cogn. Sci.* **2016**, *20*, 701–714. [CrossRef]
92. Semendeferi, K.; Armstrong, E.; Schleicher, A.; Zilles, K.; Van Hoesen, G.W. Prefrontal cortex in humans and apes: A comparative study of area 10. *Am. J. Phys. Anthropol.* **2001**, *114*, 224–241. [CrossRef]
93. Allegri, R.F. Prefrontal cortex in memory and attention. *Rev. Neurol.* **2001**, *32*, 449–453. [CrossRef]
94. Middleton, F.A.; Strick, P.L. Basal ganglia and cerebellar loops: Motor and cognitive circuits. *Brain Res. Rev.* **2000**, *31*, 236–250. [CrossRef]
95. Packard, M.G.; Knowlton, B.J. Learning and memory functions of the basal ganglia. *Annu. Rev. Neurosci.* **2002**, *25*, 563–593. [CrossRef] [PubMed]

96. Estes, A.; Shaw, D.W.W.; Sparks, B.F.; Friedman, S.; Giedd, J.N.; Dawson, G.; Bryan, M.; Dager, S.R. Basal ganglia morphometry and repetitive behavior in young children with autism spectrum disorder. *Autism Res.* **2011**, *4*, 212–220. [CrossRef] [PubMed]
97. Nayate, A.; Bradshaw, J.L.; Rinehart, N.J. Autism and Asperger's disorder: Are they movement disorders involving the cerebellum and/or basal ganglia? *Brain Res. Bull.* **2005**, *67*, 327–334. [CrossRef] [PubMed]
98. Belton, E.; Salmond, C.H.; Watkins, K.E.; Vargha-Khadem, F.; Gadian, D.G. Bilateral brain abnormalities associated with dominantly inherited verbal and orofacial dyspraxia. *Hum. Brain Mapp.* **2003**, *18*, 194–200. [CrossRef] [PubMed]
99. Liégeois, F.J.; Hildebrand, M.S.; Bonthrone, A.; Turner, S.J.; Scheffer, I.E.; Bahlo, M.; Connelly, A.; Morgan, A.T. Early neuroimaging markers of FOXP2 intragenic deletion. *Sci. Rep.* **2016**, *6*. [CrossRef] [PubMed]
100. Barsotti, J.; Mangani, G.; Nencioli, R.; Pfanner, L.; Tancredi, R.; Cosenza, A.; Sesso, G.; Narzisi, A.; Muratori, F.; Cipriani, P.; et al. Grammatical comprehension in italian children with autism spectrum disorder. *Brain Sci.* **2020**, *10*, 510. [CrossRef] [PubMed]
101. Retico, A.; Tosetti, M.; Muratori, F.; Calderoni, S. Neuroimaging-based methods for autism identification: A possible translational application? *Funct. Neurol.* **2014**, *29*, 231–239. [CrossRef]
102. Frangou, S.; Modabbernia, A.; Doucet, G.; Papachristou, E.; Williams, S.C.; Agartz, I.; Aghajani, M.; Akudjedu, T.; Albajes-Eizagirre, A.; Alnæs, D.; et al. Cortical Thickness Trajectories across the Lifespan: Data from 17,075 healthy individuals aged 3–90 years. *bioRxiv* **2020**. [CrossRef]
103. Szucs, D.; Ioannidis, J.P. Sample size evolution in neuroimaging research: An evaluation of highly-cited studies (1990–2012) and of latest practices (2017–2018) in high-impact journals. *Neuroimage* **2020**, *221*, 117164. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

Development of a New Measure for Assessing Mentalizing: The Multidimensional Mentalizing Questionnaire (MMQ)

Alessio Gori [1,*], Alessandro Arcioni [2], Eleonora Topino [3], Giuseppe Craparo [4] and Rosapia Lauro Grotto [1]

[1] Department of Health Sciences, University of Florence, Via di San Salvi 12, pad. 26, 50135 Firenze, Italy; rosapia.laurogrotto@unifi.it
[2] Department of Education, Languages, Intercultures, Literatures and Psychology (Psychology Section), University of Florence, 50121 Firenze, Italy; alessandro.arcioni25@gmail.com
[3] Department of Human Sciences, LUMSA University of Rome, Via della Traspontina 21, 00193 Rome, Italy; eleonora.topino@gmail.com
[4] Faculty of Human and Social Sciences, UKE—Kore University of Enna, Via Cittadella Universitaria s.n.c., 94100 Enna, Italy; giuseppe.craparo@unikore.it
* Correspondence: alessio.gori@unifi.it

Abstract: This research consists of two studies which aimed to: (1) evaluate the psychometric properties of a new self-report measure for the assessment of mentalizing, the Multidimensional Mentalizing Questionnaire (MMQ); and (2) investigate the ability of the instrument to discriminate between community and clinical populations. A sample of 349 participants (19% male, 81% female; M_{age} = 38.6, SD = 15.3) filled in the MMQ and other self-report measures, in order to assess the factor structure, reliability and some aspects of construct validity of the measure. Then, a clinical sample (N = 46; 52% male and 48% female; M_{age} = 33.33, SD = 12.257) and a community one (N = 50; 42% male and 58% female; M_{age} = 38.86, SD = 16.008) filled in the MMQ, to assess its clinical sensitivity. The factorial analysis identified six principal dimensions of the measure: reflexivity, ego-strength, relational attunement, relational discomfort, distrust, and emotional dyscontrol. The MMQ showed satisfactory psychometric properties and a theoretically relevant factor structure. Furthermore, significantly greater impairment in mentalizing was found in the clinical sample in respect of the community one. The findings are discussed in terms of clinical implications, emphasizing the usefulness of the MMQ in both research and clinical practice.

Keywords: mentalization; self-report measure; multilevel model; integration; assessment

Citation: Gori, A.; Arcioni, A.; Topino, E.; Craparo, G.; Lauro Grotto, R. Development of a New Measure for Assessing Mentalizing: The Multidimensional Mentalizing Questionnaire (MMQ). *J. Pers. Med.* **2021**, *11*, 305. https://doi.org/10.3390/jpm11040305

Academic Editor: Marco Costanzi

Received: 5 March 2021
Accepted: 14 April 2021
Published: 15 April 2021

1. Introduction

Even though mentalizing occurs from a very early age to give some meaning to the environment, an analysis seems to be a hard task because it is a complex construct to identify and enclose within its boundaries. Bateman and Fonagy [1] define mentalizing as "the process by which we make sense of each other and ourselves, implicitly and explicitly, in terms of subjective states and mental processes. A profoundly social construct in the sense that we are attentive to the mental states of those we are with, physically or psychologically" (p. 11). Being a "process", Allen [2] additionally supports the use of the participle "mentalizing" instead of the noun "mentalization", in order to emphasize mental activity. Seemingly, mentalizing is rooted in four different areas of psychology: first, cognitive psychology, with the identification of the construct of theory of mind [3], described as a module phylogenetically tasked with processing the others' mental states. Second, Bion [4] proposed a theory "of the thought" where imagination arises as a response to separation anxiety. The mother, thanks to her *reverie* (i.e., a specific function which allows the mother to feel the infant inside her, and to give shape and words to the infant's experience), can make sense of the raw material inside the infant (beta elements) to help them create building blocks for their emotional and intellectual development (alpha elements).

All this appears to be similar to Fonagy theory. Additionally, French-speaking psychoanalysts provided their contribution in developing the concept of *penseé operatoire* [5]. Today, we may define this concept as a "failure" in the mentalizing process, because it recalls hyper-concretism of thought deprived of its imaginative mental activity. Furthermore, Lecours and Bouchard [6] theorized a development of thought considered today similar to mentalizing. The authors focus on how human beings evolve from libidinal impulses to attribution of meaning. Finally, a relevant contribution was given by Anglo-Saxon psychoanalysis and by the Attachment Theory. Winnicott [7] introduced the concept of maternal *mirroring* within the transitional space set in the dyad with the infant; Bowlby [8] theorized that a positive caregiver-infant interaction favors an attachment system which helps the infant regulate their emotional state. Mayes [9] highlighted that this is easily detected when measuring the arousal levels in infants with differing attachment styles. Recent studies showed how different mentalizing ways are linked to distinct brain region activity and this reflects the actual conception of an environment-dependent construct based on four different polarities: automatic and controlled mentalizing, internal and external mentalizing, self-other mentalizing, and cognitive-affective mentalizing [10]. Controlled mentalizing requires a number of slow and typically verbal skills which demand mental effort, high arousal levels and focused attention; implicit or automatic mentalizing is likely to be based on simple heuristics [11]. Internal mentalizing is referred to as the process focused on thought, feelings, and internal experience of both one's Self and others, while external mentalizing is referred to as external features and behavior of both one's Self and others [10]. In normal development, the separation-individuation process drives the infant to differentiate their own experiences from those of others. In this process, reciprocity between mirroring and reflective functioning seemingly plays a crucial role: The implicit perspective-taking is strictly connected to an active giving of meaning and differentiating of the behavior of both one's Self and others [10]. Cognitive-affective mentalizing has a precursor in the Baron-Cohen dualism, i.e., the theory of mind module and empathy system [3]. Such apparent dichotomy reflects the difference between people who usually tend to interpret their environment through emotions, with the risk of ending in projective identification [12], and those who tend to rationalize their affective experience.

Given the growing interest on mentalizing in mental health, some authors have engaged in the development of assessment methods. These have been developed as experimental-observational tasks, e.g., the Reading the Mind in the Eyes Test [13], narrative-based measures, such as the Reflective Functioning Scale [14], or questionnaires, among which the Mentalization Scale [15], the Mentalization Questionnaire [16], and the Reflective Functioning Questionnaire [17] can be mentioned. Concerning this last type of measures, to the authors' knowledge, the Italian context includes clinician-report scales, i.e., the Mentalization Imbalances Scale [18], the Modes of Mentalization Scale [19], and a self-report one, i.e., the Italian version of the Reflective Functioning Scale [20]. The latter is an agile eight-item questionnaire with a satisfactory reliability and construct validity which effectively discriminates between borderline personality patients and healthy controls and consists of two subscales giving an evaluation of the respondents' hypo- and hyper-mentalizing attitudes [20]. However, mentalizing is a multifaceted construct, in which problems can be an expression of imbalance in the different polarities underpin it, while functional levels are an expression of balance between the different dimensions included in mentalizing. Therefore, the present research aims to respond to the need to enhance the framework of the evaluation methods concerning this important construct, by proposing a new self-report questionnaire for the assessment of mentalizing which also explore its subcomponents.

This research consists of two studies. The aim of the first study is to present a new measure for the assessment of mentalizing, the Multidimensional Mentalizing Questionnaire, based on an integrated perspective and inspired by the criteria of brevity, good psychometric properties, and usefulness in therapeutic activity both in the initial stages and during the process. All this, based on these aspects:

1. An assessment procedure of mentalizing based on cognitive-affective, self-other, internal-external, explicit-implicit axes;
2. An integration of both positive and negative mentalizing clusters which express on different polarities.

The aim of the second study is to investigate the clinical sensitivity of the MMQ, by assessing the ability of the measure to discriminate between community and clinical populations. In particular, based on the above-described literature and in recent evidence supporting the role of imbalances in mentalizing and in its dimensions in psychopathology (see [21] for a review), it is supposed to find higher levels of impairment in mentalizing in the clinical sample, also by exploring the subdimensions of the construct.

2. Study 1

2.1. Materials and Methods

2.1.1. Participants

This study involved a sample of 349 subjects (19.0% male, 81.0% female), with an age ranging from 16 to 20 years (M = 38.6, SD = 15.3). Most of the subjects were unmarried (59.6% single) and came from central Italy (62.7%). Concerning the professional condition, 32.5% of participants were employees, 31.2% were students and 21.1% were freelance. Furthermore, 129 subjects had a Master's degree and 131 had a high school diploma.

2.1.2. Procedure

The 33 items were elaborated to reflect the core aspects of the construct, as described in the Handbook of Mentalizing in Mental Health Practice [12]. This phase has been implemented by organizing focus groups with a pool of researchers and clinical experts, to make this step more effective. The questions were written in order to obtain answers along a five-point continuum, with a Likert scale ranging from 1 = "Not at all" to 5 = "A great deal". Cohen's Kappa coefficient was examined to determine inter-rater compliance about the goodness of each item, showing a good concordance (K = 0.80). Participants were randomly recruited through a snowball-like spreading strategy of an anonymous on-line link. They completed the self-report measures together with a demographic questionnaire (sex, age, marital status, profession and degree of study) on the Google Forms platform after they were informed about the aim of the research. Written informed consent was obtained from all subjects. They did not receive any form of compensation for their involvement in the study and were free to leave at any time. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved by the Ethical Committee of the Integrated Psychodynamic Psychotherapy Institute (IPPI) (ethical approval number 002/2020).

2.1.3. Measures

The Multidimensional Mentalizing Questionnaire (MMQ)

The Multidimensional Mentalizing Questionnaire (MMQ) is a self-report measure that consists of 33 items, covering the different core aspects the construct on four different axes: (1) Cognitive–affective; (2) self–other; (3) outside–inside; and (4) explicit–implicit. Items were reviewed for clarity and to avoid ambiguity or double negatives. Response format was on a five-point Likert scale from 1 = "Not at all" to 5 = "A great deal".

20-Item Toronto Alexithymia Scale (TAS-20)

The 20-Item Toronto Alexithymia Scale (TAS-20) [22,23] is a 20-item self-report scale designed to assess the level of alexithymia. Each item is rated on a five-point scale ranging from 1 (strongly disagree) to 5 (strongly agree), measuring three main dimensions: (1) difficulty in identifying feelings and distinguishing between feelings and bodily sensations in emotional activation, (2) difficulty in the verbal expression of emotions, and (3) externally

oriented thinking. In this study the Italian version of Bressi and colleagues [24] was used and showed a good internal consistency with a Cronbach's alpha of 0.84.

Barratt Impulsiveness Scale (BIS-11)

The Barratt Impulsiveness Scale (BIS-11) [25] is a 30-item self-repot measure designed to assess impulsiveness. Each item is rated on a four-point Likert scale ranging from 1 (rarely/never) to 4 (almost always/always), measuring six first-order factors grouped into three second-order factors: (1) Attentional impulsiveness, composed by attention and cognitive instability first-order factors; (2) motor impulsiveness, composed by motor and perseverance first-order factors; and (3) non-planning impulsiveness, composed by complexity and self-control first-order factors. In the present study the Italian version of Fossati and colleagues [26] was used, showing a satisfactory internal consistency ($\alpha = 0.75$).

Rosenberg Self-Esteem Scale (RSES)

The Rosenberg Self-Esteem Scale (RSES) [27] is a 10-item self-report questionnaire designed to measure global self-esteem. Each item is rated on a four-point scale ranging from strongly agree to strongly disagree. In this study the Italian version of Prezza and colleagues [28] was used, showing good internal consistency ($\alpha = 0.89$).

General Self-Efficacy Scale (GSE)

The General Self-Efficacy Scale (GSE) [29] is a 10-item self-report questionnaire designed to measure self-efficacy. Each item is rated on a four-point Likert scale ranging from 1 (not at all true for me) to 4 (very true for me). In this study the Italian version of Sibilia, Schwarzer, and Jerusalem [30] was used and showed a good internal consistency with a Cronbach's alpha of 0.91.

Psychological Treatment Inventory—Attachment Styles Scale (PTI-ASS)

The Psychological Treatment Inventory-Attachment Styles Scale (PTI-ASS) [31] is a 22-item self-report scale designed to assess the quality of adult attachment and is a section of the Psychological Treatment Inventory [32]. Each item is rated on a five-point Likert scale ranging from 1 (Not at All) to 5 (A Great Deal) and evaluates attachment style between in secure, preoccupied, avoidant or unresolved. The subscales' Cronbach α in the current study were of 0.80, 0.84, 0.78, and 0.68, respectively.

Italian Ten Item Personality Inventory (I-TIPI)

The Ten Item Personality Inventory (TIPI) [33] is a 10-item self-report scale designed to assess personality traits according to the Big Five model [34]. Each item is rated on a seven-point Likert scale ranging from 0 (Disagree strongly) to 7 (Agree strongly), which evaluates 5 dimensions: extraversion, agreeableness, conscientiousness, emotional stability and openness. In this study, the Italian Ten Item Personality Inventory (I-TIPI) of Di Fabio, Gori and Giannini [35] was used, with subscales' Cronbach α of 0.89, 0.70, 0.76, 0.83 and 0.79, respectively.

Insight Orientation Scale (IOS)

The Insight Orientation Scale (IOS) [36] is a seven-item self-report measure designed to assessing some of the characteristics of insight, including behaviors, feelings and opinions about this construct. Each item is rated on a five-point Likert scale ranging from 1 (not at all) to 5 (a great deal), focused on seven core aspects of insight: level of consciousness, problem solving, restructuring (behavior change), awareness, complexity (abstraction, depth), surprise, and self-reflectiveness (thoughtfulness). In the present sample, the scale showed a Cronbach α of 0.76.

2.1.4. Data Analysis

Data were analyzed with the SPSS software (IBM-SPSS 25.0 version, IBM, Armonk, NY, USA) for Windows and MPlus Version 8.1 [37]. Descriptive statistics were calculated. An exploratory factor analysis (EFA) with a principal axis factoring extraction method (Promax rotation with Kaiser normalization) was performed in order to verify the factor structure of the Multidimensional Mentalizing-Q. Then, the factor model was tested through a confirmatory factor analysis (CFA), considering the following indices: (1) the model chi-square (χ^2), indicating a good model fit when the probability value is nonsignificant [38]; (2) the root mean square error of approximation (RMSEA), with accepted values ≤ 0.08 [39]; (3) the Tucker Lewis index (TLI), for which Kline [40] considers reasonable values ≥ 0.90; (4) the Comparative Fit Index (CFI), for which reasonable values were $\geq$0.90 [40]; (5) the standardized root mean square residual (SRMR), with recommended values ≤ 0.08 [41]. Reliability of both, the scale and its factors, was calculated using the Cronbach's alpha coefficient. Finally, in order to assess some aspects of construct validity, Pearson's correlation was carried out between the MMQ subscales and the other investigated variables.

2.2. Results

Descriptive statistics for the sample were reported in Table 1, while means and standard deviations were showed for each item of the Multidimensional Mentalizing-Q in Table 2.

Table 1. Demographics variables of the sample (n = 349).

Characteristics	$M \pm SD$	n	%
Age	38.56 ± 15.27		
Sex			
Male		78	19.04
Female		319	80.96
Provenience			
Northern Italy		79	20.05
Central Italy		247	62.69
Southern Italy		68	17.26
Marital Status			
Single		235	59.64
Married		119	30.20
Separated		18	4.57
Divorced		17	4.31
Widowed		5	1.27
Professional condition			
Unemployed		6	1.52
Freelance		83	21.07
Employee		128	32.49
Trader		10	2.54
Housewife		22	5.58
Student		123	31.22
Retired		16	4.06
Artisan		6	1.52
Study degree			
Elementary school (5 years)		4	1.02
Middle School diploma		36	9.14
High School diploma		131	33.25
University degree		75	19.04
Master's degree		129	32.74
Post-Lauream Specialization		19	4.82

Table 2. Factor structure of the Multidimensional Mentalizing Questionnaire and means and standard deviations of the items.

Item	F1	F2	F3	F4	F5	F6	M(SD)
1. I often try to explain what is happening to me [a] *Provo spesso a darmi delle spiegazioni su ciò che mi accade*	0.80						4.11 (0.92)
16. I ponder over what happens to me [a] *Rifletto su quello che mi succede*	0.79						2.83 (1.25)
18. I often think about why things happen [a] *Rifletto spesso sul perché delle cose*	0.78						2.84 (1.36)
32. I'm keen on understanding why certain things happen to me [a] *M'interessa capire perché certe cose mi accadono*	0.73						3.87 (0.91)
10. I'm interested in understanding my mental processes [a] *M'interessa comprendere i miei processi mentali*	0.67						3.77 (1.02)
17. I find beneficial to analyse my behaviour [a] *Trovo beneficio ad analizzare il mio comportamento*	0.62						4.26 (0.86)
31. I am a thoughtful person [a] *Sono una persona riflessiva*	0.53						2.57 (1.24)
8. I am able to reflect on my behaviours [a] *Sono in grado di riflettere sui miei comportamenti*	0.47						4.18 (0.76)
6. Understanding what others feel is crucial in understanding their actions [a] *Capire ciò che gli altri provano è importante per comprendere le loro azioni*	0.44						1.91 (1.07)
30. I am able to cope with difficult situations [a] *Sono in grado di affrontare situazioni difficili*		0.79					4.20 (1.01)
25. I am able to bear the emotional load of stressful situations [a] *Sono in grado di sopportare il carico emotivo delle situazioni stressanti*		0.76					3.46 (0.98)
24. I am able to sort out difficult problems when life presents those to me [a] *Sono in grado di risolvere problemi anche complessi che la vita mi mette davanti*		0.71					2.51 (1.17)
11. I can tolerate frustrations of daily life [a] *Sono in grado di tollerare le frustrazioni della vita di tutti i giorni*		0.68					2.61 (1.17)
22. I can usually adapt myself to different contexts with no difficulties [a] *In generale so adattarmi a diversi contesti senza difficoltà*		0.54					4.06 (0.93)
26. When I feel an intense emotion, I can control it [a] *Quando provo un'emozione forte riesco a controllarla*		0.44					2.54 (1.27)
28. I can easily attune to other people's thinking [a] *Riesco a sintonizzarmi facilmente sul pensiero altrui*			0.83				4.23 (0.88)
5. I can tune in other other people's mental states [a] *Riesco a sintonizzarmi sugli stati mentali degli altri*			0.80				4.00 (0.98)
14. I'm able to empathize with others when they tell me something [a] *Mi immedesimo negli altri quando mi raccontano qualcosa*			0.66				4.14 (0.97)
4. I'm able to get the deepest aspects of people around me [a] *Riesco a cogliere gli aspetti più profondi delle persone a me vicine*			0.61				2.46 (1.31)
21. I am sensitive to what happens to others [a] *Sono sensibile a quello che accade agli altri*			0.49				2.61 (1.16)
12. Others don't understand me [a] *Gli altri non mi capiscono*				0.70			4.13 (0.85)
9. Relationships with other people prevent me from being myself [a] *Le relazioni con gli altri mi impediscono di essere me stesso*				0.68			3.90 (1.01)
27. People abandon me [a] *Le persone mi abbandonano*				0.59			3.39 (1.09)
15. I am afraid to open up with other people [a] *Ho paura ad aprirmi con gli altri*				0.56			3.77 (0.91)
33. Some people are the cause of my problems [a] *Alcune persone sono la causa dei miei problemi*				0.39			3.53 (1.09)
13. It's better to beware of others [a] *È meglio stare attenti agli altri*					0.76		3.06 (1.08)
29. It's better to beware of strangers [a] *Bisogna ben guardarsi dalle persone che non si conoscono*					0.73		1.97 (1.17)
20. I don't trust others [a] *Non mi fido degli altri*					0.56		3.76 (1.00)
19. For me things are either white or black [a] *Per me le cose sono o bianche o nere*					0.42		2.76 (1.21)
2. I am an impulsive person [a] *Sono una persona impulsiva*						0.65	3.89 (0.91)
7. I sometimes feel like I am losing control of my emotions [a] *A volte ho la sensazione di perdere il controllo delle mie emozioni*						0.59	4.02 (0.98)
3. I sometimes experience mood swings I can't control [a] *Talvolta ho degli sbalzi di umore che non riesco a controllare*						0.56	4.09 (1.04)
23. It happens to me to have conflicting emotions [a] *Mi capita di provare emozioni contrastanti*						0.53	2.73 (1.36)

Note: F1 = reflexivity (riflessività; $\alpha = 0.89$); F2 = ego-strength (adattamento; $\alpha = 0.81$); F3 = relational attunement (sintonizzazione relazionale; $\alpha = 0.82$); F4 = relational discomfort (disagio relazionale; $\alpha = 0.76$); F5 = distrust (sfiducia; $\alpha = 0.74$); F6 = emotional dyscontrol (discotrollo emotivo; $\alpha = 0.72$). Italics indicate the Italian version of the Multidimensional Mentalizing-Q. [a] English translation of the Multidimensional Mentalizing-Q.

Skewness and kurtosis of the MMQ total score were between −1 and +1 (0.04 and −0.06, respectively) and the mean value of the total score was 111.58 (*SD* = 11.24).

Results of an exploratory factorial analysis (EFA) using the principal axis factoring method (Promax rotation with Kaiser normalization) showed a factor structure with six principal dimensions, which combined explained 56.9% of the total variance (eigenvalue = 1.35).

The first factor accounted for 19.51% of the variance and was made up of nine items indicating reflexivity; the second one accounted for 16.64 of variance and consisted of six items related to ego-strength; the third one accounted for 6.05% of variance and included five items describing relational attunement; the forth one accounted for 5.72% of variance and was composed by five items referring to relational discomfort; finally, the remaining two factors (both of four items) accounted the 4.88% and 4.08% of variance and indicated distrust and emotional dyscontrol, respectively (see Figure 1). The factor correlation matrix showed a prominent inter-correlation among factor scales, indicating that the questionnaire subscales measured several dimensions of mentalizing, relatively distinct from each other (see Table 3).

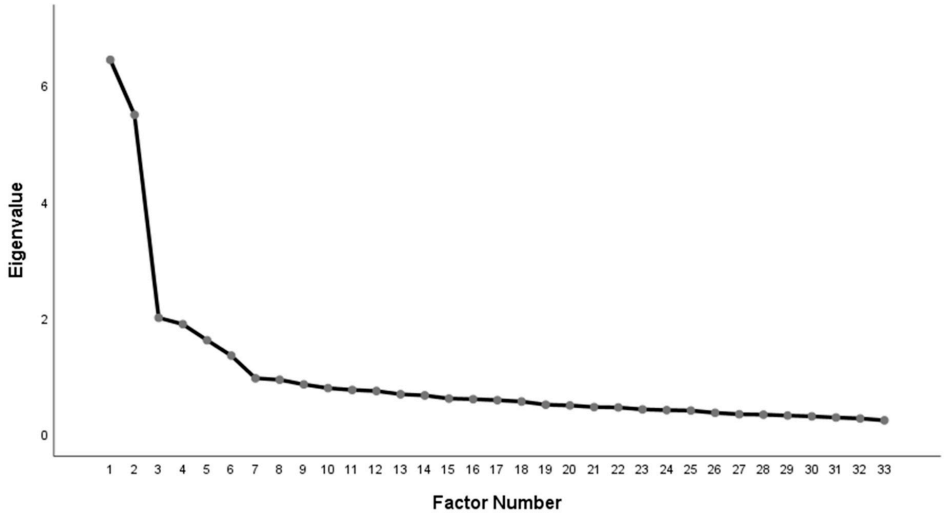

Figure 1. Scree plot.

Table 3. Descriptive statistics and factor correlation matrix.

Factors	M	SD	Skewness	Kurtosis	Factor Correlation Matrix *					
					1	2	3	4	5	6
1. Reflexivity	36.63	5.32	−0.55	−0.17	1					
2. Ego-strength	21.61	4.31	−0.57	0.75	0.10	1				
3. Relational attunement	19.60	3.58	−0.57	0.25	0.55	0.23	1			
4. Relational discomfort	11.67	4.32	0.80	0.34	0.09	−0.52	−0.08	1		
5. Distrust	10.44	3.63	0.34	−0.32	−0.04	−0.35	−0.19	0.47	1	
6. Emotional Dyscontrol	11.63	3.65	0.08	−0.70	0.11	−0.37	0.06	0.38	0.29	1

* Extraction Method: Principal axis factoring. Rotation method: Promax with Kaiser Normalization.

Concerning the confirmatory factor analysis (CFA), the goodness-of-fit indices indicated a satisfactory fit of the six-factor model. Indeed, although the Model Chi-Square was significant (χ^2 = 134.88, $p < 0.001$), the other indices showed satisfactory values (RMSEA = 0.053; TLI = 0.90; CFI = 0.90; SRMR = 0.067). Then, the reliability of the scale was calculated using the Cronbach's alpha coefficient and indicated a good level of internal consistency for both the total scale (α = 0.75) and the subscales (factor 1, α = 0.89; factor 2, α = 0.81; factor 3, α = 0.82; factor 4, α = 0.76; factor 5, α = 0.74; factor 6, α = 0.72). Finally, Pearson's correlation was carried out to assess convergent and discriminant validity (see Table 4).

Table 4. Correlation matrix.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1. MMQ (F1)	1																								
2. MMQ (F2)	0.228 **	1																							
3. MMQ (F3)	0.556 **	0.163 **	1																						
4. MMQ (F4)	−0.106*	−0.431 **	−0.068	1																					
5. MMQ (F5)	−0.168 **	−0.310 **	−0.131 **	0.509 **	1																				
6. MMQ (F6)	0.019	−0.389 **	0.034	0.411 **	0.339 **	1																			
7. TAS20	−0.491 **	−0.353 **	−0.327 **	0.535 **	0.423 **	0.432 **	1																		
8. TAS20 (F1)	−0.196 **	−0.435 **	−0.082	0.538 **	0.367 **	0.553 **	0.815 **	1																	
9. TAS20 (F2)	−0.337 **	−0.240 **	−0.219 **	0.488 **	0.353 **	0.303 **	0.800 **	0.528 **	1																
10. TAS20 (F3)	−0.632 **	−0.073	−0.491 **	0.144 **	0.223 **	0.052	0.629 **	0.199 **	0.308 **	1															
11. BIS11	−0.362 **	−0.194 **	−0.217 **	0.233 **	0.173 **	0.419 **	0.462 **	0.362 **	0.261 **	0.418 **	1														
12. BIS11 (F1)	−0.263 **	−0.240 **	−0.137 **	0.335 **	0.246 **	0.345 **	0.487 **	0.414 **	0.357 **	0.317 **	0.716 **	1													
13. BIS11 (F2)	−0.099 *	0.037	0.013	0.097	0.095	0.362 **	0.245 **	0.207 **	0.138 **	0.202 **	0.726 **	0.321 **	1												
14. BIS11 (F3)	−0.422 **	−0.226 **	−0.328 **	0.123*	0.075	0.263 **	0.333 **	0.224 **	0.129*	0.409 **	0.817 **	0.379 **	0.367 **	1											
15. RSES	0.080	0.479 **	0.046	−0.595 **	−0.352 **	−0.410 **	−0.451 **	−0.526 **	−0.387 **	−0.051	−0.214 **	−0.244 **	−0.068	−0.176 **	1										
16. GSE	0.230 **	0.686 **	0.195 **	−0.370 **	−0.207 **	−0.251 **	−0.364 **	−0.439 **	−0.231 **	−0.103*	−0.204 **	−0.200 **	0.061	−0.293 **	0.586 **	1									
17. PTI (F1)	0.253 **	0.354 **	0.223 **	−0.367 **	−0.157 **	−0.090	−0.355 **	−0.293 **	−0.326 **	−0.178 **	−0.158 **	−0.151 **	−0.020	−0.177 **	0.365 **	0.358 **	1								
18. PTI (F2)	0.085	−0.344 **	0.093	0.495 **	0.324 **	0.419 **	0.281 **	0.396 **	0.258 **	−0.072	0.079	0.194 **	0.051	−0.033	−0.481 **	−0.286 **	−0.222 **	1							
19. PTI (F3)	−0.017	0.111 *	0.059	0.102*	0.067	−0.009	0.084	0.025	0.036	0.141 **	0.088	−0.004	0.175 **	0.033	0.022	0.142 **	−0.283 **	−0.073	1						
20. PTI (F4)	−0.014	−0.179 **	0.010	0.381 **	0.152 **	0.292 **	0.212 **	0.273 **	0.146 **	0.026	0.221 **	0.149 **	0.168 **	0.181 **	−0.311 **	−0.153 **	−0.397 **	0.324 **	0.169 **	1					

Table 4. *Cont.*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
21. I-TIPI (F1)	0.105 *	0.253 **	0.097	−0.366 **	−0.157 **	0.099*	−0.231 **	−0.164 **	−0.280 **	−0.081	0.106*	0.020	0.195 **	0.035	0.278 **	0.299 **	0.236 **	−0.165 **	−0.043	−0.034	1				
22. I-TIPI (F2)	0.049	0.179 **	0.117*	−0.187 **	−0.161 **	−0.334 **	−0.204 **	−0.200 **	−0.163 **	−0.083	−0.169 **	−0.133 **	−0.174 **	−0.088	0.164 **	0.139 **	0.147 **	−0.090	−0.048	−0.120*	−0.033	1			
23. I-TIPI (F3)	0.109 *	0.280 **	0.116*	−0.262 **	−0.021	−0.286 **	−0.233 **	−0.252 **	−0.164 **	−0.087	−0.425 **	−0.305 **	−0.250 **	−0.392 **	0.300 **	0.264 **	0.160 **	−0.116*	0.037	−0.210 **	−0.071	0.284 **	1		
24. I-TIPI (F4)	0.022	0.494 **	−0.015	−0.373 **	−0.224 **	−0.476 **	−0.353 **	−0.490 **	−0.233 **	−0.007	−0.247 **	−0.306 **	−0.112*	−0.160 **	0.484 **	0.469 **	0.226 **	−0.396 **	0.083	−0.202 **	0.014	0.263 **	0.291 **	1	
25. I-TIPI (F5)	0.274 **	0.344 **	0.205 **	−0.224 **	−0.257 **	−0.064	−0.229 **	−0.138 **	−0.162 **	−0.230 **	−0.019	−0.101*	0.163 **	−0.091	0.137 **	0.250 **	0.169 **	−0.101*	0.109*	−0.122*	0.251 **	0.046	−0.050	0.149 **	1
26. IOS	0.514 **	0.507 **	0.408 **	−0.324 **	−0.177 **	−0.213 **	−0.504 **	−0.418 **	−0.359 **	−0.351 **	−0.359 **	−0.307 **	−0.108 *	−0.378 **	0.407 **	0.578 **	0.361 **	−0.191 **	0.047	−0.122*	0.145 **	0.158 **	0.247 **	0.354 **	0.229 **

** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed). MMQ = Multidimensional Mentalizing Questionnaire; MMQ (F1) = reflexivity; MMQ (F2) = ego-strength; MMQ (F3) = relational attunement; MMQ (F4) = relational discomfort; MMQ (F5) = distrust; MMQ (F6) = emotional dyscontrol; TAS20= 20-item Toronto Alexithymia Scale; TAS20 (F1)= difficulty identifying feelings and distinguishing between feelings and bodily sensations in emotional activation; TAS20 (F2) = difficulty in the verbal expression of emotions; TAS20 (F3): externally oriented thinking; BIS11= Barratt Impulsiveness Scale 11; BIS11 (F1) = attentional impulsiveness; BIS11 (F2) = motor impulsiveness; BIS11 (F3) = non-planning impulsiveness; RSES = Rosenberg self-esteem scale; GSE = general self-efficacy scale; PTI (F1) = secure attachment; PTI (F2) = preoccupied attachment; PTI (F3) = avoidant attachment; PTI (F4) = unresolved attachment; I-TIPI (F1) = extroversion; I-TIPI (F2) = agreeableness; I-TIPI (F3) = conscientiousness; I-TIPI (F4) = emotional stability; I-TIPI (F5) = openness; IOS= insight orientation scale.

The MMQ subscales showed significant correlations with most of the measures used to assess construct validity.

3. Study 2

3.1. Materials and Methods

3.1.1. Participants and Procedure

This study involved a community sample and a clinical one. The latter consisted of 46 individuals (52% male and 48% female), with an age ranging from 18 to 62 years (M_{age} = 33.33, SD = 12.257). They were recruited in various private clinical settings and received a diagnosis in line with the International Classification of Diseases-11th Edition [42]: Schizophrenia or other primary psychotic disorders (10.9%), mood disorders (28.3%), anxiety or fear-related disorders (19.6%), obsessive-compulsive or related disorders (19.6%) and personality disorders and related traits (21.7%). The community sample consists of 50 individuals (42% male and 58% female), with a mean age of 38.86 (SD = 16.008; ranging from 20 to 76). All participants completed a paper-pencil questionnaire, and written informed consent was obtained from all subjects. Privacy and anonymity were guaranteed. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved by the Ethical Committee of the Integrated Psychodynamic Psychotherapy Institute (IPPI) (ethical approval number 002/2020).

3.1.2. Measures

The Multidimensional Mentalizing–Q (MMQ)

The *Multidimensional Mentalizing–Q* (MMQ) was used to assess the level of mentalizing, considering the multifaced nature of the construct. Indeed, this 33-item self-report measure permits a multidimensional assessment, with scores on the positive (Reflexivity, Ego-strength and Relational Attunement) and negative (Relational discomfort, Distrust and Emotional dyscontrol) subscales, as well as an overall MMQ score, by summing all the items after having reversed those included in the negative subscales.

3.1.3. Data Analysis

Data were analyzed with the SPSS software (IBM-SPSS 25.0 version, IBM, Armonk, NY, USA) for Windows and MPlus Version 8.1 [37]. The MMQ scores and those of its subscales were compared in the community and clinical samples, by using an independent samples t test. A two-tailed p value of less than 0.05 was considered as statistically significant.

3.2. Results

The independent-samples t-test showed significant differences in the MMQ total score and its subscales between the community and clinical samples, except for the factors of relational attunement and emotional dyscontrol (see Table 5).

Table 5. Independent samples t-test results for Multidimensional Mentalizing Questionnaire and its subscales' scores between the community and clinical samples.

	Community Sample (n = 50)		**Clinical Sample (n = 46)**		t	df	p	**95% Confidence Interval of the Difference**	
	M	***SD***	***M***	***SD***				**Lower**	**Upper**
MMQ total score	113.08	11.127	105.70	10.673	3.314	94	0.001	2.960	11.809
Reflexivity	37.20	4.567	32.85	5.428	4.262	94	0.001	2.325	6.380
Ego-strength	21.30	4.432	17.15	5.011	4.303	94	0.001	2.234	6.062
Relational attunement	18.92	4.174	18.83	3.542	0.118	94	0.906	−1.482	1.670
Relational discomfort	11.90	3.955	14.30	4.857	−2.669	94	0.009	−4.193	−0.615
Distrust	11.54	3.655	15.02	3.073	−5.029	94	0.001	−4.856	−2.107
Emotional Dyscontrol	12.22	3.710	11.80	4.539	0.489	87.101	0.626	−1.275	2.106

4. General Discussion

The aim of this research was the development of a new measure for the assessment of mentalizing, the Multidimensional Mentalizing Questionnaire (MMQ), and to evaluate its psychometric properties and clinical sensitivity, also illustrating and supporting a new integrated and multilevel model of mentalizing (see Figure 2). This conceptualization, in fact, moves on four axes that intertwine and explicate in the different factors found in MMQ: cognitive-affective, self-other, outside-inside, explicit-implicit. Good levels of mentalizing presuppose the balance of these polarities, which must be used flexibly according to the needs and the environment [12]. This, therefore, implies an alternation of implicit (fast insight) and explicit (metacognition) modalities with harmony between cognitive and affective aspects, without one persistently dominating the other. Furthermore, mentalizing guides the interpersonal relationships, through the ability to proactively focus on internal elements while maintaining a functional awareness of the external world and with the oscillation between the perspective of the self and that of the other: it requires the ability to see "ourselves from the outside" and "others from the inside" [43] (p. 347). The imbalance of these dimensions could be seen as a core aspect in mental illness [12] and is, therefore, important to pay attention to them during assessment phases, evaluating their combinations in setting treatment programs to improve outcomes.

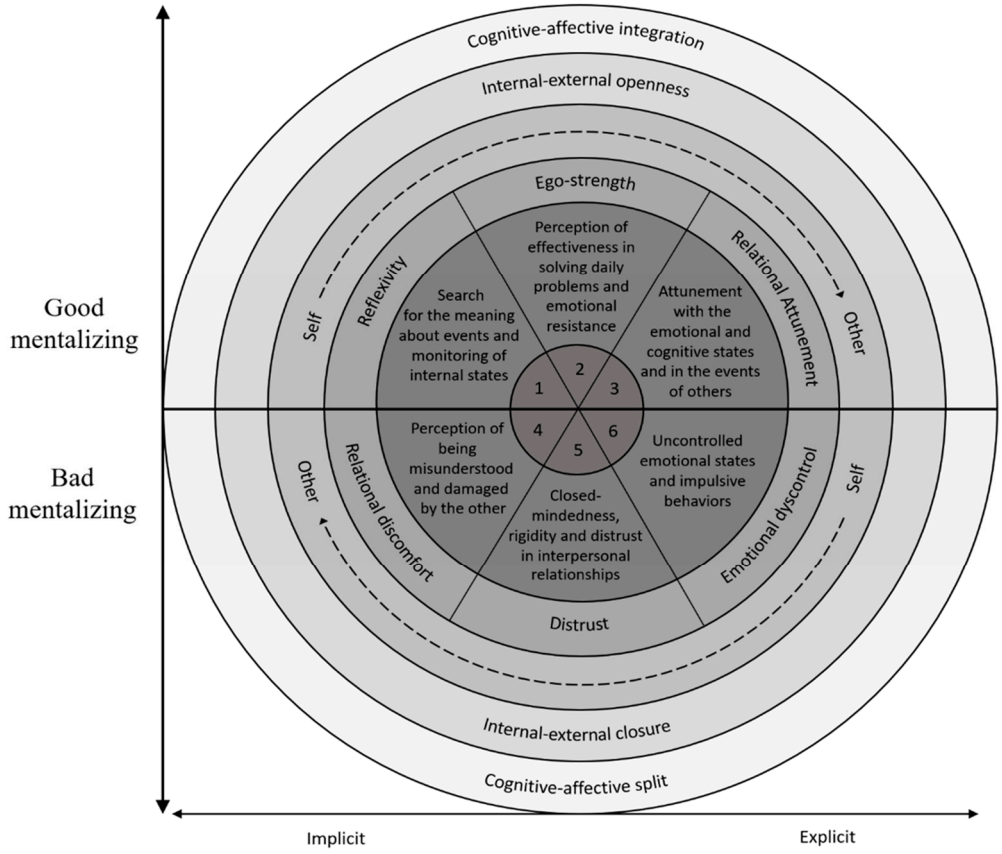

Figure 2. Integrated and multilevel model of mentalizing.

The MMQ showed satisfactory psychometric properties, with a clear and theoretically relevant factor structure, an adequate internal consistency and good construct validity. Mentalizing is a broad and multifaceted concept that encompasses and combines multiple constructs involved in treating others and ourselves as social agents [44]. As a reflection of this, the exploratory factor analysis (EFA) indicates a six-factor structure, also confirmed by the confirmatory factor analysis (CFA): the first three subscales (reflexivity, ego-strength, and relational attunement) are "positive" and highlight functional components of mentaliz-

ing, while the last three (relational discomfort, distrust, and emotional dyscontrol) are the "negative" opposites, referring to failures and distortions. Reflexivity appears to be strictly linked meta-cognition, introspection, and critical thinking [45], indicating a propensity towards the search for a deep understanding of one's experiences: It manifests itself with interest and curiosity for the exploration of one's mental states and with the desire to analyze behaviors and events. It is conceptually opposed to emotional dyscontrol, that refers to the difficulty to manage own affective states and to a tendency to impulsiveness. Dysregulated activations undermine the ability to mentalize [44]: Indeed, hyperarousal states can lead to a temporary "blindness" linked to the prevalence of limbic activity over cortical one, hindering the ability to minding the mind, which is closely related to the prefrontal cortex elaborations [46]. The second positive factor, ego-strength, is a key component of resilience [47] and concerns the perception to be able to face everyday problems with an emotional resistance to stress and frustrations: "Ego strength is the foundation from which a person can move forward into the environment" (ibidem, p. 21). It favors the metabolization of painful experiences without these damaging the self, keeping realistic trust and a sense of efficacy [44]. Its opposite is distrust, described as an attitude of closed-mindedness, distrust in relationships and tendency to have a black or white view of the world. This leads to the immersion of the subject in sturdy vicious circles where the conceptions of himself as fragile and the other as threatening are repeatedly confirmed, with a strong mistrust regarding external and unknown experiences that instead could instead expand and enrich the social understanding of the individual: this absence of trust, therefore, impairs the ability to change [48]. Finally, relational attunement indicates the ability to tune in the emotional and cognitive states of the others and deeply understand their experiences. It is a component of empathy [49], a necessary side of mentalizing [44] that focuses most on understanding the others, acquiring their perspective with a subject-object state matching [50]. It finds its opposite in relational discomfort, characterized by interpersonal difficulties and the perception of being misunderstood and damaged by others: this determines relational insecurity, fear of abandonment and pessimistic closure in one's own self. It could also be seen as a typical manifestation of the non-mentalizing state of psychic equivalence, in which thoughts are experienced as facts, without the modulation of mental processes of higher levels and, therefore, with unshakable strength and intensity: one's own painful mental state, in other words, means that others are bad [51]. Thus, in the present model, the positive subdimensions and negative ones (each with good values of internal consistency) could be conceptualized as opposite poles of a continuum of good-bad mentalizing. The former, as shown by the correlations, are all significantly and positively associated with secure attachment, openness, and self-efficacy, contrasting instead with alexithymia and impulsiveness. The negative poles, on the other hand, present a diametrically opposed framework. This could be read in light of the clinical research suggesting the role of attachment patterns in being facilitators or inhibitors of mentalizing [1,44,52]. In secure one, the mental states are discovered through mirroring and contingent interactions with the caregiver [53]: their reaction to the communicative manifestations of the child lead the latter to understand the effects of their behavior and develop a perception of themselves as effective [54]. Furthermore, this also allows to increase and confident disposition towards the exploration, identification and expression of one's mental states, favoring greater openness to experience. In this way, the subject who grew up in an environment capable of satisfying basic human needs will develop adaptive social mentalities [55] and this will make them able to use the information capacity of their feelings [56] in the interpersonal sphere promoting positive and healthy relationships, also having a greater effectiveness in the management of conflicts [44]. On the other hand, insecure attachment patterns are associated with difficulties in understanding and regulating emotions, due to unresolved past events that continue to keep a trace in the present [57]. The lack of awareness of one's feelings limits access to effective regulatory strategies, thus resulting in impulsive and destructive reactions [58]. All this negatively impacts the subject in many spheres of his

life, with a tendency to instability, affective lability, suspiciousness, relational insecurity, and social avoidance.

Consistently, results showed significantly lower mentalizing skills in the clinical sample than in the community one, also demonstrating the clinical sensitivity of the MMQ. This is in line with the scientific literature highlighting the association between imbalances of mentalizing and psychopathology (see [21] for a review), and this was further confirmed by the exploration of the MMQ sub-dimensions. Concerning the positive ones, indeed, the clinical sample showed significant lower levels in Reflexivity and Ego-strength than the community one. Such findings are consistent with previous research showing severe deficits in metacognition, that "capitalizes on the reflexive nature of consciousness" [59] (p. 50), in several kind of psychopathology, such as personality disorders, schizophrenia and bipolar disorders (see [60] for a review). This implies an abstract and generic reflexive modality that fail to explain related thoughts, feelings or intentions of one's self or of others, with a deficit on critical thinking manifesting itself with the tendency to have rigid beliefs considered indisputably true about themselves and others [61]. Furthermore, the reflexive process is related to the strength of the ego and its organizing skills, acquired by means of the containing relationship with the caregiver [62]: the awareness of being an individual having a mind and the consequent reflexive functioning develops within the secure attachment, strongly associated with important aspects for the constitution of the self, including cognitive competence, exploratory ability, ego resilience and ego control, and frustration tolerance [52]. On the contrary, attachment insecurity is a major contributor to mental disorders [63]. It is characterized by a caregiver failure in responding to the child's emotional and physical needs and this leads to compromised mentalizing abilities and epistemic mistrust [53,59]. Especially, previous research [64] showed that the mentalizing process is the mental activity linking the internal working models to the perception of strain in interpersonal contact and problems in the interpersonal functioning, which is compromised in many forms of psychopathology. This is reflected in the findings of the present research, in the exploration of negative mentalizing subdimensions: indeed, results showed significantly higher levels in relational discomfort and distrust in the clinical sample than in the community one.

This research presents some limitation that should be identified and discussed. Firstly, the differences between the different psychopathologies in the total levels of mentalizing or in the different subcomponents were not investigated, also due to the size of the clinical sample. This could be of great interest for future research, also using the MMQ to explore mentalizing from a multidimensional perspective. Furthermore, data were collected by the use of only self-reported measures, that could be subject to upward and social desirability bias. Finally, the MMQ requires a self-evaluation of aspects of which one might not have a full awareness. In future research, the use of a multimodal approach (e.g., with the integration of structured interview) could permit to have a more complete and accurate assessments, overcoming these issues.

5. Conclusions

Important implications can be drawn from the current study. The MMQ showed good psychometric properties, and the rapid and easy administration of the measure allow a comprehensive assessment of mentalizing, also considering its dimensionality. Moreover, this research highlighted the clinical sensitivity of the MMQ, further supporting the association between the imbalances of mentalizing and psychopathology. Such findings may contribute to underline the centrality of the mentalizing construct in different forms of pathology. Indeed, previous research showed that neural injuries may affect mentalization abilities in a body-specific manner [65], and that targeted physiotherapy can improve such mentalizing deficits, possibly in association with physical improvements [66]. Furthermore, the integration of ours results with evidences in the field of neuro-psychology may also offer a new reading key to favor a broader understanding of the psychiatric aspects of the neuro-cognitive relationship between mentalizing and cortico-spinal excitability [67]. On that

basis, this study may favor insights on possible homologies between mentalization-related consequences of the psychiatric conditions and previous evidences on neurological injuries. Therefore, the MMQ can be usefully adopted in both research and clinical practice.: the theoretical framework of mentalizing proposed here, the integrated and multilevel model of mentalizing, can provide important suggestions for psychotherapy and treatments. In conclusion, the MMQ may be a valuable self-report for repeated measurement of client status over the course of therapy, favoring tailored interventions and supporting clinical research.

Author Contributions: Conceptualization: A.G.; methodology: A.G.; formal analysis: A.G. and E.T.; investigation: A.G., A.A., and G.C.; data curation: A.G. and E.T.; writing—original draft preparation: A.G., A.A., and E.T.; writing—review and editing: A.G., A.A., and E.T.; supervision: A.G. and R.L.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Integrated Psychodynamic Psychotherapy Institute (IPPI; protocol code 002/2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bateman, A.; Fonagy, P. Mentalization based treatment for borderline personality disorder. *World Psychiatry* **2010**, *9*, 11–15. [CrossRef]
2. Allen, J.G. Mentalizing. *Bull. Menn. Clin.* **2003**, *67*, 91–112. [CrossRef]
3. Baron-Cohen, S. *Mindblindness: An Essay on Autism and Theory of Mind*; MIT Press: Cambridge, UK, 1997.
4. Bion, W.R. A theory of thinking. *Int. J. Psychoanal.* **1962**, *43*, 306–310.
5. Luquet, P. Le changement dans la mentalisation. *Rev. Fr. Psychanal.* **1981**, *45*, 1023–1028.
6. Lecours, S.; Bouchard, M.A. Dimensions of mentalization: Outlining levels of mental elaboration. *Int. J. Psychoanal.* **1997**, *78*, 855–875.
7. Winnicott, D.W. *Playing and Reality*; Travistock: London, UK, 1971.
8. Bowlby, J. *Attachment and Loss: Attachment*; Basic Books: London, UK, 1969; Volume 1.
9. Mayes, L.C. Arousal regulation, emotional flexibility, medial amygdala function, and the impact of early experience: Comments on the paper of Lewis et al. *Ann. N. Y. Acad. Sci.* **2006**, *1094*, 178–192. [CrossRef]
10. Luyten, P.; Fonagy, P. The Neurobiology of Attachment and Mentalizing: A Neurodevelopmental Perspective. In *Neurobiology of Personality Disorders*; Schmahl, C., Phan, K.L., Friedel, R.O., Eds.; Oxford University Press: Oxford, UK, 2018.
11. Luyten, P.; Jurist, E.L.; Slade, A.; Bergner, S. (Eds.) Mind to Mind: Infant Research, Neuroscience, and Psychoanalysis. *Clin. Soc. Work J.* **2011**, *39*, 116–118. [CrossRef]
12. Bateman, A.; Fonagy, P.E. *Handbook of Mentalizing in Mental Health Practice*; American Psychiatric Publishing, Inc.: Washington, DC, USA, 2012.
13. Baron-Cohen, S.; Wheelwright, S.; Hill, J.; Raste, Y.; Plumb, I. The "Reading the Mind in the Eyes" Test revised version: A study with normal adults, and adults with Asperger syndrome or high-functioning autism. *J. Child Psychol. Psychiatry* **2001**, *42*, 241–251. [CrossRef] [PubMed]
14. Fonagy, P.; Target, M.; Steele, H.; Steele, M. *Reflective-Functioning Manual: Version 5 for Application to Adult Attachment Interview*; Unpublished Manual; University College: London, UK, 1998.
15. Dimitrijević, A.; Hanak, N.; Altaras Dimitrijević, A.; Jolić Marjanović, Z. The Mentalization Scale (MentS): A self-report measure for the assessment of mentalizing capacity. *J. Pers. Assess.* **2018**, *100*, 268–280. [CrossRef] [PubMed]
16. Hausberg, M.C.; Schulz, H.; Piegler, T.; Happach, C.G.; Klöpper, M.; Brütt, A.L.; Sammet, I.; Andreas, S. Is a self-rated instrument appropriate to assess mentalization in patients with mental disorders? Development and first validation of the Mentalization Questionnaire (MZQ). *Psychother. Res.* **2012**, *22*, 699–709. [CrossRef] [PubMed]
17. Fonagy, P.; Luyten, P.; Moulton-Perkins, A.; Lee, Y.-W.; Warren, F.; Howard, S.; Ghinai, R.; Fearon, P.; Lowyck, B. Development and validation of a self-report measure of mentalizing: The Reflective Functioning Questionnaire. *PLoS ONE* **2016**, *11*, e0158678. [CrossRef]
18. Gagliardini, G.; Gullo, S.; Caverzasi, E.; Boldrini, A.; Blasi, S.; Colli, A. Assessing mentalization in psychotherapy: First validation of the Mentalization Imbalances Scale. *Res. Psychother. Psychopathol. Process Outcome* **2018**, *21*, 164–177. [CrossRef] [PubMed]

19. Gagliardini, G.; Colli, A. Assessing mentalization: Development and preliminary validation of the Modes of Mentalization Scale. *Psychoanal. Psychol.* **2019**, *36*, 249. [CrossRef]
20. Morandotti, N.; Brondino, N.; Merelli, A.; Boldrini, A.; De Vidovich, G.Z.; Ricciardo, S.; Abbiati, V.; Ambrosi, P.; Caverzasi, E.; Fonagy, P.; et al. The Italian version of the Reflective Functioning Questionnaire: Validity data for adults and its association with severity of borderline personality disorder. *PLoS ONE* **2018**, *13*, e0206433. [CrossRef] [PubMed]
21. Luyten, P.; Campbell, C.; Allison, E.; Fonagy, P. The mentalizing approach to psychopathology: State of the art and future directions. *Annu. Rev. Clin. Psychol.* **2020**, *16*, 297–325. [CrossRef] [PubMed]
22. Bagby, R.M.; Parker, J.D.; Taylor, G.J. The twenty-item Toronto Alexithymia Scale—I. Item selection and cross-validation of the factor structure. *J. Psychosom. Res.* **1995**, *38*, 23–32. [CrossRef]
23. Bagby, R.M.; Taylor, G.J.; Parker, J.D. The twenty-item Toronto Alexithymia Scale—II. Convergent, discriminant, and concurrent validity. *J. Psychosom. Res.* **1994**, *38*, 33–40. [CrossRef]
24. Bressi, C.; Taylor, G.; Parker, J.; Bressi, S.; Brambilla, V.; Aguglia, E.; Allegranti, I.; Bongiorno, A.; Giberti, F.; Bucca, M.; et al. Cross validation of the factor structure of the 20-item Toronto Alexithymia Scale: An Italian multicenter study. *J. Psychosom. Res.* **1996**, *41*, 551–559. [CrossRef]
25. Patton, J.H.; Stanford, M.S.; Barratt, E.S. Factor structure of the Barratt impulsiveness scale. *J. Clin. Psychol.* **1995**, *51*, 768–774. [CrossRef]
26. Fossati, A.; Di Ceglie, A.; Acquarini, E.; Barratt, E.S. Psychometric properties of an Italian version of the Barratt Impulsiveness Scale-11 (BIS-11) in nonclinical subjects. *J. Clin. Psychol.* **2001**, *57*, 815–828. [CrossRef]
27. Rosenberg, M. Rosenberg self-esteem scale (RSE). *PsycTESTS Dataset* **1965**, *61*, 52. [CrossRef]
28. Prezza, M.; Trombaccia, F.R.; Armento, L. La scala dell'autostima di Rosenberg: Traduzione e validazione italiana [The Rosenberg Self-Esteem Scale: Italian translation and validation]. *Boll. Psicol. Appl.* **1997**, *223*, 35–44.
29. Schwarzer, R.; Jerusalem, M. Generalized self-efficacy scale. *PsycTESTS Dataset* **1995**, *1*, 35–37. [CrossRef]
30. Sibilia, L.; Schwarzer, R.; Jerusalem, M. Italian Adaptation of the General Self-Efficacy Scale. 1995. Available online: http://userpage.fu-berlin.de/~{}health/italian.htm (accessed on 6 December 2020).
31. Giannini, M.; Gori, A.; De Sanctis, E.; Schuldberg, D. Attachment in psychotherapy: Psychometric properties of the Psychological Treatment Inventory Attachment Styles Scale (PTI-ASS). *J. Psychother. Integr.* **2011**, *21*, 363–381. [CrossRef]
32. Gori, A.; Giannini, M.; Schuldberg, D. *PTI—Psychological Treatment Inventory*; Giunti OS–Organizzazioni Speciali: Florence, Italy, 2015.
33. Gosling, S.; Rentfrow, P.J.; Swann, W.B. Ten-Item Personality Inventory-(TIPI). *J. Res. Pers.* **2003**, *37*, 504–528. [CrossRef]
34. Costa, P.T.; McCrae, R.R. Four ways five factors are basic. *Pers. Individ. Differ.* **1992**, *13*, 653–665. [CrossRef]
35. Di Fabio, A.; Gori, A.; Giannini, M. Analysing the psychometric properties of a Big Five measure with an alternative method: The example of the Ten Item Personality Inventory (TIPI). [Analysing the psychometric properties of a Big Five measurement tool with an alternative method: The example of the Ten Item Personality Inventory (TIPI)]. *Couns. G. Ital. Ric. Appl.* **2016**, *9*, 1–6.
36. Gori, A.; Craparo, G.; Giannini, M.; Loscalzo, Y.; Caretti, V.; La Barbera, D.; Manzoni, G.M.; Castelnuovo, G.; Tani, F.; Ponti, L.; et al. Development of a new measure for assessing insight: Psychometric properties of the insight orientation scale (IOS). *Schizophr. Res.* **2015**, *169*, 298–302. [CrossRef]
37. Muthén, L.K.; Muthén, B.O. *Mplus User's Guide*, 8th ed.; Muthén & Muthén: Los Angeles, CA, USA, 2017.
38. Hooper, D.; Coughlan, J.; Mullen, M.R. Structural equation modelling: Guidelines for determining model fit. *Elecron. J. Bus. Res. Methods* **2008**, *6*, 53–60.
39. Marsh, H.W.; Hau, K.T.; Wen, Z. In search of golden rules: Comment on hypothesis-testing approaches to setting cutoff values for fit indexes and dangers in overgeneralizing Hu and Bentler's (1999) findings. *Struct. Equ. Modeling* **2005**, *11*, 320–341. [CrossRef]
40. Kline, R.B. *Principles and Practice of Structural Equation Modeling*, 2nd ed.; Guilford: New York, NY, USA, 2005.
41. Hu, L.T.; Bentler, P.M. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Struct. Equ. Modeling* **1999**, *6*, 1–55. [CrossRef]
42. World Health Organization. ICD-11 for Mortality and Morbidity Statistics. 2018. Available online: https://www.who.int/classifications/classification-of-diseases (accessed on 6 December 2009).
43. Asen, E.; Fonagy, P. Mentalization-based therapeutic interventions for families. *J. Fam. Ther.* **2012**, *34*, 347–370. [CrossRef]
44. Allen, J.G.; Fonagy, P. *The Handbook of Mentalization-Based Treatment*; John Wiley & Sons: Hoboken, NJ, USA, 2006.
45. Burgess, S.; Rhodes, P.; Wilson, V. Exploring the in-session reflective capacity of clinical psychology trainees: An interpersonal process recall study. *Clin. Psychol.* **2013**, *17*, 122–130. [CrossRef]
46. Gallagher, H.L.; Happé, F.; Brunswick, N.; Fletcher, P.C.; Frith, U.; Frith, C.D. Reading the mind in cartoons and stories: An fMRI study of 'theory of mind'in verbal and nonverbal tasks. *Neuropsychologia* **2000**, *38*, 11–21. [CrossRef]
47. Kadner, K.D. Resilience: Responding to adversity. *J. Psychosoc. Nurs. Ment. Health Serv.* **1989**, *27*, 20–25. [CrossRef] [PubMed]
48. Fonagy, P.; Luyten, P.; Bateman, A. Translation: Mentalizing as treatment target in borderline personality disorder. *Pers. Disord. Theory Res. Treat.* **2015**, *6*, 380. [CrossRef] [PubMed]
49. Davis, J.L.; Love, T.P. Self-in-Self, Mind-in-Mind, Heart-in-Heart: The Future of Role-Taking, Perspective Taking, and Empathy. In *Advances in Group Processes*; Thye, S.R., Lawler, E.J., Eds.; Emerald Publishing Limited: Bingley, UK, 2017; pp. 151–174.

50. De Waal, F. Morally evolved: Primate social instincts, human morality and the rise and fall of "veneer theory". In *Primates and Philosophers: How Morality Evolved*; de Waal, F., Macedo, S.E., Ober, J.E., Eds.; Princeton University Press: Princeton, NJ, USA, 2006; pp. 1–80.
51. Fonagy, P.; Allison, E. What is mentalization? The concept and its foundations in developmental research. In *Minding the Child: Mentalization-Based Interventions with Children, Young People and Their Families*; Midgley, N., Vrouva, I., Eds.; Routledge: London, UK, 2013; pp. 25–48.
52. Fonagy, P.; Gergely, G.; Jurist, E.; Target, M. *Affect Regulation, Mentalization, and the Development of the Self*; Other Press: New York, NY, USA, 2002.
53. Gergely, G. The social construction of the subjective self: The role of affect-mirroring, markedness, and ostensive communication in self-development. In *Developments in Psychoanalysis. Developmental Science and Psychoanalysis: Integration and Innovation*; Mayes, L., Fonagy, P., Target, M., Eds.; Karnac Books: London, UK, 2007; pp. 45–88.
54. Neisser, U. Five kinds of self-knowledge. *Philos. Psychol.* **1988**, *1*, 35–59. [CrossRef]
55. Brasini, M.; Tanzilli, A.; Pistella, J.; Gentile, D.; Di Marco, I.; Mancini, F.; Lingiardi, V.; Baiocco, R. The Social Mentalities Scale: A new measure for assessing the interpersonal motivations underlying social relationnships. *Pers. Individ. Differ.* **2020**, *167*, 110236. [CrossRef]
56. Damasio, A.R. *Looking for Spinoza: Joy, Sorrow, and the Feeling Brain*; Houghton Mifflin Harcourt: New York, NY, USA, 2003.
57. Main, M. The organized categories of infant, child, and adult attachment: Flexible vs. inflexible attention under attachment-related stress. *J. Am. Psychoanal.* **2000**, *48*, 1055–1096. [CrossRef]
58. Gratz, K.L.; Roemer, L. Multidimensional assessment of emotion regulation and dysregulation: Development, factor structure, and initial validation of the difficulties in emotion regulation scale. *J. Psychopathol. Behav.* **2004**, *26*, 41–54. [CrossRef]
59. Allen, J.G.; Fonagy, P.; Bateman, A.W. *Mentalizing in Clinical Practice*; American Psychiatric Pub: Washington, DC, USA, 2008.
60. Wells, A. Metacognition and psychological therapy. In *Social Metacognition*; Brinol, P., DeMarree, K.G., Eds.; Psychology Press: New York, NY, USA, 2012; pp. 323–342.
61. Dimaggio, G.; Lysaker, P.H. Metacognition and mentalizing in the psychotherapy of patients with psychosis and personality disorders. *J. Clin. Psychol.* **2015**, *71*, 117–124. [CrossRef] [PubMed]
62. Winkler, A. Resilience as reflexivity: A new understanding for work with looked-after children. *J. Soc. Work Pract.* **2014**, *28*, 461–478. [CrossRef]
63. Mikulincer, M.; Shaver, P.R. An attachment perspective on psychopathology. *World Psychiatry* **2012**, *11*, 11–15. [CrossRef] [PubMed]
64. Hayden, M.C.; Müllauer, P.K.; Gaugeler, R.; Senft, B.; Andreas, S. Mentalization as mediator between adult attachment and interpersonal distress. *Psychopathology* **2019**, *52*, 10–17. [CrossRef]
65. Ionta, S.; Villiger, M.; Jutzeler, C.R.; Freund, P.; Curt, A.; Gassert, R. Spinal cord injury affects the interplay between visual and sensorimotor representations of the body. *Sci. Rep.* **2016**, *6*, 20144. [CrossRef]
66. Scandola, M.; Dodoni, L.; Lazzeri, G.; Arcangeli, C.A.; Avesani, R.; Moro, V.; Ionta, S. Neurocognitive benefits of physiotherapy for spinal cord injury. *J. Neurotrauma* **2019**, *36*, 2028–2035. [CrossRef]
67. Perruchoud, D.; Fiorio, M.; Cesari, P.; Ionta, S. Beyond variability: Subjective timing and the neurophysiology of motor cognition. *Brain Stimul.* **2018**, *11*, 175–180. [CrossRef] [PubMed]

Journal of *Personalized Medicine*

MDPI

Article

Screening for Early Signs of Paternal Perinatal Affective Disorder in Expectant Fathers: A Cluster Analysis Approach

Sonia Mangialavori [1,*,†], Michele Giannotti [2,†], Marco Cacioppo [1], Federico Spelzini [3] and Franco Baldoni [4]

[1] Department of Human Sciences, LUMSA University of Rome, 00193 Rome, Italy; m.cacioppo@lumsa.it
[2] Department of Psychology and Cognitive Sciences, University of Trento, 38068 Rovereto, Italy; michele.giannotti@unitn.it
[3] Department of Obstetrics & Gynecology, Hospital of Infermi of Rimini, 47923 Rimini, Italy; federico.spelzini@gmail.com
[4] Department of Psychology, University of Bologna, 40127 Bologna, Italy; franco.baldoni@unibo.it
* Correspondence: s.mangialavori@lumsa.it; Tel.: +39-0668422910
† These authors contributed equally to this work.

Abstract: Previous studies documented gender-related differences in the expression of Perinatal Affective Disorders. However, little attention has been paid to screening the male population during the perinatal period. This study was based on three aims: (1) to investigate the mental health of expectant fathers based on their levels of depression, anxiety, addiction, anger attacks/hostility, and somatization, identifying psychological profiles; (2) to analyze the association between these profiles and the individual variable of perceived stress; (3) and to examine the association between these profiles and the couple's variable of marital adjustment. A total of 350 Italian expectant fathers in the last trimester of pregnancy were asked to fill in questionnaires concerning perceived stress, dyadic adjustment, psychiatric symptomatology, and depression. Three different clusters were found: "psychologically healthy men" (68%) with low levels of symptoms on all the scales; "men at risk of externalized behavioral problems" (17.1%), characterized by one or more addictive or risky behaviors and moderate levels of scales scores; and "men experiencing psychological distress" (14.9%), with the highest scores on all the scales. A significant association emerged among the perceived stress, marital adjustment, and cluster membership. These results highlight the importance of screening fathers in perinatal health services, which are still predominantly mother-centered, and underscore the necessity to create tailored and personalized interventions.

Keywords: affective disorder; perinatal period; fatherhood; prevention; gender; screening

Citation: Mangialavori, S.; Giannotti, M.; Cacioppo, M.; Spelzini, F.; Baldoni, F. Screening for Early Signs of Paternal Perinatal Affective Disorder in Expectant Fathers: A Cluster Analysis Approach. *J. Pers. Med.* **2021**, *11*, 10. https://dx.doi.org/10.3390/jpm11010010

Received: 6 November 2020
Accepted: 20 December 2020
Published: 23 December 2020

1. Introduction

Although being a father for most men is a joyful and fulfilling journey [1], the transition to parenthood, or the arrival of an additional child, can also be perceived as overwhelming and demanding [2]. Indeed, it has been widely recognized that adjustment to fatherhood may negatively affect the men's mental health, increasing psychological distress, depression, and anxiety from the prenatal period [3,4].

In the last decades, an ever-growing number of studies have addressed the impact of transition to parenthood on fathers' mental health [5–7]; however, evidence to propose an appropriate gender-based screening for fathers is lacking [1–8]. In this regard, Walsh, Davis, and Garfield [9] highlighted the urgency of increased attention to screening for Paternal Perinatal Depression (PPND), stating that it is inappropriate to consider the identification, prevention, and treatment interventions of PPND as optional.

PPND is considered a specific disorder that many fathers may suffer from between pregnancy and the first year after childbirth. PPND is related to maternal perinatal depression [10–12] and poor outcomes in offspring, including externalizing and internalizing symptoms [13–15].

Several studies identified significant associations between PPND and some individual variables such as high levels of perceived stress [16,17], multiparity [2,18,19], having a previous history of psychiatric disorders [20], and experiencing stressful life events (e.g., job loss, divorce, mourning) [21,22]. Other studies have highlighted the positive correlation between PPND and risk of perinatal depression in their partners [23,24] and the negative association between PPND and marital adjustment [11,25,26].

Two recent meta-analyses showed a PPND prevalence in the world ranging from 8.4% [27] to 10.4% [23]. In addition, longitudinal studies have shown that pregnancy is a period of high risk for the onset of depressive symptoms in both expectant parents [19,28].

1.1. PPND Clinical Expression

According to the masked depression framework, PPND signs and clinical expression are different from those observed in Maternal Perinatal Depression (MPND), since men often exhibit externalizing symptoms defined as depressive equivalents to hide their depression condition [8,29]. In fact, depressive symptoms can be milder and less defined and are often comorbid with anxiety, somatic symptoms and complaints, hostility and/or anger attacks, substance use (alcohol and drugs), or other addictions or risky behaviors (e.g., gambling, compulsive use of computer/smartphone, or internet, driving very fast, extra marital affairs) [8,30,31]. For this reason, Baldoni [32] proposed to replace the term PPND with Paternal Perinatal Affective Disorder (PPAD) using a more inclusive definition to embrace the broad range of depressive symptoms related to male psychological perinatal distress. Clinicians treating men for depression have also confirmed, based on their clinical experience, that the men's tendency to externalize their distress and provoke interpersonal conflict are "masculine-specific manifestations of depression" [33].

Since perinatal depression risks and psychological responses differ significantly based on gender [31,34,35], it would be helpful to consider the wide array of paternal affective symptoms. Thus, identifying fathers' psychological distress profiles could help mental health professionals better recognize the condition of these men and to develop gender-sensitive screening tools and treatment options tailored to fathers.

1.2. Screening for Early Signs of PPND

Previous studies documented gender-related differences in the manifestation of perinatal depression, [31,36]; however, little attention has been paid to the screening practice in the male population, especially during the perinatal period [5,37]. However, during the occasional perinatal screening visits for expectant fathers, when participants are interviewed to assess if their symptomatology truly indicates depression, the researchers and clinicians use the Diagnostic and Statistical Manual of Mental Disorders(DSM) diagnostic criteria of five or more symptoms from the list of nine potential symptoms for depression [38]. These symptoms are identical for both men and women. Thus, to date, there is no acknowledgement in this diagnostic system that the two genders may experience and/or exhibit depression differently.

Although measures to assess male-type depressive symptomatology are available, such as the Gotland Male Depression Scale (GMDS) [39], they have not been specifically developed for the perinatal period. Indeed, research and screening of perinatal affective disorders are based almost exclusively on self-report scales that only consider symptoms associated with MPND. In this regard, recent findings highlighted several limitations of traditional scales in capturing paternal psychological distress.

For instance, even if the Edinburgh Postnatal Depression Scale (EPDS) [40] has been validated in fathers [41–44], there is not yet a shared consensus on the optimal cut-off scores for depression and anxiety, which change across studies. Moreover, Nishimura and Ohashi [45] revealed different rates of at-risk fathers using the CES-D (Center for Epidemiological Study Depression Scale) (7.5%; cut-off $\geq$ 16) and the EPDS (11.6%; cut-off $\geq$ 9). A Danish study [46] revealed that 20.6% of the at-risk fathers exceed the cut-off value on the GMDS but not on the EPDS. Similarly, Carlberg et al. [47] found that EPDS and GMDS were

related to different risk factors and prevalence of PPND. Interestingly, a specific subgroup of fathers only showed externalizing symptomatology without conventional depressive symptoms, proving that a multidimensional and gender-based screening should be used to cover different clinical features of paternal perinatal distress. Considering these limitations, the number of at-risk fathers may be often underestimated, especially when the screening process does not include the assessment of male-type depressive symptoms.

The analysis of different profiles of psychological distress during pregnancy has only been investigated in primiparous women [48]. In this study, three different profiles were found: (1) "psychologically healthy women" with low levels of symptoms of depression, anxiety and fear of childbirth; (2) "women experiencing pregnancy- and childbirth-related anxiety", with an average state anxiety above the clinical value; and (3) "psychologically distressed women", that included women who reported high levels of depressive and anxious symptoms, some above the clinical cut-offs. These findings underlined the importance of early psychological screening in order to understand the diverse experience of expectant parents and to develop person-centered interventions [48].

Hence, based on an integrative and gender-based perspective, the present study was based on three aims: (1) to investigate the mental health of expectant fathers based on their levels of depression, anxiety, addiction, anger attacks/hostility, and somatization by identifying psychological profiles; (2) to analyze the association between the emergent psychological profiles and the individual variable of perceived stress; and (3) to examine the association between these profiles and the couple's dimension of marital adjustment.

2. Materials and Methods

2.1. Procedure and Participants

We initially recruited 423 expectant fathers. After this preliminary recruitment, 21 were excluded for not giving informed consent, 38 were excluded because they did not complete the questionnaire entirely, 9 were excluded because the participants had poor knowledge of Italian and, after a screening by the gynecologist, 14 were excluded because the partner had a pregnancy at risk. We decided to exclude those with a partner with a high-risk pregnancy because the literature highlights that these fathers may have greater psychological distress due to this partner's condition [49,50].

In total, this cross-sectional study involved 350 Italian expectant fathers (Mean age = 35.63, Standard Deviation = 6.32, range = 20–58) in the last trimester of pregnancy. Participants were recruited at the OB/GYN Department of the "Infermi" hospital of Rimini, and of the "Santo Spirito" and San "Filippo Neri" hospitals of Rome where they attended antenatal classes or routine visits between 2016 and 2019. Expectant fathers were informed about the aims and methodology of the study before signing the written consent form. Informed consent was obtained from all subjects involved in the study.

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Infermi Hospital (N° 3691/2016).

Study inclusion criteria were being 18 years or older, in a de facto or marital relationship, and in the third trimester of pregnancy. Exclusion criteria were having a partner with a high-risk pregnancy defined as the presence of one or more maternal and/or fetal health problems including pregnancy-induced hypertension, multiple gestations, medical disorder complicating pregnancy (such as diabetes), previous miscarriages, chromosomal abnormalities in the fetus, pregnancy complications (such as abnormal placenta position, fetal growth restriction) and threatened premature labor; refusal to provide informed consent; presence of cognitive disability and/or current psychiatric diagnosis; poor knowledge of Italian, or other verbal communication limitations that compromised the participant's ability to follow the research protocol.

2.2. Measures

The Center for Epidemiologic Studies Depression Scale (CES-D) [51] is a 20-item self-report measure used to assess depressive symptomatology in the last week measured

on a 4-point Likert scale, ranging from 0 to 3. Summing responses to all items formed the depression score, with higher scores indicating more depressive symptoms. The CES-D has been used extensively in community settings and among expectant parents [52]. The Italian version of CES-D [53] was used in this study, showing a satisfactory level of internal consistency ($\alpha = 0.71$).

The Symptom Checklist-90-Revised (SCL-90-R) [54] is a well-known 90-item questionnaire, scored on a Likert scale from 0 to 4, that is used to assess psychiatric symptomatology. In this study, Anxiety (ANX); Somatization (SOM); and Hostility (HOS) subscales were used, with higher scores indicating higher symptoms frequency. The Italian version of SCL-90-R [55] was used, showing a fair level of internal consistency for all the subscales respectively $\alpha = 0.72$ for ANX, $\alpha = 0.78$ for SOM, and $\alpha = 0.75$ for HOS.

The Perceived Stress Scale (PSS) [56] was used to measure the perception of stress in the last six months. It is a measure of the degree to which situations in one's life are appraised as stressful. It contains 10 items that are rated on a 5-point scale that ranges from never to very often. High total scores indicate greater perceived stress. The PSS was widely used during the perinatal period both for mothers and fathers [57]. In this study, the Italian validation [58] was used, showing a good level of internal consistency ($\alpha = 0.76$).

The Dyadic Adjustment Scale (DAS) [59] was used to assess a couple's functioning. It is composed of 32 items, 31 of which are related to the specific dimension of marital adjustment while one item refers to the overall perceived happiness with the relationship. In this study, the Italian validated version [60] showed a very good internal consistency ($\alpha = 0.89$).

Addictions and other risky behaviors were assessed with ad hoc categorical (yes or no) item "In the previous two weeks, I smoked, drank alcohol, used drugs, gambled or used the internet more than usual; or I have taken risks more than usual (e.g., driving very fast, doing dangerous sports, unnecessary risks at work, etc.) (one or more of these)".

Finally, Sociodemographic information (age, education, occupation, number of children) and individual information about the previous history of psychiatric disorders and the presence of stressful life events (e.g., job loss, divorce, mourning) in the previous six months were investigated.

2.3. Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences, version 23 (SPSS Inc., Chicago, IL, USA) and are presented as means, standard deviations (SD), ranges and percentages (%). The correlation index between study variables (CES-D, ANX, SOM, HOS, PSS, and DAS) was calculated.

As suggested by Kent, Jensen and Kongsted [61], in order to identify different subgroups of psychological distressed men characterized by high within-cluster homogeneity and high between-cluster heterogeneity, a Two-Step cluster analysis was performed on the continuous variables of CES-D, ANX, SOM, and HOS together with the categorical addiction/risky behaviors variable.

The Two-Step cluster analysis is a statistical approach that first uses a distance measure to separate groups and then a probabilistic approach to select the optimal sub-group model [61]. Two-Step cluster analysis is also considered more reliable and accurate when compared to traditional clustering methods such as the k-means clustering algorithm [62,63]. This technique presents several advantages compared to more traditional techniques, such as determining automatically the number of clusters based on a statistical measure of fit (AIC or BIC) rather than on an arbitrary choice, using categorical and continuous variables simultaneously, analyzing atypical values (i.e., outliers), and being able to handle large datasets [61,64]. Comparative studies regarded Two-Step cluster analysis as one of the most reliable in terms of the number of subgroups detected, the classification probability of individuals to subgroups, and the reproducibility of findings on clinical data [61,65]. In the first step (pre-clustering), a sequential approach is used to pre-cluster the cases with the aim to reduce the size of the matrix that contains distances between all possible

pairs of cases. In the second step (clustering), the pre-clusters are clustered using the hierarchical clustering algorithm. No prescribed number of clusters was suggested, and the log-likelihood criterion was used for distance measure. Schwarz's Bayesian criterion (BIC) and the silhouette coefficient were used to compare cluster solutions. Silhouette measures of less than 0.2 were classified as poor; between 0.2 and 0.5 were classified as fair; and greater than 0.5 were classified as good solution quality, with fair or higher considered acceptable clustering [64].

Regarding the second and third aims of the study, the association among psychological profiles, perceived stress (PSS), and dyadic adjustment (DAS) was tested through two univariate ANOVAs with the Bonferroni correction in the post hoc tests.

The level of statistical significance was set at $p < 0.05$.

Moreover, to provide a more comprehensive descriptive analysis, the association between psychological profiles and some individual variables (being or not a primiparous parent, previous psychiatric conditions, and the presence of stressful life events) was investigated through chi-square statistics with the standard residual method, as post hoc, to identify those specific cells making the greatest contribution to the chi-square test result [66]. In line with Field [67], since, in our case, the inspection of residuals was used as a guide to what cells might be of interest, we preferred to choose a more conservative alpha value than 0.05 such 0.01 (z value $+/-$ 2.58).

3. Results

Descriptive variables of the study sample (sociodemographic characteristics, being or not a primiparous parent, previous psychiatric diagnosis, presence of stressful life events) are presented in Table 1. Descriptive statistics of the psychological dimensions (CES-D, ANX, SOM, HOS, PSS, DAS, addiction/risky behavior item) are presented in Table 2. All the variables were normally distributed. Correlation coefficients among the variables of interests are reported in Table 3. All the variables were significant for each cluster (Table 4). The composition of the clusters and the importance of variables within a cluster have been examined.

When we only consider the CES-D cut-off [51], the rate of men at risk of depression was 8.2% (n = 29; cut-off $\geq$ 16).

Regarding the SCL-90 mean scores, when we compared the mean scores of the subscales anxiety (ANX), somatization (SOM), and anger/hostility (HOS) to the Italian norms, only the anxiety mean score was higher than the general male population mean score, but it did not reach clinical significance (T < 45) [55].

With respect to the first aim of the study, the Two-Step cluster analysis yielded three clusters (BIC = 817.04; ratio of distance measure = 2.28), with no exclusion of cases. The Schwarz BIC was selected as the final clustering criterion because it provides a more precise cluster estimate [63] and the three-cluster solution provided a silhouette coefficient S(i) of 0.6, which indicates a good amount of separation and cohesion between data points within the clusters and overall goodness of fit cluster solution [64,68,69].

In term of predictive variables, depressive, anxious, and somatic symptomatology together with anger/hostility and addictive/risky behaviors were the five input variables for the generation of the clusters.

The first cluster included 68% of the total sample (n = 238), and it was characterized by low levels of anxiety, depression, hostility, somatization, and the absence of any reported addictive or risky behaviors. We defined it as a "psychologically healthy men" cluster. In the second cluster (14.9% of the study sample; n = 52), expectant fathers reported the higher scores for anxious and depressive symptoms, hostility as well as somatization, whereas the majority of them (n = 43, 82.7%) did not fit in the addictive and risky behaviors category. Thus, this cluster was named "men experiencing psychological distress". The third cluster included 60 expectant fathers (17.1% of the total sample), and it comprised primarily the presence of one or more addictive or risky behaviors in the last two weeks with perceived anxiety, depression, hostility, and somatization represented to a moderate degree. We

named this cluster as "men at-risk of externalized behavioral problems". The ratio of sizes, largest cluster to smallest cluster, was 4.68.

For the first cluster, anxious symptoms emerged as main predictor for the group membership with a predictor importance (PI) of 0.93, followed by hostility (PI = 0.50), somatization (PI = 0.49), addictive/risky behaviors (PI = 1.00), and depressive symptoms (PI = 0.38). For the second cluster, anxious symptoms emerged as the main predictor (PI = 0.93), followed by depressive symptoms (PI = 0.38), somatization (PI= 0.49), hostility (PI = 0.50), and addictive/risky behaviors (PI = 1.00). Considering the third cluster, the main predictor was addictive/risky behaviors dimension (PI = 1.00), followed by hostility (PI = 0.50), depressive symptoms (PI = 0.38), anxious symptoms (PI = 0.93), and somatization (PI = 0.49).

According to our second aim, the findings revealed a significant association between cluster membership and perceived stress ($F(2, 347) = 56.53$, $p < 0.001$). In particular, perceived stress was significantly different between psychologically healthy men and psychologically distressed men, with men in the first cluster reporting an average score on PSS that was significantly lower than psychologically distressed men (mean difference = −7.52; standard error = 0.75; $p < 0.001$) and men at-risk of externalized behavioral problems (m.d. = −3.94; s.e. = 0.71; $p < 0.001$). Moreover, men in the second cluster obtained a higher average score on the PSS than men at-risk of externalized behavioral problems (m.d. = 3.58; s.e. = 0.93; $p < 0.001$).

Finally, as regards the third research aim, findings revealed a significant association between marital adjustment and cluster membership ($F(2, 347) = 16.88$, $p < 0.001$). Specifically, psychologically healthy men reported an average DAS score that is significantly higher than men at-risk of externalized behavioral problems (m.d. = 10.30; s.e. = 2.22; $p < 0.001$) and psychologically distressed men (m.d. = 9.05; s.e. = 2.09; $p < 0.001$); whereas no differences emerged between men at-risk of externalized behavioral problems and psychologically distressed men.

Regarding the descriptive analysis between the three emergent psychological profiles and individual variable of being or not a primiparous parent, the chi square test was not significant ($\chi^2(2) = 1.44$, $p = 0.48$). The association between the three clusters and the presence of previous psychiatric disorders was statistically significant ($\chi^2(2) = 19.22$, $p < 0.01$), while most of the individuals in the cluster of "psychologically healthy men" did not have previous psychiatric disorders ($n = 220$, 92.43%). The highest percentage of those who had previous psychiatric history was from individuals in the cluster of "psychologically distressed men" ($n = 15$, 28.84%), while the percentage of individuals who had previous psychiatric history of cluster of "men at-risk of externalized behavioral problems" was 16.66% ($n = 10$). A chi-square post-hoc test via the standard residual method confirmed that the standard residuals in the "psychologically healthy men" group category with previous psychiatric disorders significantly contributed to a significant omnibus chi-square statistic ($\chi^2 = 15.37$; $p < 0.001$). In addition, the inspection of standard residuals in the "psychologically distressed men" group category with the presence of previous psychiatric disorders significantly contributed to a significant omnibus chi-square statistic ($\chi^2 = 15.52$; $p < 0.001$), while it was observed that the standard residuals of "men at-risk of externalized behavioral problems" group with the variable of previous psychiatric disorders did not contribute to significant omnibus chi-square statistic ($\chi^2 = 1.28$; $p = 0.77$).

Furthermore, the association between the three clusters and the presence of stressful life events was statistically significant ($\chi^2(2) = 18.27$, $p < 0.01$) with individuals of cluster "psychologically distressed men" had a higher percentage of negative past events than the other two groups ($n = 31$, 59.61%), whereas the men in the third cluster had a percentage of 31.66% ($n = 19$). Most of the men in the first cluster (66.80%; $n = 159$) had reported no presence of stressful life events in the previous six months. A chi-square post-hoc test via the standard residual method showed that only the standard residuals in the "psychologically distressed men" category with the stressful life events variable significantly contributed to significant omnibus chi-square statistic ($\chi^2 = 13.59$; $p < 0.001$).

Table 1. Sample's descriptive characteristics.

(*n* = 350)	
Education	%
Elementary school	0.6%
Middle school diploma	12.2%
High school diploma	53.1%
Graduate degree	34.1%
Occupation	
Unemployed	0.9%
Student	1.5%
White/Blue collar	69.3%
Self-employed (professional/business owner)	26.9%
Executive/manager	1.2%
Marital status	
Married	50.6%
Cohabitant	49.4%
Number of children	
Primiparous	72.2%
Not Primiparous	27.8%
Stressful life events [a]	
None	63.4%
One	32.4%
More than two	4.3%
Previous psychiatric diagnosis	
No	87.5%
Yes	12.5%

[a] (job loss, serious financial problems, serious problems at work, divorce, mourning, family conflicts, fights, own illness, illness of loved ones).

Table 2. Descriptive statistics of our study variables.

	Mean	**SD**	**Range**
CES-D	8.13	4.95	0–30
ANX	2.10	2.65	0–17
SOM	3.48	4.02	0–29
HOS	1.63	2.44	0–17
PSS	10.97	5.66	0–30
DAS	124.47	15.27	0–151
Addiction/risky behaviors	%		
No	80.1%		
Yes	19.9%		

Note. CES-D, The Center for Epidemiological Studies Depression Scale; ANX, Anxiety; SOM, Somatization; HOS, Hostility; PSS, The Perceived Stress Scale; DAS, the Dyadic Adjustment Scale; SD, Standard Deviation.

Table 3. Bivariate correlations among the variables.

	CES-D	ANX	SOM	HOS	PSS	DAS
CES-D	1	0.575 **	0.447 **	0.450 **	0.551 **	−0.341 **
ANX		1	0.572 **	0.533 **	0.584 **	−0.274 **
SOM			1	0.386 **	0.365 **	−0.149 **
HOS				1	0.496 **	−0.354 **
PSS					1	−0.381 **
DAS						1

Note. CES-D, The Center for Epidemiological Studies Depression Scale; ANX, Anxiety; SOM, Somatization; HOS, Hostility; PSS, The Perceived Stress Scale; DAS, The Dyadic Adjustment Scale. ** $p < 0.01$.

Table 4. Cluster analysis: ANOVA and chi-squared test.

	Cluster		Error		F–χ^2	Sig.
	Mean Square	df	Mean Square	df		
CES-D	1215.91	2	17.63	347	68.80	<0.001
ANX	683.85	2	3.16	347	215.71	<0.001
SOM	990.45	2	10.66	347	92.87	<0.001
HOS	371.1	2	3.90	347	95.14	<0.001
Addiction/risky behaviors		2			302.97	<0.001

Note. CES-D, The Center for Epidemiological Studies Depression Scale; ANX, Anxiety; SOM, Somatization; HOS, Hostility; df, degree of freedom.

4. Discussion

The expression of father psychological distress during the perinatal period tends to be multifaceted compared to maternal depressive symptomatology, including a wide range of symptoms as depressive equivalents. Thus, the conventional self-report questionnaires used for the screening of perinatal depression in mothers may be not sufficient to capture paternal psychological distress during transition to parenthood. In particular, the manifestation of male-type symptoms may be overlooked, leading to an underestimation of at-risk fathers. Therefore, it becomes essential to consider depressive equivalents, especially externalizing behaviors, for the screening of early signs of PPND. To this purpose, the current study examined psychological distress profiles in expectant fathers, using a cluster-analysis approach and testing their associations with individual and couple dimensions.

Firstly, the percentage of at-risk fathers in our sample is relatively in line with the rates of PPND emerged in previous studies [27,70]. Notably, we found that a greater number of fathers (32%) might be at-risk of developing a paternal affective disorder when other types of symptoms related to the expression of paternal perinatal distress were considered. Therefore, in these cases, a prevalence of depression in mothers and fathers can be similar, consistently with a previous study showing no differences between gender in rates of depression [31].

It has been argued that the underestimation of perinatal depression in men compared to women could be related to the type of measurements, which have been developed to address maternal mental health issues. This discrepancy highlighted the need to cover a wide range of clinical manifestations in fathers to address the impact of transition to fatherhood on paternal mental health [8,37].

Specifically, we found three profiles of paternal psychological distress during the prenatal period. The larger group included expectant fathers who reported lower levels of symptoms across the different investigated domains (anxiety, depression, hostility, and somatization). None of the expectant fathers of the "psychologically healthy men" reported addictive or risky behaviors during the last two weeks before the assessment. This finding confirms that most men perceived the transition to fatherhood as an adaptive process, without reporting specific symptoms of clinical significance during the screening process.

Focusing on the at-risk groups, the third cluster of expectant fathers defined as "men at risk of externalizing behaviors" is characterized primarily for the manifestation of one of more addictive or risky behaviors during the third trimester of pregnancy. Thus, expectant fathers may feel the need to express their psychological distress reacting with externalizing symptoms such as substance use, gambling, internet addiction, self-disruptive, and other risky behaviors as highlighted by previous research [1,31,37]. A possible explanation is that the adherence to traditional masculinity norms may pose a challenge for men who are less likely to express their psychological vulnerabilities through internalizing symptoms or clear expression of weakness. This finding supports the idea that males may often mask their depression condition showing a wide range of alternative symptoms, in particular externalizing behavior [71,72]. In particular, a large body of research revealed that substance use, including smoking, during pregnancy is one of the most relevant associated factors with PPND [17,73,74] and should be considered as a fundamental aspect in the screening of early signs and symptoms of paternal affective disorder. Substance use disorder in new parents has been linked to adverse effects for parenting, which may compromise adequate caregiving. Research has widely documented the association between substance abuse and child negative outcome, including insecure attachment, maltreatment as well as emotional, behavioral, and health problems [75,76]. Moreover, in the group of "men at risk of externalizing behaviors", hostility emerged as an important predictor to discriminate groups. Prior research highlighted the significance of the hostility, resentment, anger, and irritability as a relevant clinical manifestation of depression in men [33,77]. In this regard, it has been documented that irritability in men is associated with poor impulse control, anger attacks and aggression, substance misuse, and risk-taking or escape behaviors [78,79]. Hostility and substance use in fathers could also negatively affect parenting and couple relationships, leading to poor father–child interaction, aggressive parenting behaviors, and increasing the risk for engaging in intimate partner violence [80].

With respect to the second cluster defined as "psychologically distressed men", we found that one father out of ten reported higher levels of depression and anxiety before childbirth.

Interestingly, anxiety rather than depressive symptoms emerged as the most important predictor for this group. Evidence has shown that anxious symptoms during the perinatal period are common in men, suggesting the need to assess both depression and anxiety in expectant fathers [81]. A recent systematic review showed that the rates of anxiety disorders during the prenatal period ranged from 4.1% to 16% and remain substantially stable across the transition to parenthood [82]. This finding underlined that anxiety may be frequent in men who experience internalizing symptoms before childbirth, including those without significant depressive symptoms. Importantly, even in the case of men who experience internalizing distress, the assessment of depression could be limited, since anxiety is not adequately addressed. Both depression and anxiety in fathers have been associated with an increased risk for maternal and child health [81,83]. According to our results, fathers in this cluster could also show somatization symptoms experiencing the perception of physical dysfunction. This is consistent with previous studies showing that new fathers can express physical distress through somatic complaints and abnormal illness behaviors (the so-called Couvade Syndrome), which are considered to be part of the complex clinical picture of paternal perinatal distress [8,37].

Moreover, the association between the emerged psychological profiles and perceived stress was significant, with psychological health men reported a lower score in the scale of perceived stress than the other two clusters. Moreover, our results showed that psychologically distressed men reported higher perceived stress than the men at risk of externalized behavioral problems. According to previous studies, high perceived stress is associated to paternal affective disorders, especially with depressive and anxious symptomatology [3,26,27,84].

Finally, focusing on the association between the psychological profiles and marital adjustment, our findings revealed a significant relationship, with psychologically healthy

men reporting the highest levels of marital adjustment and psychologically distressed men reporting the lowest levels. The lack of differences on dyadic adjustment between men at risk of externalized behavioral problems and psychologically distressed men, suggests that a poor intimate relationship is a common thread among men experiencing perinatal affective symptomatology. This result highlights the relationship between individual and couple's functioning during pregnancy [11,25,85,86] and confirms the importance to consider dyadic and relational aspects as potential risk for men's health both in case of externalizing and internalizing symptoms. Indeed, other authors have focused on the negative impact that perinatal affective disorders had on marital quality, especially on marital and sexual satisfaction [87–89].

Furthermore, in our sample, the presence of symptoms of psychological distress is not related to be a first-time father. Whereas some studies have revealed that multiparous parents exhibit a higher level of anxiety, depression symptoms, and a poor health-related quality of life than primiparous parents [18,19], others have reported that parity was unassociated with an increased risk of anxiety and depression or lower health-related quality of life scores during the perinatal period [27,90].

With the respect to the association between the psychological profiles and previous psychiatric disorders, our findings revealed a significant relationship, with psychologically distressed men reporting the highest percentage of previous psychiatric disorders compared with psychologically healthy men. These findings are consistent with previous studies that have identified the presence of previous psychiatric history related to the onset or the exacerbation of affective symptomatology during the perinatal period [84,91,92].

Similarly, the association between our psychological profiles and the presence of stressful life events in the preceding six months was statistically significant, with individuals of cluster "psychologically distressed men" having a higher percentage of stressful life events than the other two groups. This finding is supported by previous studies that identified the presence of stressful life events as a potential risk factor for perinatal affective disorders [20,27,85,93].

Our findings have relevant clinical implications. Prevention programs should be implemented including both parents from the prenatal period. Given that the quality of marital adjustment can be negatively affected by perinatal affective symptoms, a partner inclusive approach needs to be adopted throughout perinatal period [94]. For the screening and diagnosis, it is essential to consider the manifestation of externalizing behavior as depressive equivalents. We encourage extending the assessment by including non-traditional symptoms of perinatal affective disorder, following a gender-sensitive perspective. In this regard, it becomes crucial to raise the awareness of perinatal practitioners with respect to the clinical expression of paternal psychological distress. Fathers at risk of externalizing behavioral problems require a more in-depth diagnostic assessment, and a personalized treatment if needed. Interventions should be tailored to specific needs and clinical manifestations of the fathers, promoting partner reciprocal support.

5. Conclusions

The present study has strengths and limitations that should be addressed. This is the first pioneering study to examine the mental health of expectant fathers based on their levels of depression, anxiety, addiction, anger attacks/hostility and somatization by identifying psychological profiles. Second, in doing this, we also examined the association among these psychological profiles, perceived stress, and marital adjustment. Third, most of the studies on PPND have focused on first-time fathers and postnatal period, whereas we examined paternal mental health before childbirth, also including fathers with one or more children.

Despite these strengths, the findings of the present study should be interpreted with caution. Indeed, the cross-sectional nature of the data prevents us from drawing conclusions about causal direction. In the future, it could be useful for the research to implement a longitudinal design that makes it possible to expand the study to the postpartum period,

analyzing the association between these psychological profiles and individual and couple variables during the postnatal period. Furthermore, it could be useful to anticipate the assessment during pregnancy to the first trimester. Indeed, data about prevalence rates of depression and anxiety and changes over time during the perinatal period vary widely [6,70]; thus, an early screening could make it possible to identify not just the presence of a symptomatology but also the trajectories of change over time [94]. Moreover, since our study was conducted on expectant fathers in their third trimester of partner's pregnancy, it could be useful in the future to also obtain information on gestation weeks to assess if expectant fathers in their final weeks are at greater risk of PPAD than others.

Another limitation of the study was to have few subjects with psychiatric history and stressful life events; future studies should better investigate the association between these variables and men at risk of PPAD.

Finally, we used self-report instruments that are not specifically developed to assess men's perinatal distress. Future studies could include, for example, clinical interviews that can better capture the complexity and the variety of early signs of paternal perinatal affective symptomatology. Moreover, it is essential to develop new measures to evaluate a broad range of depressive equivalents increasing the sensitivity and specificity of the screening in the perinatal period. [1,8,37,41]. In this perspective, a team of researchers recently created the Perinatal Assessment of Paternal Affectivity (PAPA) [32,95] a self-report instrument for the screening of affective symptomatology in fathers based on recent research on perinatal affective disorders. This tool assesses different dimensions of paternal perinatal distress (anxiety, depression, irritability/anger, couple and relational difficulties, somatic complaints, risky behaviors, and addictions). Above all, an early diagnosis of Paternal Perinatal Affective Disorder (PPAD) may reflect a more comprehensive viewpoint to assess mental health of fathers during the perinatal period and avoid potential consequences on mothers' mental health and children's development [8].

In conclusion, our findings highlight the need to design an effective and also inclusive perinatal service for fathers' psychological care, and they point out the importance of an appropriate gender-sensitive screening for detecting fathers' affective symptoms given the impact of men psychological distress on the whole family well-being.

Author Contributions: Conceptualization, F.B.; Methodology, S.M. and M.G.; Formal Analysis, S.M and M.G.; Writing—Original Draft Preparation, S.M. and M.G.; Writing—Review and Editing, M.C. and F.B.; Resources, F.S.; Supervision, F.B. and F.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Infermi Hospital (Nº 3691/2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Madsen, S.A. Men and perinatal depression. *Trends Urol. Men's Health* **2019**, *10*, 7–9. [CrossRef]
2. Philpott, L.F.; Savage, E.; Leahy-Warren, P.; Fitzgearld, S. Paternal Perinatal Depression: A Narrative Review. *Int. J. Men's Soc. Community Health* **2020**, *3*, e1–e15. [CrossRef]
3. Bruno, A.; Celebre, L.; Carmela, M.; Rizzo, A.; Silvestri, M.C.; De Stefano, R.; Zoccali, R.A.; Muscatello, M.R.A. When Fathers Begin to Falter: A Comprehensive Review on Paternal Perinatal Depression. *Int. J. Environ. Res. Public Health* **2020**, *17*, 1139. [CrossRef] [PubMed]
4. Field, T.; Diego, M.; Hernandez-Reif, M.; Figueiredo, B.; Deeds, O.; Contogeorgos, J.; Ascencio, A. Prenatal paternal depression. *Infant Behav. Dev.* **2006**, *29*, 579–583. [CrossRef]
5. Baldoni, F. Attachment, danger and role of the father in family life span. *Transilv. J. Psychol.* **2010**, *4*, 375–402.
6. Field, T. Paternal Prenatal, Perinatal and Postpartum Depression: A narrative review. *J. Anxiety Depress.* **2018**, *1*, 102–118. [CrossRef]

7. Fletcher, R.; Matthey, S.; Marley, C.G. Addressing depression and anxiety among new fathers. *Med. J. Aust.* **2006**, *185*, 461–463. [CrossRef]
8. Baldoni, F.; Giannotti, M. Perinatal Distress in Fathers: Toward a Gender-Based Screening of Paternal Perinatal Depressive and Affective Disorders. *Front. Psychol.* **2020**, *11*, 1892. [CrossRef]
9. Walsh, T.B.; Davis, R.N.; Garfield, C. A Call to Action: Screening Fathers for Perinatal Depression. *Pediatrics* **2019**, *145*, e20191193. [CrossRef]
10. Baldoni, F.; Baldaro, B.; Benassi, M. Affective disorders and illness behaviour in perinatal period: Correlations between fathers and mothers. *Child Dev. Disabil.* **2009**, *36*, 25–44.
11. Mangialavori, S.; Terrone, G.; Cantiano, A.; Franquillo, A.C.; Di Scalea, G.L.; Ducci, G.; Cacioppo, M. Dyadic Adjustment and Prenatal Parental Depression: A Study with Expectant Mothers and Fathers. *J. Soc. Clin. Psychol.* **2019**, *38*, 860–881. [CrossRef]
12. Paulson, J.F.; Bazemore, S.D.; Goodman, J.H.; Leiferman, J.A. The course and interrelationship of maternal and paternal perinatal depression. *Arch. Women's Ment. Health* **2016**, *19*, 655–663. [CrossRef]
13. Gentile, S.; Fusco, M.L. Untreated perinatal paternal depression: Effects on offspring. *Psychiatry Res.* **2017**, *252*, 325–332. [CrossRef]
14. Letourneau, N.; Apron the APrON Team; Leung, B.; Ntanda, H.; Dewey, D.; Deane, A.J.; Giesbrecht, G.F. Maternal and paternal perinatal depressive symptoms associate with 2- and 3-year-old children's behaviour: Findings from the APrON longitudinal study. *BMC Pediatr.* **2019**, *19*, 1–13. [CrossRef]
15. Sweeney, S.; Macbeth, A. The effects of paternal depression on child and adolescent outcomes: A systematic review. *J. Affect. Disord.* **2016**, *205*, 44–59. [CrossRef]
16. Chhabra, J.; McDermott, B.; Li, W. Risk factors for paternal perinatal depression and anxiety: A systematic review and meta-analysis. *Psychol. Men Masc.* **2020**, *21*, 593–611. [CrossRef]
17. Underwood, L.; Waldie, K.E.; Peterson, E.; D'Souza, S.; Verbiest, M.; McDaid, F.; Morton, S. Paternal Depression Symptoms During Pregnancy and After Childbirth Among Participants in the Growing Up in New Zealand Study. *JAMA Psychiatry* **2017**, *74*, 360–410. [CrossRef]
18. Chen, Y.-H.; Huang, J.-P.; Au, H.-K. High risk of depression, anxiety, and poor quality of life among experienced fathers, but not mothers: A prospective longitudinal study. *J. Affect. Disord.* **2019**, *242*, 39–47. [CrossRef]
19. Figueiredo, B.; Conde, A. Anxiety and depression in women and men from early pregnancy to 3-months postpartum. *Arch. Women's Ment. Health* **2011**, *14*, 247–255. [CrossRef]
20. Wee, K.Y.; Skouteris, H.; Pier, C.; Richardson, B.; Milgrom, J. Correlates of ante- and postnatal depression in fathers: A systematic review. *J. Affect. Disord.* **2011**, *130*, 358–377. [CrossRef]
21. Da Costa, D.; Zelkowitz, P.; Dasgupta, K.; Sewitch, M.; Lowensteyn, I.; Cruz, R.; Hennegan, K.; Khalifé, S. Dads Get Sad Too: Depressive Symptoms and Associated Factors in Expectant First-Time Fathers. *Am. J. Men's Health* **2017**, *11*, 1376–1384. [CrossRef]
22. Philpott, L.F.; Corcoran, P. Paternal postnatal depression in Ireland: Prevalence and associated factors. *Midwifery* **2018**, *56*, 121–127. [CrossRef]
23. Paulson, J.F.; Bazemore, S.D. Prenatal and Postpartum Depression in Fathers and Its Association with Maternal Depression. *JAMA* **2010**, *303*, 1961–1969. [CrossRef]
24. Pinheiro, R.T.; Magalhaes, P.V.S.; Horta, B.L.; Pinheiro, K.A.T.; A Da Silva, R.; Pinto, R.H. Is paternal postpartum depression associated with maternal postpartum depression? Population-based study in Brazil. *Acta Psychiatr. Scand.* **2006**, *113*, 230–232. [CrossRef]
25. Terrone, G.; Mangialavori, S.; Di Scalea, G.L.; Cantiano, A.; Temporin, G.; Ducci, G.; Gori, A.; Cacioppo, M.; Schimmenti, A.; Caretti, V. The relationship between dyadic adjustment and psychiatric symptomatology in expectant couples: An actor–partner interdependency model approach. *J. Affect. Disord.* **2020**, *273*, 468–475. [CrossRef]
26. Baldoni, F.; Giannotti, M.; Casu, G.; Luperini, V.; Spelzini, F. A Dyadic Study on Perceived Stress and Couple Adjustment During Pregnancy: The Mediating Role of Depressive Symptoms. *J. Fam. Issues* **2020**, *41*, 1935–1955. [CrossRef]
27. Cameron, E.E.; Sedov, I.D.; Tomfohr-Madsen, L.M. Prevalence of paternal depression in pregnancy and the postpartum: An updated meta-analysis. *J. Affect. Disord.* **2016**, *206*, 189–203. [CrossRef]
28. Nakano, M.; Sourander, A.; Luntamo, T.; Chudal, R.; Skokauskas, N.; Kaneko, H. Early risk factors for postpartum depression: A longitudinal Japanese population-based study. *J. Affect. Disord.* **2020**, *269*, 148–153. [CrossRef]
29. O'Brien, A.P.; McNeil, K.A.; Fletcher, R.; Conrad, A.; Wilson, A.J.; Jones, D.; Chan, S.W. New Fathers' Perinatal Depression and Anxiety-Treatment Options: An Integrative Review. *Am. J. Men's Health* **2016**, *11*, 863–876. [CrossRef]
30. Kim, P.; Swain, J.E. Sad Dads: Paternal Postpartum Depression. *Psychiatry* **2007**, *4*, 35–47.
31. Martin, L.A.; Neighbors, H.W.; Griffith, D.M. The Experience of Symptoms of Depression in Men vs Women. *JAMA Psychiatry* **2013**, *70*, 1100–1106. [CrossRef]
32. Baldoni, F.; Matthey, S.; Agostini, F.; Schimmenti, A.; Caretti, V. Perinatal assessment of paternal affectivity (PAPA): Preliminary report on a new screening tool. *Infant Ment. Health J.* **2016**, *37*, 132–133.
33. Cochran, S.V.; Rabinowitz, F.E. Gender-sensitive recommendations for assessment and treatment of depression in men. *Prof. Psychol. Res. Pract.* **2003**, *34*, 132–140. [CrossRef]
34. Danielsson, U.; Johansson, E.E. Beyond weeping and crying: A gender analysis of expressions of depression. *Scand. J. Prim. Health Care* **2005**, *23*, 171–177. [CrossRef]

35. Habib, C. Paternal perinatal depression: An overview and suggestions towards an intervention model. *J. Fam. Stud.* **2012**, *18*, 4–16. [CrossRef]
36. Magovcevic, M.; Addis, M.E. The Masculine Depression Scale: Development and psychometric evaluation. *Psychol. Men Masc.* **2008**, *9*, 117–132. [CrossRef]
37. Psouni, E.; Agebjörn, J.; Linder, H. Symptoms of depression in Swedish fathers in the postnatal period and development of a screening tool. *Scand. J. Psychol.* **2017**, *58*, 485–496. [CrossRef]
38. American Psychiatric Association (APA). (Ed.) *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, 5th ed.; American Psychiatric Association: Washington, DC, USA, 2013.
39. Zierau, F.; Bille, A.; Rutz, W.; Bech, P. The Gotland Male Depression Scale: A validity study in patients with alcohol use disorder. *Nord. J. Psychiatry* **2002**, *56*, 265–271. [CrossRef]
40. Cox, J.L.; Holden, J.M.; Sagovsky, R. Detection of Postnatal Depression: Development of the 10-item Edinburgh Postnatal Depression Scale. *Br. J. Psychiatry* **1987**, *150*, 782–786. [CrossRef]
41. Matthey, S.; Barnett, B.; Kavanagh, D.J.; Howie, P. Validation of the Edinburgh Postnatal Depression Scale for men, and comparison of item endorsement with their partners. *J. Affect. Disord.* **2001**, *64*, 175–184. [CrossRef]
42. Edmondson, O.J.; Psychogiou, L.; Vlachos, H.; Netsi, E.; Ramchandani, P.G. Depression in fathers in the postnatal period: Assessment of the Edinburgh Postnatal Depression Scale as a screening measure. *J. Affect. Disord.* **2010**, *125*, 365–368. [CrossRef] [PubMed]
43. Lai, B.P.; Tang, A.K.; Lee, D.T.; Yip, A.S.; Chung, T.K. Detecting postnatal depression in Chinese men: A comparison of three instruments. *Psychiatry Res.* **2010**, *180*, 80–85. [CrossRef] [PubMed]
44. Loscalzo, Y.; Giannini, M.; Contena, B.; Gori, A.; Benvenuti, P. The Edinburgh Postnatal Depression Scale for Fathers: A contribution to the validation for an Italian sample. *Gen. Hosp. Psychiatry* **2015**, *37*, 251–256. [CrossRef] [PubMed]
45. Nishimura, A.; Ohashi, K. Risk factors of paternal depression in the early postnatal period in Japan. *Nurs. Health Sci.* **2010**, *12*, 170–176. [CrossRef] [PubMed]
46. Madsen, S.A.; Juhl, T. Paternal depression in the postnatal period assessed with traditional and male depression scales. *J. Men's Health Gend.* **2007**, *4*, 26–31. [CrossRef]
47. Carlberg, M.; Edhborg, M.; Lindberg, L. Paternal Perinatal Depression Assessed by the Edinburgh Postnatal Depression Scale and the Gotland Male Depression Scale: Prevalence and Possible Risk Factors. *Am. J. Men's Health* **2018**, *12*, 720–729. [CrossRef]
48. Molgora, S.; Fenaroli, V.; Saita, E. Psychological distress profiles in expectant mothers: What is the association with pregnancy-related and relational variables? *J. Affect. Disord.* **2020**, *262*, 83–89. [CrossRef]
49. Azam Hajikhani, N.; Mahmoodi, Z.; Nasiri, M.; Rahnemaei, F.A.; Ozgoli, G. Predictors of Fathers' Worry about High Risk Pregnancy of Their Wife: A Path Analysis. *Int J Pediatr.* **2020**. [CrossRef]
50. McCain, G.C.; Deatrick, J.A. The Experience of High-Risk Pregnancy. *J. Obstet. Gynecol. Neonatal Nurs.* **1994**, *23*, 421–427. [CrossRef]
51. Radloff, L.S. The CES-D Scale. *Appl. Psychol. Meas.* **1977**, *1*, 385–401. [CrossRef]
52. Field, T.; Hernandez-Reif, M.; Diego, M. Risk factors and stress variables that differentiate depressed from nondepressed pregnant women. *Infant Behav. Dev.* **2006**, *29*, 169–174. [CrossRef]
53. Fava, G.A. Assessing depressive symptoms across cultures: Italian validation of the CES-D self-rating scale. *J. Clin. Psychol.* **1983**, *39*, 249–251. [CrossRef]
54. Derogatis, L.R. *Symptom Checklist-90-R (SCL-90-R): Administration, Scoring and Procedures Manual*, 3rd ed.; National Computer Systems: Minneapolis, MN, USA, 1984.
55. Prunas, A.; Sarno, I.; Preti, E.; Madeddu, F.; Perugini, M. Psychometric properties of the Italian version of the SCL-90-R: A study on a large community sample. *Eur. Psychiatry* **2012**, *27*, 591–597. [CrossRef]
56. Cohen, S.; Kamarck, T.; Mermelstein, R. A global measure of perceived stress. *J. Health Soc. Behav.* **1983**, *24*, 385. [CrossRef]
57. Gao, L.-L.; Chan, S.W.-C.; Mao, Q. Depression, perceived stress, and social support among first-time Chinese mothers and fathers in the postpartum period. *Res. Nurs. Health* **2009**, *32*, 50–58. [CrossRef]
58. Mondo, M.; Sechi, C.; Cabras, C. Psychometric evaluation of three versions of the Italian Perceived Stress Scale. *Curr. Psychol.* **2019**, 1–9. [CrossRef]
59. Spanier, G.B. Measuring Dyadic Adjustment: New Scales for Assessing the Quality of Marriage and Similar Dyads. *J. Marriage Fam.* **1976**, *38*, 15. [CrossRef]
60. Gentili, P.; Contreras, L.; Cassaniti, M.; D Arista, F. A measurement of dyadic adjustment: The Dyadic Adjustment Scale. *Minerva Psichiatr.* **2002**, *43*, 107–116.
61. Kent, P.; Jensen, R.K.; Kongsted, A. A comparison of three clustering methods for finding subgroups in MRI, SMS or clinical data: SPSS TwoStep Cluster analysis, Latent Gold and SNOB. *BMC Med. Res. Methodol.* **2014**, *14*, 113. [CrossRef]
62. Norusis, M. *Cluster Analysis*; Prentice Hall: Upper Saddle River, NJ, USA, 2008; pp. 361–391.
63. Tkaczynski, A. *Segmentation in Social Marketing*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2016; pp. 109–125.
64. Sarstedt, M.; Mooi, E. *A Concise Guide to Market Research*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2014.

65. Gelbard, R.; Goldman, O.; Spiegler, I. Investigating diversity of clustering methods: An empirical comparison. *Data Knowl. Eng.* **2007**, *63*, 155–166. [CrossRef]
66. Sharpe, D. Chi-Square Test Is Statistically Significant: Now What? *Pract. Assess. Res. Eval.* **2015**, *20*, 8. [CrossRef]
67. Discovering Statistics Using IBM SPSS Statistics. And Sex and Drugs and Rock'n'Roll. *Pflege* **2014**, *27*, 430. [CrossRef]
68. Cohen, J.R.; Spiro, C.N.; Young, J.F.; Gibb, B.E.; Hankin, B.L.; Abela, J.R.Z. Interpersonal Risk Profiles for Youth Depression: A Person-Centered, Multi-Wave, Longitudinal Study. *J. Abnorm. Child Psychol.* **2015**, *43*, 1415–1426. [CrossRef] [PubMed]
69. Kaufman, L.; Rousseeuw, P.J. *Finding Groups in Data: An Introduction to Cluster Analysis*; Wiley Series in Probability and Mathematical Statistics; Wiley: Hoboken, NJ, USA, 2005.
70. Rao, W.-W.; Zhu, X.-M.; Zong, Q.-Q.; Zhang, Q.; Hall, B.J.; Ungvari, G.S.; Xiang, Y.-T. Prevalence of prenatal and postpartum depression in fathers: A comprehensive meta-analysis of observational surveys. *J. Affect. Disord.* **2020**, *263*, 491–499. [CrossRef]
71. Rutz, W.; Von Knorring, L.; Pihlgren, H.; Rihmer, Z.; Wålinder, J. Prevention of male suicides: Lessons from Gotland study. *Lancet* **1995**, *345*, 524. [CrossRef]
72. Lynch, J.; Kilmartin, C. *The Pain behind the Mask: Overcoming Masculine Depression*; The Haworth Press Advances in Psychology and Mental Health; Haworth Press: New York, NY, USA, 1999.
73. Epifanio, M.S.; Genna, V.; De Luca, C.; Roccella, M.; La Grutta, S. Paternal and Maternal Transition to Parenthood: The Risk of Postpartum Depression and Parenting Stress. *Pediatr. Rep.* **2015**, *7*, 38–44. [CrossRef]
74. Recto, P.; Champion, J.D. Psychosocial Factors Associated with Paternal Perinatal Depression in the United States: A Systematic Review. *Issues Ment. Health Nurs.* **2020**, *41*, 608–623. [CrossRef]
75. Osborne, C.; Berger, L.M. Parental Substance Abuse and Child Well-Being. *J. Fam. Issues* **2009**, *30*, 341–370. [CrossRef]
76. Eiden, R.D.; Edwards, E.P.; Leonard, K.E. Mother–infant and father–infant attachment among alcoholic families. *Dev. Psychopathol.* **2002**, *14*, 253–278. [CrossRef]
77. Oliffe, J.L.; Phillips, M.J. Men, depression and masculinities: A review and recommendations. *J. Men's Health* **2008**, *5*, 194–202. [CrossRef]
78. Winkler, M.U.D.; Pjrek, E.; Kasper, S. Anger Attacks in Depression-Evidence for a Male Depressive Syndrome. *Psychother. Psychosom.* **2005**, *74*, 303–307. [CrossRef]
79. Cavanagh, A.; Wilson, C.J.; Kavanagh, D.; Caputi, P. Differences in the Expression of Symptoms in Men Versus Women with Depression. *Harv. Rev. Psychiatry* **2017**, *25*, 29–38. [CrossRef]
80. Stover, C.S.; Kiselica, A. Hostility and substance use in relation to intimate partner violence and parenting among fathers. *Aggress. Behav.* **2015**, *41*, 205–213. [CrossRef]
81. Matthey, S.; Barnett, B.; Howie, P.; Kavanagh, D.J. Diagnosing postpartum depression in mothers and fathers: Whatever happened to anxiety? *J. Affect. Disord.* **2003**, *74*, 139–147. [CrossRef]
82. Leach, L.; Poyser, C.; Cooklin, A.R.; Giallo, R. Prevalence and course of anxiety disorders (and symptom levels) in men across the perinatal period: A systematic review. *J. Affect. Disord.* **2016**, *190*, 675–686. [CrossRef]
83. Ramchandani, P.G.; Psychogiou, L. Paternal psychiatric disorders and children's psychosocial development. *Lancet* **2009**, *374*, 646–653. [CrossRef]
84. Ramchandani, P.G.; O'Connor, T.G.; Evans, J.; Heron, J.; Murray, L.; Stein, A. The effects of pre- and postnatal depression in fathers: A natural experiment comparing the effects of exposure to depression on offspring. *J. Child Psychol. Psychiatry* **2008**, *49*, 1069–1078. [CrossRef]
85. Boyce, P.; Condon, J.T.; Barton, J.; Corkindale, C.J. First-Time Fathers' Study: Psychological Distress in Expectant Fathers During Pregnancy. *Aust. N. Z. J. Psychiatry* **2007**, *41*, 718–725. [CrossRef]
86. De Montigny, F.; Girard, M.-E.; Lacharité, C.; Dubeau, D.; DeVault, A. Psychosocial factors associated with paternal postnatal depression. *J. Affect. Disord.* **2013**, *150*, 44–49. [CrossRef]
87. Banker, J.E.; Lacoursiere, D.Y. Postpartum Depression: Risks, Protective Factors, and the Couple's Relationship. *Issues Ment. Health Nurs.* **2014**, *35*, 503–508. [CrossRef]
88. Małus, A.; Szyluk, J.; Galińska-Skok, B.; Konarzewska, B. Incidence of postpartum depression and couple relationship quality. *Psychiatr. Pol.* **2016**, *50*, 1135–1146. [CrossRef]
89. Sipsma, H.L.; Callands, T.; Desrosiers, A.; Magriples, U.; Jones, K.; Albritton, T.; Kershaw, T. Exploring Trajectories and Predictors of Depressive Symptoms Among Young Couples During Their Transition to Parenthood. *Matern. Child Health J.* **2016**, *20*, 2372–2381. [CrossRef]
90. Breitkopf, C.R.; Primeau, L.A.; Levine, R.E.; Olson, G.L.; Wu, Z.H.; Berenson, A.B. Anxiety symptoms during pregnancy and postpartum. *J. Psychosom. Obstet. Gynecol.* **2006**, *27*, 157–162. [CrossRef]
91. Condon, J.T.; Boyce, P.; Corkindale, C.J. The First-Time Fathers Study: A prospective study of the mental health and wellbeing of men during the transition to parenthood. *Aust. N. Z. J. Psychiatry* **2004**, *38*, 56–64. [CrossRef]
92. Matthey, S.; Barnett, B.; Ungerer, J.; Waters, B. Paternal and maternal depressed mood during the transition to parenthood. *J. Affect. Disord.* **2000**, *60*, 75–85. [CrossRef]
93. Da Costa, D.; Danieli, C.; Abrahamowicz, M.; Dasgupta, K.; Sewitch, M.; Lowensteyn, I.; Zelkowitz, P. A prospective study of postnatal depressive symptoms and associated risk factors in first-time fathers. *J. Affect. Disord.* **2019**, *249*, 371–377. [CrossRef]

94. Kiviruusu, O.; Pietikäinen, J.T.; Kylliäinen, A.; Pölkki, P.; Saarenpää-Heikkilä, O.; Marttunen, M.; Paunio, T.; Paavonen, E.J. Trajectories of mothers' and fathers' depressive symptoms from pregnancy to 24 months postpartum. *J. Affect. Disord.* **2020**, *260*, 629–637. [CrossRef]
95. Baldoni, F.; Matthey, S.; Agostini, F.; Schimmenti, A.; Caretti, V. Perinatal Assessment of Paternal Affectivity (PAPA). First validation in Italian samples. 16th World Association for Infant Mental Health (WAIMH) Congress. *Infant Ment. Health J.* **2018**, *39*, 311.

Journal of *Personalized Medicine*

MDPI

Article

The Relationship between Psychological Distress during the Second Wave Lockdown of COVID-19 and Emotional Eating in Italian Young Adults: The Mediating Role of Emotional Dysregulation

Anna Guerrini Usubini [1,2], Roberto Cattivelli [1,2], Giorgia Varallo [1,2], Gianluca Castelnuovo [1,2], Enrico Molinari [1,2], Emanuele Maria Giusti [1,2,*], Giada Pietrabissa [1,2], Tommaso Manari [3], Maria Filosa [4], Christian Franceschini [4] and Alessandro Musetti [3]

1 Istituto Auxologico Italiano IRCCS, Psychology Research Laboratory, 20145 Milan, Italy; u.guerrini@auxologico.it (A.G.U.); r.cattivelli@auxologico.it (R.C.); g.varallo@auxologico.it (G.V.); gianluca.castelnuovo@auxologico.it (G.C.); molinari@auxologico.it (E.M.); g.pietrabissa@auxologico.it (G.P.)
2 Department of Psychology, Catholic University of Milan, 20123 Milan, Italy
3 Department of Humanities, Social Sciences and Cultural Industries, University of Parma, 43121 Parma, Italy; tommaso.manari@unipr.it (T.M.); alessandro.musetti@unipr.it (A.M.)
4 Department of Medicine and Surgery, University of Parma, 43121 Parma, Italy; filosa.maria@hotmail.it (M.F.); Christian.franceschini@unipr.it (C.F.)
* Correspondence: e.giusti@auxologico.it; Tel.: +39-0323-514246

Citation: Guerrini Usubini, A.; Cattivelli, R.; Varallo, G.; Castelnuovo, G.; Molinari, E.; Giusti, E.M.; Pietrabissa, G.; Manari, T.; Filosa, M.; Franceschini, C.; et al. The Relationship between Psychological Distress during the Second Wave Lockdown of COVID-19 and Emotional Eating in Italian Young Adults: The Mediating Role of Emotional Dysregulation. *J. Pers. Med.* **2021**, *11*, 569. https://doi.org/10.3390/jpm11060569

Academic Editor: Marco Costanzi

Received: 3 May 2021
Accepted: 14 June 2021
Published: 17 June 2021

Abstract: This cross-sectional study aims to investigate the impact of psychological distress experienced during the second wave of the COVID-19 pandemic on emotional eating and to assess the mediating role of emotional dysregulation in a sample of Italian young adults (20–35). A total of 437 participants provided demographical data and were assessed using the Depression Anxiety Stress Scale, the Difficulties in Emotion Regulation Scale, and the Emotional Eating subscale of the Dutch Eating Behavior Questionnaire. Correlational analyses were performed to assess the relationship between continuous variables, while ANOVA was conducted to detect differences between males and females for emotional eating. To assess whether demographic and clinical data predicted emotional eating, hierarchical linear regression was performed. Then, a mediation analysis was conducted to assess whether emotional dysregulation was a mediator between psychological distress and emotional eating. Emotional eating was associated with psychological distress and emotional dysregulation. Moreover, higher levels of emotional eating were found in females than in males. Predictors of emotional eating were sex, psychological distress, and emotional dysregulation. Mediation analyses showed that the indirect effect of psychological distress on emotional eating through emotional dysregulation was significant (b = 0.0069; SE = 0.0024; CI = 0.0024–0.0118), confirming that the relationship between psychological distress and emotional eating was mediated by emotional dysregulation, controlling for sex. The model explained 26.8% (R^2 = 0.2680) of the variance. These findings may help to plan and develop psychological interventions aimed at addressing emotional eating in young adults by targeting emotional dysregulation.

Keywords: COVID-19; young adults; social isolation; psychological distress; emotional eating; emotional dysregulation

1. Introduction

Coronavirus disease 19 (COVID-19), a new form of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was firstly identified in Wuhan City (China) in December 2019. Since then, COVID-19 has rapidly spread throughout China and has quickly become a global health concern. After China, Italy was one of the first countries in which COVID-19 spread. The first infections were recorded at the end of January 2020, and over the next

few months the number of cases grew exponentially. Facing an increasing number of cases, the Italian government has implemented extraordinary preventive measures based on social distancing, limitation of movement and physical interaction, and unprecedented quarantine measures. Citizens were asked to isolate themselves and were not allowed to leave their homes except for well-documented reasons. Non-essential activities and schools were closed, and most workers were restricted to working from home or stopping work. On 11 March 2020, Italy was locked down. This extreme measure was adopted until 4 May 2020. Then, a subsequent reduction in case numbers allowed the Italian government to reduce the imposed containment measures in summer. Unfortunately, after a period of decreased case numbers, a second wave of COVID-19 began, and Italy once more faced a series of restrictive measures, even if there was different severity in different areas.

Although it was necessary to halt the contagion curve, the prolonged restrictive measures harmed the physical and mental health of the Italian general population, generating a variety of psychological problems. Recent studies reported increased anxiety and depressive symptoms, post-traumatic stress, digestive symptoms [1,2], compulsive and addictive behaviors [3–5], and poor sleep quality [6,7] in the Italian population during the lockdown.

The negative impact of the COVID-19 pandemic and the associated restrictive measures was particularly marked among specific populations, including young adults who typically report negative psychological consequences during health emergencies [8,9]. Young adults reported negative psychological effects related to the pandemic [7–9]. In Italy, a study [10] showed that during the first four weeks of lockdown (from 16 March to 16 April, 2020), Italian young adults (19–29) reported an increase in internalizing problems, including depression, anxiety, withdrawal, and somatic complaints, and externalizing problems, such as aggressive and rule-breaking behaviors. Conversely, the perception of personal strengths decreased.

Stress, anxiety, and depression due to the COVID-19 pandemic and subsequent restrictive measures had a negative impact on eating behaviors [9]. In a recent study aimed at exploring changes in eating habits during the lockdown using an Italian sample, individuals reported eating more than usual and eating unhealthy food. Participants also attributed changes in their eating habits to increased anxiety caused by COVID-19 and subsequent lockdowns [10]. In another Italian cross-sectional study, the authors found that participants reported eating in response to negative feelings of anxiety and increasing their food intake [11].

This scenario reflects emotional eating. Emotional eating is defined as "the tendency to eat in response to negative emotions" [12]. Emotional eating could be problematic for both physical and psychological health since it has been associated with consuming unhealthy food and, therefore, weight gain, as well as with poorer psychological well-being, depression [13], and eating disorders [14,15]. Psychological distress, in particular depression and anxiety, was found to be a risk factor for the onset of eating disorders. For instance, it has been found that individuals with low mood engage in disordered eating behaviors to feel comfort from aversive emotional states [16]. Even though it is well recognized that emotional eating is triggered by psychological distress and negative mood, the mechanisms underlying this relationship are yet to be addressed [17].

In the literature, one of the key factors associated with emotional eating is emotional dysregulation [18]. Gratz and Roemer [19] proposed a multidimensional conceptualization of emotional regulation that included the awareness and acceptance of experienced emotions and the control of impulsive behaviors when experiencing negative emotions in order to behave in accordance with desired goals. In addition, it included the ability to use appropriate emotional regulation strategies to flexibly modulate emotional responses to situations. Based on this model, emotional regulation strategies allow individuals to act in accordance with personal goals, even in the presence of negative emotions, while controlling impulsive behaviors. On the other hand, emotional eating is generally used to regulate negative feelings when emotional regulation abilities are lacking. In light of this model, a recent study found that psychological distress, in particular anxiety, was related

to "drunkorexia"—an eating disorder that is characterized by indulging in weight control behaviors in relation to drinking alcohol—in the presence of higher levels of emotional dysregulation in a sample of non-clinical adolescents [20]. On the basis of the same model of emotion dysregulation, Squires and colleagues [21] found a positive and significant correlation between psychological distress and emotional dysregulation. These findings were also reported in previous studies [22–24].

Emotional dysregulation was found to be an underlying mechanism of emotional eating. McAtamney and colleagues [25] recently explored the mediating role of emotional dysregulation between the difficulty in describing feelings (alexithymia) and emotional eating in a sample of 136 participants recruited from the general population in the United Kingdom in July 2020 after a period of lockdown. Results showed an indirect effect of emotional dysregulation by which difficulties in identifying and describing emotions predicted emotional eating. By outlining the mechanism underpinning emotional eating, findings from that study increased awareness about how eating behaviors changed in the context of the pandemic.

To the best of the authors' knowledge, no studies have been carried out to assess emotional eating in Italian young adults during the COVID-19 pandemic.

Therefore, the current study aimed to explore the relationship between psychological distress related to the second COVID-19 lockdown and emotional eating. Moreover, the second aim of the study was to investigate the mediating role of emotional dysregulation in the link between psychological distress and emotional eating.

In particular, we hypothesized that the relationships between psychological distress and emotional dysregulation and between emotional dysregulation and emotional eating would be significant. In addition, we hypothesized that there would be a significant relationship between psychological distress and emotional eating. Finally, we hypothesized that the relationship between psychological distress and emotional eating would be mediated by emotional dysregulation.

2. Materials and Methods

2.1. Participants and Procedures

This cross-sectional study is part of a larger research project called "Effects of the second wave COVID-19 on general population: sleep quality and hyperconnectivity". Data were collected from 1 December 2020 to 31 January 2021 during the second wave of COVID-19 in Italy. A convenience sample of 437 Italian young adults completed an anonymous online survey via the Microsoft Azure platform after providing written informed consent.

Inclusion criteria were a) age between 20 and 35 years, b) Italian mother tongue, and c) living in Italy during the second wave of COVID-19 lockdown.

The Ethical Committee of the Center for Research and Psychological Intervention (CERIP) of the University of Messina approved the study (protocol number: 17758). All procedures were conducted in accordance with the Declaration of Helsinki and its later advancements.

2.2. Measures

The survey involved demographical and clinical measures. Demographical data included sex, age, nationality, work status, marital status, weight, and height. Body mass index (BMI = kg/m^2) was obtained by dividing weight expressed in kilograms by the square of height in meters. To assess clinical variables, we used the Italian validated questionnaires discussed below.

Psychological distress. The *Depression Anxiety Stress Scale* (DASS-21) [26] was administered to measure psychological distress. It is a self-report questionnaire composed of 21 items, rated on a 4-point Likert scale, ranging from 0 to 3, which explores three subscales: depression, anxiety, and stress. The total score of DASS-21 was used as a measure of psychological distress. We used the Italian version validated by Bottesi and colleagues [27] that showed good psychometric properties (Cronbach's alpha values of subscales ranged

from 0.83 to 0.91. The Cronbach's alpha of the total score was = 0.92. In our sample, the Cronbach's alpha of the total score was excellent (Cronbach's alpha = 0.94).

Emotional dysregulation. The Difficulties in Emotion Regulation Scale (DERS) [19] was administered to assess difficulties in emotional regulation. This is a self-report questionnaire consisting of 36 items, rated on a 5-point Likert scale, ranging from 1 (almost never) to 5 (almost always), which explores the following subscales: non-acceptance of negative emotions, inability to undertake purposeful behavior when experiencing negative emotions, difficulty in controlling impulsive behavior when experiencing negative emotions, limited access to emotion regulation strategies that are considered effective, lack of awareness of one's emotions, and lack of understanding of the nature of one's emotional responses. We used the Italian version validated by Giromini and colleagues [28] that showed good psychometric properties. The Cronbach's alpha of the total score was 0.92. In our sample, the Cronbach's alpha of the total score was excellent (Cronbach's alpha = 0.90)

Emotional eating. The Emotional Eating subscale of the Dutch Eating Behavior Questionnaire (EE_DEBQ) [15] was administered to assess emotional eating. The DEBQ is a self-report questionnaire used to assess eating behaviors. The Emotional Eating subscale consists of 13 items, rated on a 5-step Likert scale, ranging from 0 (never) to 4 (almost always). We used the Italian version validated by Dakanalis and colleagues [29] that showed good psychometric properties (Cronbach's alpha = 0.97). In our sample, the Cronbach's alpha of the subscale was excellent (Cronbach's alpha = 0.95)

2.3. Statistical Analysis

Frequencies and percentages for categorical variables and means and standard deviations for continuous variables were computed. To assess normal distribution of variables, skewness and kurtosis were evaluated. Parameters outside the limit of $+1.5/-1.5$ range were considered an index of non-normality. Bivariate Pearson's correlations were calculated to assess the correlations between all the continuous demographical (age and BMI) and clinical (psychological distress, emotional dysregulation, and emotional eating) variables. Univariate analysis of variance (ANOVA) was performed to assess whether males and females differed in emotional eating. A hierarchical linear regression model was used to determine which factors were predictors of emotional eating. Mediation analysis was performed to assess the mediating role of emotional dysregulation in the relationship between psychological distress and emotional eating using Model 4 of PROCESS Macro for SPSS [30]. An estimation of the indirect effect was obtained using the bias-corrected bootstrapping method (5,000 samples). Then, 95% bias-corrected confidence intervals (BC-CIs) were calculated to determine the significance of the mean indirect effects. The indirect effect was considered statistically significant at $p < 0.05$ when 95% BC-CIs did not include zero.

Analyses were performed using Jamovi (1.6.15) and IBM Statistical Package for the Social Sciences SPSS version 26 (Armonk, NY: IBM Corp).

3. Results

3.1. Descriptive Statistics of the Sample and Relations to Emotional Eating

After subjects who did not meet inclusion criteria were excluded, 592 subjects filled out the online survey. In order to have a normal-weight sample, we excluded participants with BMI less than 18.5 and more than 25 (WHO, 2000). The final sample was composed of 437 participants. There were 213 (48.7%) males and 224 (51.3%) females; the mean age was 25.2 (SD = 5.12). Most of participants were Italian (97.3%), had a high school degree (68.2%), were students (38%), and were single (69.8%). Missing data were less than 5% and so were considered negligible [29]. The descriptive statistics of the sample are presented in Table 1. A flow chart of the recruitment of the sample is shown in Figure 1.

Table 1. Descriptive statistics of the sample.

	N (%)	Mean ± SD	Range
Sex			
Male	213 (48.7%)		
Female	224 (51.3%)		
Age (in years)		25.2 ± (5.12)	20–35
BMI (Kg/m2)		21.9 ± (1.73)	18.5–25
Nationality			
Italian	425 (97.3%)		
Non-Italian	12 (2.7%)		
Educational level			
Primary school	0 (0%)		
Secondary school	11 (2.5%)		
Higher school	298 (68.2%)		
Bachelor's degree	120 (27.5%)		
Master's degree	8 (1.8%)		
Marital status			
Single	305 (69.8%)		
Married	131 (30%)		
Divorced	1 (0.2%)		
Work status			
Student	166 (38%)		
Student and employed	82 (18.8%)		
Employed	161 (36.8%)		
Unemployed	16 (3.7%)		
Other	12 (2.7%)		
DASS-21		40.5 ± (12.8)	21–80
DERS		89.7 ± (19.5)	36–156
EE_DEBQ		2.07 ± (0.9)	1–5

Note: BMI: body mass index; DASS-21: Depression Anxiety and Stress scale; DERS: Difficulties in Emotional Regulation Scale; EE_DEBQ: Emotional Eating subscale of the Dutch Eating Behavior Questionnaire.

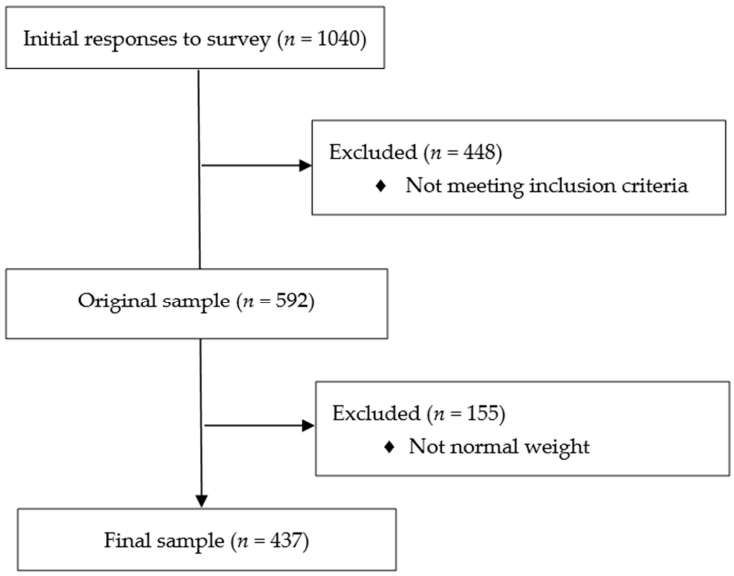

Figure 1. Flow chart. Bivariate correlations revealed that the demographic variables of age and BMI were not significantly correlated with emotional eating. As far as psychological distress is concerned, there was a significant and positive correlation between the total score of DASS-21 (r = 0.395; $p < 0.001$) and emotional eating. Emotional dysregulation, assessed with the total score of DERS, was significantly and positively associated with emotional eating (r = 0.348; $p < 0.001$). All correlations are presented in Table 2.

Table 2. Relationships between all the variables of interest.

	Age	BMI	DASS-21	DERS	EE_DEBQ	F	*p*
Age							
BMI	0.196 ***						
DASS-21	−0.160 ***	−0.078					
DERS	−0.137 ***	−0.086	0.580 ***				
EE_DEBQ	−0.074	0.024	0.395 ***	0.348 ***			
Sex						64.84	<0.001

Note: BMI: body mass index; DASS-21: Depression Anxiety and Stress scale; DERS: Difficulties in Emotional Regulation Scale; EE_DEBQ: Emotional Eating subscale of the Dutch Eating Behavior Questionnaire;*** $p < 0.001$.

ANOVA test results indicated that there was a significant difference between males and females in emotional eating (F(1,413) = 64.84; $p < 0.001$). Females reported greater emotional eating (M = 2.38; SD = 0.94) than males did (M = 1.74; SD = 0.71).

3.2. Predictors of Emotional Eating

To assess whether demographic and clinical variables were predictors of emotional eating, a multiple hierarchical linear regression model was performed. The model was built to detect the effect of psychological distress and emotional dysregulation on emotional eating controlling for sex, the only demographical variable related to emotional eating. Sex was added as a control variable at the first block; the total score of DASS-21 at the second block; and the total score of DERS at the third.

The first model accounted for a significant amount of variance in emotional eating ($R^2 = 0.13$; $p < 0.001$; F(1434) = 64.1; $p < 0.001$). Then, the total score of DASS-21 was added at the second block. The model explained 25% of the variance for emotional eating ($R^2 = 0.25$; $p < 0.001$; F(2433) = 72.4; $p < 0.001$). Finally, the total score of DERS was added at the third block. The final model accounted for 27% of the variance for emotional eating ($R^2 = 0.27$; $p < 0.001$; F(3432) = 52.7; $p < 0.001$). The results are presented in Table 3.

Table 3. Hierarchical multiple regression with emotional eating as a dependent variable.

	Predictor	R^2	*Adj* R^2	F	*p*	B	SE B	β	*p*
Model 1		0.129	0.127	64.1	<0.001				
	Constant					2.385	0.0561		<0.001
	Sex					−0.644	0.0805	−0.717	<0.001
Model 2		0.251	0.247	72.4	<0.001				
	Constant					1.3403	0.13488		<0.001
	Sex					−0.5583	0.07540	−0.621	<0.001
	DASS-21					0.0248	0.00295	0.353	<0.001
Model 3		0.268	0.263	52.7	<0.001				
	Constant					0.93462	0.18392		<0.001
	Sex					−0.55264	0.07463	−0.615	<0.001
	DASS-21					0.01789	0.00362	0.255	<0.001
	DERS					0.00760	0.00237	0.165	0.001

Note: BMI: body mass index; DASS-21: Depression Anxiety and Stress scale; DERS: Difficulties in Emotional Regulation Scale; EE_DEBQ: Emotional Eating subscale of the Dutch Eating Behavior Questionnaire.

3.3. Mediation Analysis

To assess the hypothesis that psychological distress might influence emotional eating through emotional dysregulation, mediation analysis was performed. The independent variable was psychological distress, the outcome variable was emotional eating, and the mediator was emotional dysregulation. In order to take the impact of sex into account, it was added as a covariate in the model.

The results showed that the total effect of psychological distress on emotional eating was significant (b = 0.0248; SE = 0.0030; $p < 0.001$; CI = 0.0190–0.0306). In addition, with the

inclusion of the mediator, the direct effect of psychological distress on emotional eating was significant (b = 0.0179; SE = 0.0036; $p < 0.001$; CI = 0.0108–0.0250). Again, the indirect effect of psychological distress on emotional eating through emotional dysregulation was found to be significant (b = 0.0069; SE = 0.0024; CI = 0.0024–0.0118). The results also suggest that the indirect mediated effect accounted for 26.8% ($R^2 = 0.2680$) of the variance. This evidence suggests that the relationship between psychological distress and emotional eating is partially mediated by emotional dysregulation.

The model is presented in Figure 2.

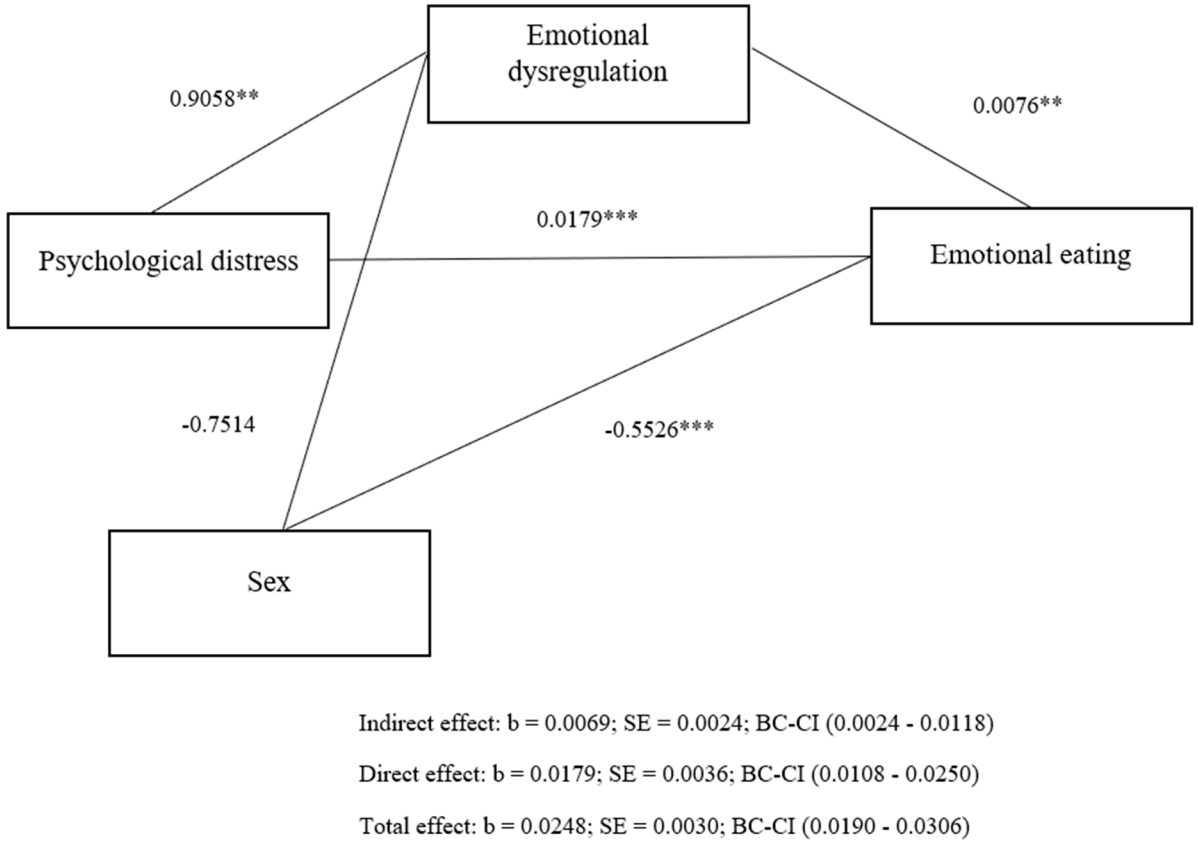

Figure 2. Mediation model for the relationship between psychological distress, emotional, and emotional eating, with sex as a covariate. Note: DASS-21: Depression Anxiety and Stress scale; DERS: Difficulties in Emotional Regulation Scale; EE_DEBQ: Emotional Eating subscale of the Dutch Eating Behavior Questionnaire; ** $p < 0.05$, *** $p < 0.001$.

4. Discussion

The restrictive measures introduced to counter the COVID-19 outbreak dramatically affected the physical and psychological health of the Italian population. Among others, Italian young adults were seriously harmed by the COVID-19 outbreak [11], with an increase in both internalizing and externalizing problems during the first lockdown.

The pandemic strongly affected the daily habits and changed the lifestyle of the Italian population [14]. In particular, there was an increase in disordered eating, including emotional eating, provoked by the COVID-19 outbreak [12–14]. However, the underlying mechanism explaining the relationship between psychological distress related to the pandemic and emotional eating was unclear.

This study was conceived to explore the impact of psychological distress due to the lockdown measures on emotional eating in a sample of Italian young adults by assessing the mediating role of emotional dysregulation.

As hypothesized, psychological distress during the second wave of lockdown was related to emotional eating, and this relationship was partially mediated by emotional dysregulation. Specifically, according to our results, emotional eating was found to be

related to emotional dysregulation and psychological distress, particularly depression, anxiety, and stress. Moreover, higher levels of emotional eating were reported in women than in men. These findings were in line with previous studies illustrating that emotional eating was triggered by psychological distress, including anxiety, depression, stress, and emotional dysregulation [31–35]. In addition, the difference we identified between males and females in regard to emotional eating behavior was supported by previous findings in the literature [15,29]. Sex differences in eating may be due to several factors, including medical and psychological differences between males and females. In particular, females generally show higher levels of anxiety, depression, and stress than men, as well as higher body dissatisfaction, all of which correlate with disordered eating [36].

We did not find significant associations between emotional eating and age or emotional eating and BMI. It could be hypothesized that these inconsistent relationships-that were previously demonstrated [29]-were most likely due to the composition of our sample, which included only young adults with normal weight.

Our findings are consistent with the affect regulation model, which suggests that maladaptive behaviors, such as eating in response to negative feelings, function as an attempt to alleviate negative emotions [36]. It also extends the model using a sample of Italian young adults dealing with the psychological consequences of the COVID-19 pandemic and the related restrictive measures.

From a clinical point of view, our results have important implications. The global pandemic requires researchers and clinicians not only to assess and monitor the psychological implications of the pandemic but also to plan and develop efficient psychological interventions to take care of citizens' mental health, with a particular emphasis on high-risk groups, such as young adults. By assessing the role of emotional dysregulation on the link between psychological distress and emotional eating, the current study could inform interventions aimed at mitigating the negative effects of COVID-19 on eating habits by promoting emotional regulation strategies. Such interventions may help individuals to notice and regulate their internal states without using food to deal with their emotions.

Several limitations of the present study must be considered. Firstly, the cross-sectional nature of this study does not allow us to carry out causal explanations of relations among variables. Control group, manipulation, and longitudinal measures are lacking. Moreover, additional variables that were not taken into account in this study could play a role in emotional eating, such as alexithymia [25,37]. However, in the present study, possible confounder variables, such as age and BMI, were addressed. Secondly, all the measurements were self-reported and could therefore be affected by bias. Another limitation is related to the sample. This study used a convenience sample, a type of non-probability sample that confers many advantages, such as a quick and inexpensive data collection, as well as disadvantages, such as selection bias and reduced representativity. Future replications of the study would be helpful to reduce bias in convenience sampling by using probability sampling. Future studies should examine the role of other variables that could play a key role in influencing emotional eating. In addition, future research could consider samples of under- or over-weight young adults to extend the findings.

Author Contributions: Conceptualization, A.G.U., R.C., and G.V.; methodology, T.M., M.F., E.M.G., and G.P.; formal analysis, C.F. and A.M.; writing—original draft preparation, A.G.U., R.C., and G.V.; writing—review and editing, G.C. and E.M.; supervision, C.F. and A.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Center for Research and Psychological Intervention (CERIP) of the University of Messina (protocol code 17758).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The collected in this study are available on request from the author A.G.U. The data are not publicly available due to privacy/ethical restrictions.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lenzo, V.; Quattropani, M.C.; Musetti, A.; Zenesini, C.; Freda, M.F.; Lemmo, D.; Vegni, E.; Borghi, L.; Plazzi, G.; Castelnuovo, G.; et al. Resilience Contributes to Low Emotional Impact of the COVID-19 Outbreak Among the General Population in Italy. *Front. Psychol.* **2020**, *11*, 576485. [CrossRef] [PubMed]
2. Rogers, J.P.; Chesney, E.; Oliver, D.; Pollak, T.A.; McGuire, P.; Fusar-Poli, P.; Zandi, M.; Lewis, G.; David, A.S. Psychiatric and neuropsychiatric presentations associated with severe coronavirus infections: A systematic review and meta-analysis with comparison to the COVID-19 pandemic. *Lancet Psychiatry* **2020**, *7*, 611–627. [CrossRef]
3. Boursier, V.; Gioia, F.; Musetti, A.; Schimmenti, A. Facing Loneliness and Anxiety During the COVID-19 Isolation: The Role of Excessive Social Media Use in a Sample of Italian Adults. *Front. Psychiatry* **2020**, *11*, 586222. [CrossRef]
4. Boursier, V.; Musetti, A.; Gioia, F.; Flayelle, M.; Billieux, J.; Schimmenti, A. Is watching TV series an adaptive coping strategy during the COVID-19 pandemic? Insights from an Italian community sample. *Front. Psychiatry* **2021**, *12*, 599859. [CrossRef]
5. Musetti, A.; Franceschini, C.; Pingani, L.; Freda, M.F.; Saita, E.; Vegni, E.; Zenesini, C.; Quattropani, M.C.; Lenzo, V.; Margherita, G.; et al. Maladaptive Daydreaming in an Adult Italian Population During the COVID-19 Lockdown. *Front. Psychol.* **2021**, *12*, 631979. [CrossRef]
6. Franceschini, C.; Musetti, A.; Zenesini, C.; Palagini, L.; Scarpelli, S.; Quattropani, M.C.; Lenzo, V.; Freda, M.F.; Lemmo, D.; Vegni, E.; et al. Poor sleep quality and its consequences on mental health during the COVID-19 lockdown in Italy. *Front. Psychol.* **2020**, *11*, 574475. [CrossRef] [PubMed]
7. Rossi, R.; Socci, V.; Talevi, D.; Mensi, S.; Niolu, C.; Pacitti, F.; Di Marco, A.; Rossi, A.; Siracusano, A.; Di Lorenzo, G. COVID-19 Pandemic and Lockdown Measures Impact on Mental Health Among the General Population in Italy. *Front. Psychiatry* **2020**, *11*, 790. [CrossRef] [PubMed]
8. Mei, S.L.; Yu, J.X.; He, B.W.; Li, J.Y. Psychological investigation of university students in a university in Jilin province. *Med. Soc.* **2011**, *24*, 84–86.
9. Cao, W.; Fang, Z.; Hou, G.; Han, M.; Xu, X.; Dong, J.; Zheng, J. The psychological impact of the COVID-19 epidemic on college students in China. *Psychiatry Res.* **2020**, *287*, 112934. [CrossRef]
10. Huang, Y.; Zhao, N. Mental health burden for the public affected by the COVID-19 outbreak in China: Who will be the high-risk group? *Psychol. Health Med.* **2021**, *26*, 23–34. [CrossRef]
11. Parola, A.; Rossi, A.; Tessitore, F.; Troisi, G.; Mannarini, S. Mental Health Through the COVID-19 Quarantine: A Growth Curve Analysis on Italian Young Adults. *Front. Psychol.* **2020**, *11*, 567484. [CrossRef] [PubMed]
12. Cecchetto, C.; Aiello, M.; Gentili, C.; Ionta, S.; Osimo, S.A. Increased emotional eating during COVID-19 associated with lockdown, psychological and social distress. *Appetite* **2021**, *160*, 105122. [CrossRef]
13. Scarmozzino, F.; Visioli, F. Covid-19 and the subsequent lockdown modified dietary habits of almost half the population in an Italian sample. *Foods* **2020**, *9*, 675. [CrossRef] [PubMed]
14. Di Renzo, L.; Gualtieri, P.; Pivari, F.; Soldati, L.; Attinà, A.; Cinelli, G.; Leggeri, C.; Caparello, G.; Barrea, L.; Scerbo, F.; et al. Eating habits and lifestyle changes during COVID-19 lockdown: An Italian survey. *J. Transl. Med.* **2020**, *18*, 229. [CrossRef] [PubMed]
15. Van Strien, T.; Frijters, J.E.R.; Bergers, G.P.A.; Defares, P.B. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *Int. J. Eat. Disord.* **1986**, *5*, 295–315. [CrossRef]
16. Ouwens, M.A.; Van Strien, T.; Van Leeuwe, J.F.J. Possible pathways between depression, emotional and external eating. A structural equation model. *Appetite* **2009**, *53*, 245–248. [CrossRef]
17. Braden, A.; Musher-Eizenman, D.; Watford, T.; Emley, E. Eating when depressed, anxious, bored, or happy: Are emotional eating types associated with unique psychological and physical health correlates? *Appetite* **2018**, *125*, 410–417. [CrossRef]
18. Van Strien, T.; Herman, C.P.; Verheijden, M.W. Eating style, overeating and weight gain. A prospective 2-year follow-up study in a representative Dutch sample. *Appetite* **2012**, *59*, 782–789. [CrossRef]
19. Gratz, K.L.; Roemer, L. Multidimensional Assessment of Emotion Regulation and Dysregulation: Development, Factor Structure, and Initial Validation of the Difficulties in Emotion Regulation Scale. *J. Psychopathol. Behav. Assess.* **2004**, *26*, 41–54. [CrossRef]
20. Laghi, F.; Pompili, S.; Bianchi, D.; Lonigro, A.; Baiocco, R. Exploring the association between psychological distress and drunkorexia behaviors in non-clinical adolescents: The moderating role of emotional dysregulation. *Eat. Weight. Disord.* **2021**, *26*, 797–806. [CrossRef]
21. Squires, L.R.; Hollett, K.B.; Hesson, J.; Harris, N. Psychological Distress, Emotion Dysregulation, and Coping Behaviour: A Theoretical Perspective of Problematic Smartphone Use. *Int. J. Ment. Health Addict.* **2020**, 1–16. [CrossRef]
22. Frayn, M.; Knäuper, B. Emotional Eating and Weight in Adults: A Review. *Curr. Psychol.* **2018**, *37*, 924–933. [CrossRef]
23. Abdi, R.; Pak, R. The mediating role of emotion dysregulation as a transdiagnostic factor in the relationship between pathological personality dimensions and emotional disorders symptoms severity. *Personal. Individ. Differ.* **2019**, *142*, 282–287. [CrossRef]
24. Hallion, L.S.; Steinman, S.A.; Tolin, D.F.; Diefenbach, G.J. Psychometric properties of the difficulties in emotion regulation scale (DERS) and its short forms in adults with emotional disorders. *Front. Psychol.* **2018**, *9*, 539. [CrossRef]

25. McAtamney, K.; Mantzios, M.; Egan, H.; Wallis, D.J. Emotional eating during COVID-19 in the United Kingdom: Exploring the roles of alexithymia and emotion dysregulation. *Appetite* **2021**, *161*, 105120. [CrossRef] [PubMed]
26. Lovibond, S.; Lovibond, P. *Manual for the Depression Anxiety Stress Scales*; Psychology Foundation of Australia: Sydney, Australia, 1996.
27. Bottesi, G.; Ghisi, M.; Altoè, G.; Conforti, E.; Melli, G.; Sica, C. The Italian version of the Depression Anxiety Stress Scales-21: Factor structure and psychometric properties on community and clinical samples. *Compr. Psychiatry* **2015**, *60*, 170–181. [CrossRef] [PubMed]
28. Giromini, L.; Velotti, P.; De Campora, G.; Bonalume, L.; Zavattini, G.C. Cultural Adaptation of the Difficulties in Emotion Regulation Scale: Reliability and Validity of an Italian Version. *J. Clin. Psychol.* **2012**, *68*, 989–1007. [CrossRef] [PubMed]
29. Dakanalis, A.; Zanetti, M.A.; Clerici, M.; Madeddu, F.; Riva, G.; Caccialanza, R. Italian version of the Dutch Eating Behavior Questionnaire. Psychometric proprieties and measurement invariance across sex, BMI-status and age. *Appetite* **2013**, *71*, 187–195. [CrossRef]
30. Hayes, A.F. PROCESS: A Versatile Computational Tool for Observed Variable Mediation, Moderation, and Conditional Process Modeling. Available online: http://claudiaflowers.net/rsch8140/Hayesprocess.pdf (accessed on 17 June 2021).
31. Goossens, L.; Braet, C.; Van Vlierberghe, L.; Mels, S. Loss of control over eating in overweight youngsters: The role of anxiety, depression and emotional eating. *Eur. Eat. Disord. Rev.* **2009**, *17*, 68–78. [CrossRef]
32. Nguyen-Rodriguez, S.T.; Unger, J.B.; Spruijt-Metz, D. Psychological determinants of emotional eating in adolescence. *Eat. Disord.* **2009**, *17*, 211–224. [CrossRef]
33. Pidgeon, A.; Lacota, K.; Champion, J. The moderating effects of mindfulness on psychological distress and emotional eating behaviour. *Aust. Psychol.* **2013**, *48*, 262–269. [CrossRef]
34. Tan, C.C.; Chow, C.M. Stress and emotional eating: The mediating role of eating dysregulation. *Personal. Individ. Differ.* **2014**, *66*, 1–4. [CrossRef]
35. Gianini, L.M.; White, M.A.; Masheb, R.M. Eating pathology, emotion regulation, and emotional overeating in obese adults with binge eating disorder. *Eat. Behav.* **2013**, *14*, 309–313. [CrossRef] [PubMed]
36. Yu, Z.; Indelicato, N.A.; Fuglestad, P.; Tan, M.; Bane, L.; Stice, C. Sex differences in disordered eating and food addiction among college students. *Appetite* **2018**, *129*, 12–18. [CrossRef] [PubMed]
37. Casagrande, M.; Boncompagni, I.; Forte, G.; Guarino, A.; Favieri, F. Emotion and overeating behavior: Effects of alexithymia and emotional regulation on overweight and obesity. *Eat. Weight Disord. Stud. Anorex. Bulim. Obes.* **2020**, *25*, 1333–1345. [CrossRef]

Journal of *Personalized Medicine*

MDPI

Article

Predictors of the Intention to Be Vaccinated against COVID-19 in a Sample of Italian Respondents at the Start of the Immunization Campaign

Alessandro Santirocchi [1], Pietro Spataro [2], Marco Costanzi [3], Fabrizio Doricchi [1], Clelia Rossi-Arnaud [1] and Vincenzo Cestari [1,*]

[1] Department of Psychology, Sapienza University, 00185 Rome, Italy; alessandro.santirocchi@uniroma1.it (A.S.); fabrizio.doricchi@uniroma1.it (F.D.); clelia.rossi-arnaud@uniroma1.it (C.R.-A.)
[2] Department of Economy, Universitas Mercatorum, 00100 Rome, Italy; pietro.spataro@unimercatorum.it
[3] Department of Human Sciences, Lumsa University, 00193 Rome, Italy; m.costanzi@lumsa.it
* Correspondence: vincenzo.cestari@uniroma1.it

Abstract: COVID-19 vaccines are the most promising means of limiting the pandemic. The present study aims at determining the roles of several psychological variables in predicting vaccination intention in Italy. An online questionnaire was disseminated between 9 March and 9 May 2021. The sample included 971 participants. Results showed that most of the participants were willing to vaccinate. Acceptance rates were correlated with age, marital status, and area of residence. Intention to be vaccinated was positively correlated with perceived risk, pro-sociality, fear of COVID-19, use of preventive behaviors, and trust in government, in science, and in medical professionals. Intention to be vaccinated was negatively associated with belief in misinformation. The degree of acceptance is likely to be a result of the campaign tailored to address people's negative attitudes towards vaccines. Trust in government and trust in science were among the strongest psychological predictors of vaccination intention. Fear of COVID-19, but not perceived risk, was associated with increased vaccine uptake, suggesting that the affective component of risk perception was more important than the cognitive component in predicting participants' behaviors. Belief in misinformation was associated with reduced vaccination intention. Future studies will take into consideration these variables, to better understand the multifaceted process underlying vaccination intention.

Keywords: COVID-19 vaccine; vaccine acceptance; vaccine intention

Citation: Santirocchi, A.; Spataro, P.; Costanzi, M.; Doricchi, F.; Rossi-Arnaud, C.; Cestari, V. Predictors of the Intention to Be Vaccinated against COVID-19 in a Sample of Italian Respondents at the Start of the Immunization Campaign. *J. Pers. Med.* **2022**, *12*, 111. https://doi.org/10.3390/jpm12010111

Academic Editor: Rajendra D Badgaiyan

Received: 25 November 2021
Accepted: 11 January 2022
Published: 14 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

1. Introduction

To date, different vaccines against COVID-19 have been approved by regulatory agencies and are currently in use. Worldwide differences among countries exist in the type of vaccine approved and administered. Further, in some countries, the type of vaccine administered varies according to the age range of the people inoculated. As new vaccines were commercialized, the intention to get vaccinated rose in many countries. For example, a survey conducted in April 2021 on more than 10,000 respondents [1] showed that the percentage of people who declared to accept the COVID-19 vaccine was very high in Brazil (93%), Mexico (88%), Spain (83%), and China (81%), fairly high in Italy (79%), Canada (78%), Japan (73%), South Korea (72%), and Germany (71%), moderate in Australia (66%), South Africa (62%), and France (58%), and low in the United States (46%) and Russia (41%). Nevertheless, a meta-analysis of 28 nationally representative samples from 13 countries concluded that, as the pandemic progressed, the percentage of people intending to vaccinate decreased (being about 60%) and the percentage of people intending to refuse vaccination increased (being about 20%) [2]. At the moment in which we are writing (15 December 2021), about 8.59 billion doses have been administered globally, but only 56.5% of the world population has received at least one dose of a COVID-19 vaccine [3]. Shares of people

vaccinated are high in countries such as United Arab Emirates (99%), Cuba (90%), Portugal (89%), and China (84%), moderate in countries such as France (77%), United Kingdom (75%), Germany, and United States (both 72%), low in countries such as Russia (48%), South Africa (31%), and Egypt (28%), and extremely low in countries such as Kenya (10%), Ethiopia (7%), and Nigeria (4%). In Italy, more than 104 million doses have been administrated, and 85% of the population (47 million people) has been fully immunized with two doses.

Overall, these data suggest that understanding the factors that determine and promote the intention to be vaccinated represents an enduring mission for psychological research. The present study sought to contribute to this field by investigating the intention to get vaccinated in Italy in the period between March and May 2021—that is, shortly after the beginning of the vaccination campaign. There were three primary aims. First, we sought to provide up-to-date information about vaccine acceptance rates (i.e., the percentage of people who are willing to be vaccinated against COVID-19) in Italy. In a previous study by Palamenghi et al. [4] conducted during the early days of the Italian reopening after the first lockdown (May 2020), a sample of 1004 Italian citizens were asked to report their willingness to be vaccinated against COVID-19 "if a vaccine was found" on a scale ranging from 1 (not likely at all) to 5 (absolutely likely). The results showed that about 59% of the respondents were "likely" or "absolutely likely" to vaccinate. Given the recent emphasis in enhancing public trust in COVID-19 vaccination, we expected this estimate to be substantially higher at the beginning of 2021, see [5,6]. In this respect, we must note that the policy adopted by the Italian government to address vaccine hesitancy has been one of the most fruitful, at least in Europe. Generally speaking, Italy has a long-standing tradition of high coverage of vaccinations. However, in the last decade, the frequency of infant immunization has decreased alarmingly, leading to the introduction of a new law, the "Italian National Immunization Prevention Plan 2017–19" (n. 119/2017), which prescribes mandatory vaccinations against ten diseases for preschool and school-aged children [7]. The implementation of the law contributed to an increased awareness of the importance of vaccination in the Italian population [8]. During the COVID-19 pandemic, this awareness was further boosted by the broad diffusion of science-supporting messages from experts about vaccine safety and effectiveness. Pro-vaccine messages are now common in mass media, including TV, radio, magazines, newspapers, and the Internet. In addition, the Italian government has recently approved two types of green COVID-19 certificates: the Basic Green Pass (proving vaccination, recovery from COVID-19 within the last six months, or a negative result for a molecular or antigenic swab in the last couple of days) and the Super Green Pass (only granted to the vaccinated and those who have recovered from the coronavirus in the last six months). The fact that the Super Green Pass is now compulsory for certain categories (including healthcare workers, school teachers, soldiers, and police officers), as well as for accessing an increasing number of activities and services, has produced an additional boost in vaccination rates.

Our second aim was to determine the impact of individual differences in demographic variables on vaccine acceptance rates. In this respect, common findings are that the intention to vaccinate was higher in males than in females [2,6], and higher in older than in younger people [9,10]. However, in the study by Kerr et al. [6], neither age nor gender were found to be significant predictors of vaccine acceptance in a sample of 700 Italian respondents interviewed between March and October 2020. Lastly, the third aim was to determine the roles of a number of psychological variables in predicting the intention to be vaccinated against COVID-19. Following a theoretical framework originally applied to the study of risk perception [11], psychological predictors were selected in order to assess the cognitive (risk perception, pro-sociality), the emotional (fear of COVID-19), the experiential (direct experience, use of preventative measures, misinformation), and the sociocultural (trust in government, trust in science, trust in medical professionals) aspects of the current pandemic [12].

Previous research has provided evidence in support of the involvement of these variables in the prediction of vaccine hesitancy and/or the intention to be vaccinated [6,9,10,13–15],

however, to our knowledge, few studies have compared the relative importance of each of them within a single study. For example, trust in government and in medical professionals has been repeatedly demonstrated to play a key role in determining COVID-19 vaccine acceptance. A cross-sectional study in 19 countries showed that willingness to get vaccinated ranged from 88.6% (China) to 55.8% (Russia) and was positively and significantly associated with trust in the government [16] (see [17] for similar results in a Canadian sample). However, as suggested by Kerr et al. [6], current research has not considered the possible overlap between different types of trust (trust in government, trust in science, and trust in medical professionals). Perceived risk (i.e., the subjective likelihood of getting the virus) is another variable which has been often called into question in predicting the adoption of preventative behaviors and the acceptance of COVID-19 vaccines [18,19], in line with the predictions following from the Health Belief Model [20] and the Protection Motivation Theory [21]. However, these studies have typically failed to disentangle the roles of the cognitive and affective components of risk perception and many of them did not evaluate fear or worry of COVID-19 [22]. Lastly, for other predictors such as pro-sociality, available evidence is mixed, with some studies reporting significant associations with vaccination intent [23,24], and other studies reporting no association [25].

The aim of the present study was to provide an updated assessment of vaccine acceptance rates in Italy in the period between March and May 2021 at the launch of the vaccination campaign and further investigate the impact of a broad array of demographical and psychological factors in increasing (or decreasing) participants' willingness to be vaccinated against COVID-19.

2. Materials and Methods

2.1. Participants

Table 1 reports the demographic characteristics of our sample.

Table 1. Demographic properties of the sample recruited for the present study, as compared to the Italian population.

	Our Sample	**Italian Population [a]**
Gender		
Females	558 (57.6%)	51.3%
Males	411 (42.4%)	49.7%
Age		
18–30 years	641 (66.0%)	14.9%
31–40 years	91 (9.4%)	11.3%
41–50 years	97 (10.0%)	14.7%
51–60 years	94 (9.7%)	15.9%
>61 years	48 (4.9%)	30.2%
Education		
High school or less	465 (47.8%)	85.1%
Bachelor's degree	157 (16.2%)	3.8%
Master's degree	223 (23.0%)	10.7%
Postgraduate	126 (13.0%)	0.4%
Marital status		
Single	681 (70.1%)	42.9%
Married	251 (25.8%)	46.6%
Divorced/widowed	39 (4.0%)	10.5%
Living condition		
Alone	109 (11.2%)	32.9%
Family/Partner	795 (81.9%)	63.2%
Friends/Housemates	67 (6.9%)	3.9%

Table 1. *Cont.*

	Our Sample	Italian Population [a]
Region		
Central Italy	713 (73.4%)	19.9%
North Italy	148 (15.2%)	46.4%
South Italy	110 (11.3%)	33.7%
Type of area		
White/Yellow	350 (36.0%)	-
Orange	128 (13.2%)	-
Red	493 (50.8%)	-

Note [a]: Data taken from https://www.istat.it/it/censimenti (accessed on 29 December 2021).

Overall, we recruited 971 Italian-speaking participants, 411 males and 558 females and 2 participants not reporting their gender. Most of our participants were between 18 and 30 years (N = 641), were unmarried (N = 681), lived with relatives or partners (N = 795), resided in Central Italy (N = 713), and many had a university degree (N = 380, considering both Bachelor's and Master's degrees). At the time the study was conducted, 350 participants lived in a white or yellow area (low risk), 128 lived in an orange area (intermediate risk), and 493 lived in a red area (high risk). Shortly after the 2020 lockdown, the Italian government introduced a classification of regions based on white (minimum risk), yellow, orange, and red (maximum risk) color codes. Each color corresponds to the adoption of a gradually increasing number of preventative measures regulating travel possibilities within a single region and between regions, the opening of businesses, restaurants, and places of sports and culture.

When compared with the general Italian population, participants older than 61 years of age, with a high school diploma (or less), married, and living alone in Northern or Southern Italy were underrepresented in our sample (see Table 1).

2.2. Instruments and Measures

2.2.1. Intention to Be Vaccinated

Intention to be vaccinated was measured with two questions taken from Palamenghi et al. [4]: "Are you willing to be vaccinated against COVID-19?" and "Do you think that your family members should be vaccinated against COVID-19?". For both questions, participants responded on a five-point Likert scale, ranging from "not at all likely" (1) to "absolutely likely" (5). Scores were summed and therefore could range between 2 and 10. Cronbach's α was good (α = 0.88).

2.2.2. Perceived Risk

Perceived risk was assessed with three questions taken from Dryhurst et al. [12]: "How likely do you think it is that you will be directly and personally affected by the following in the next 6 months?—Catching the coronavirus/COVID-19", "How likely do you think it is that your friends and family in the country you are currently living in will be directly affected by the following in the next 6 months?—Catching the coronavirus/COVID-19", and "How much do you agree or disagree with the following statements?—Getting sick with the coronavirus/COVID-19 can be serious". For the first two questions, participants responded on a seven-point Likert scale, ranging from "not at all likely" (1) to "very likely" (7). For the third question, participants responded on a five-point Likert scale, ranging from "strongly disagree" (1) to "strongly agree" (5). Scores were summed and could therefore range between 3 and 19. Cronbach's α was acceptable (α = 0.62).

2.2.3. Pro-Sociality

Pro-sociality was investigated with a single item taken from Dryhurst et al. [12]: "To what extent do you think it's important to do things for the benefit of others and society

even if they have some costs to you personally?". Participants responded on a five-point Likert scale going from "not at all" (1) to "very much so" (5).

2.2.4. Fear of COVID-19

Feelings of anxiety towards COVID-19 were measured with the Italian version of the Fear of COVID-19 Scale FCV-19S [26,27]. This seven-item scale includes items such as "I am most afraid of Coronavirus-19", "My hands become clammy when I think about Coronavirus-19", and "When watching news and stories about Coronavirus-19 on social media, I become nervous or anxious". Participants responded on a 5-point scale ranging from 1 (strongly disagree) to 5 (strongly agree); thus, total scores ranged from 7 to 35. Cronbach's α was good ($\alpha = 0.86$).

2.2.5. Direct Experience

Direct experience with Coronavirus was examined with a single item: "Have you ever had, or thought you might have, the Coronavirus/COVID-19?". Participants had three response options: "Yes, I had COVID-19", "I thought I might have COVID-19, but I have been tested as negative", and "No, I never had COVID-19". Following Dryhurst et al. [12], this item was dichotomized by considering the first two options as "yes" responses (1) and the last option as a "no" response (0).

2.2.6. Use of Preventive Behaviors

The frequency of use of COVID-19 preventive behaviors during the past three months was assessed with 10 items that were extracted from the COVID-19 preventive methods recommended by the WHO and were previously used by Lee et al. [28]. Examples of the items were: "Washed hands regularly using alcohol-based cleanser or soap and water", "Avoided social gatherings of more than 4 people", and "Avoided hand-shaking, hugging, and kissing". Participants indicated the frequency of use of each behavior on a 5-point scale ranging from 1 (Never) to 5 (Always). Thus, total scores ranged from 10 to 50. Cronbach's α was excellent ($\alpha = 0.92$).

2.2.7. Misinformation

COVID-19 misinformation was assessed with 12 items taken from Lee et al. [28] and extracted from COVID-19 misinformation reports by the WHO. Examples of the items were: "Masks can be sterilized and reused after steaming with hot water", "Drinking tea can prevent COVID-19", "Taking antibiotics can prevent or treat COVID-19", "Only the elderly would become infected with the COVID-19", and "COVID-19 is artificially developed". Participants first indicated whether they had encountered each statement in the last three months (binary responses: yes/no; total scores ranged from 0 to 12). Then, they answered the following question: "Which of the above information do you believe is correct?". Responses were provided on a 4-point scale including "none" (1), "some are correct" (2), "most are correct" (3), and "all are correct" (4).

2.2.8. Trust in Government

Trust in government was assessed with a single item taken from Dryhurst et al. [12]: "How much do you trust the country's politicians to deal effectively with the pandemic?". Participants responded on a seven-point Likert scale going from "not at all" (1) to "very much" (7).

2.2.9. Trust in Science

Trust in science was assessed with a single item taken from Dryhurst et al. [12]: "How much do you trust each of the following?—Scientists". Participants responded on a five-point Likert scale going from "cannot be trusted at all" (1) to "can be trusted a lot" (5).

2.2.10. Trust in Medical Professionals

Trust in medical professionals was assessed with a single item taken from Dryhurst et al. [12]: "How much do you trust each of the following?—Medical doctors and nurses". Participants responded on a five-point Likert scale going from "cannot be trusted at all" (1) to "can be trusted a lot" (5).

2.3. Procedure

The questionnaire was prepared using Google Forms and disseminated through different social media (including Facebook, Instagram, Twitter, LinkedIn, Telegram, and WhatsApp), in line with the Italian government's recommendations on limiting face-to-face interactions. All data were collected between 9 March and 9 May 2021—but note that 829 participants (85% of the whole sample) completed the questionnaire by 31 March. We used a snowball sampling strategy: the links were initially shared with a sample of university students who were encouraged to pass them on to others, with a focus on recruiting the general public. The research was approved by the Ethical Committee of the University Sapienza of Rome (Protocol N.0000476) and all respondents signed an informed consent before participating.

2.4. Statistical Analyses

Since our variables resulted from the combination of a different number of questions, they had different ranges and needed to be preliminarily standardized by transforming them into z-scores. Z-scores are measured in terms of standard deviations from the mean and thus inform on how many standard deviations a raw score is away from the mean. Positive scores indicate that the participant's raw score falls above the mean, whereas negative scores indicate that it falls below the mean.

Statistical analyses were performed in three successive steps. First, we investigated whether participants' intention to be vaccinated (measured in terms of z-scores) differed as a function of the demographic properties of our sample (gender, age, education, marital status, living condition, region, and type of area). A *t*-test for independent samples was used for gender (because it involved only two categories) while between-subject univariate ANOVAs were used for all other variables: when a significant result was obtained, the main analysis was followed by post-hoc pairwise comparisons (using the Bonferroni adjustment), to determine which pairs of the factor categories were significantly different from each other. Second, Pearson's correlations were computed between the main variables, to assess which factors were associated with participants' intention to be vaccinated. Lastly, the correlational analysis was followed by a hierarchical regression analysis to determine which variables predicted participants' intention to be vaccinated. Demographic factors were entered in the first step as a series of dummy variables, to control for their influence. A dummy variable is a numerical variable used in regression analyses to represent different treatment groups. Specifically, participants were given a value of 0 if they were in the reference group (for example "female" for gender) or a 1 if they were in the other group ("male"). For a variable having k levels, $k - 1$ dummy variables were necessary to represent all groups. For example, to represent marital status, which has three different levels (single, married, divorced/widowed), two dummy variables were required. Since we chose the "single" category as the reference level, the first dummy variable was coded 1 if participants belonged to the "married" category and 0 if they belonged to the "single" or "divorced/widowed" categories. The second dummy variable was instead coded 1 if participants belonged to the "divorced/widowed" category and 0 if they belonged to the "single" or "married" categories. Psychological variables (perceived risk, pro-sociality, fear of COVID-19, direct experience, use of preventive behaviors, misinformation, trust in government, trust in science, and trust in medical professionals) were included in the model in the second step, to ascertain whether they explained additional variance, over and above the contribution provided by demographic variables. As usual, the results of the regression analysis are presented in terms of β coefficients, which describe the

mathematical relationships between each independent variable and the dependent variable. More technically, they represent the mean change in the dependent variable for one unit of change in the predictor variable while holding other predictors constant. Each coefficient was associated with a *t*-test and a *p*-value, which indicated whether the relationship between the predictor and dependent variables was statistically significant. If the *t*-test was not significant ($p > 0.05$), then the predictor had no correlation with the dependent variable, i.e., there was no association between the changes in the independent variable and the shifts in the dependent variable. Otherwise, if the *t*-test was significant ($p \leq 0.05$), then data favored the hypothesis that there was a non-zero correlation, i.e., that changes in the independent variable were associated with changes in the dependent variable at the population level. For all analyses, the α level was set to 0.05.

3. Results

Descriptive measures for the variables examined in the present study are reported in Table 2.

Table 2. Descriptive statistics for the variables measured in the present study.

Measures	M	SD	Min	Max
Intention to vaccinate	9.13	1.79	2.00	10.00
Perceived risk	12.00	2.87	3.00	19.00
Pro-sociality	5.77	1.45	1.00	7.00
Fear of COVID-19	14.94	5.52	7.00	35.00
Direct experience	0.48	0.49	0.00	1.00
Use of preventive behaviors	38.23	8.03	10.00	50.00
Misinformation (number)	2.52	1.49	1.00	10.00
Misinformation (belief)	1.26	0.50	1.00	4.00
Trust in government	3.32	1.54	1.00	7.00
Trust in science	4.17	0.90	1.00	5.00
Trust in medical professionals	4.34	0.78	1.00	5.00

In relation to our first aim, acceptance of COVID-19 vaccines was substantially high. In fact, 762 participants (78.5%) responded that they were absolutely likely to be vaccinated against COVID-19, whereas only 35 participants (3.6%) responded that they were not at all likely to get vaccination. Pooling together the first two categories (i.e., "not at all likely" and "very unlikely"), a total of 67 participants (6.9%) were hesitant about their own vaccination. Similarly, when asked about their family members, 750 participants (77.2%) responded that they should absolutely be vaccinated, whereas only 19 participants (2.0%) responded that they should absolutely not be vaccinated. Collapsing the first two categories, a total of 55 participants (5.7%) were hesitant about vaccination for their family members.

With respect to our second aim, we found that participants' intention to be vaccinated differed as a function of three demographic variables. As reported in Table 3, significant differences were observed for: (i) age (post-hoc comparisons revealed that acceptance rates were lower for participants 41–50 years old than for those who were 18–30 years old, $p = 0.025$, or older than 61 years, $p = 0.039$; all other $p > 0.32$), (ii) marital status (post-hoc comparisons revealed that acceptance rates were lower for participants who were married or divorced/widowed than for those who were single, $p = 0.003$ and $p = 0.004$), and (iii) type of area (post-hoc comparisons revealed that acceptance rates were lower for participants who resided in an orange area than for those who resided in white/yellow or red areas, $p = 0.043$ and $p = 0.010$).

Table 3. Means (and standard deviations) for intention to vaccinate (z-scores), as a function of gender, age, education, marital status, living condition, region, and type of area, together with the results of statistical analyses (*t*-test or F-test).

Categories	Intention to Vaccinate (z-Scores)	*t*-Test/F-Test
Gender		
Females (N = 558)	0.04 (0.97)	1.57
Males (N = 411)	−0.05 (1.01)	
Age		
18–30 years (N = 641)	0.05 (0.89)	3.77 **
31–40 years (N = 91)	−0.14 (1.12)	
41–50 years (N = 97)	−0.26 (1.32)	
51–60 years (N = 94)	−0.11 (1.21)	
>61 years (N = 48)	0.23 (0.81)	
Education		
High school or less (N = 465)	−0.01 (1.03)	0.27
Bachelor's degree (N = 157)	−0.03 (1.01)	
Master's degree (N = 223)	0.01 (0.95)	
Postgraduate (N = 126)	0.06 (0.94)	
Marital status		
Single (N = 681)	0.08 (0.87)	9.43 ***
Married (N = 251)	−0.15 (1.17)	
Divorced/widowed (N = 39)	−0.44 (1.56)	
Living condition		
Alone (N = 109)	0.07 (0.97)	1.55
Family/Partner (N = 795)	−0.02 (1.01)	
Friends/Housemates (N = 67)	0.17 (0.84)	
Region		
Central Italy (N = 713)	0.02 (0.97)	1.03
North Italy (N = 148)	−0.10 (1.15)	
South Italy (N = 110)	0.01 (0.92)	
Type of area		
White/Yellow (N = 350)	0.01 (0.94)	4.14 **
Orange (N = 128)	−0.23 (1.21)	
Red (N = 493)	0.05 (0.97)	

Note. **: $p \leq 0.01$; ***: $p \leq 0.001$.

For our third aim, Table 4 reports Pearson's correlations between the main variables assessed in the present study.

Table 4. Pearson's correlations between all variables (N = 978).

Total Sample	1	2	3	4	5	6	7	8	9	10	11
1. Intention to vaccinate	1.00										
2. Perceived risk	0.15 **	1.00									
3. Pro-sociality	0.23 **	0.16 **	1.00								
4. Fear of COVID-19	0.12 **	0.36 **	0.08 **	1.00							
5. Direct experience	0.02	0.18 **	0.01	0.07 *	1.00						
6. Use of preventive behaviors	0.20 **	0.12 **	0.20 **	0.16 **	−0.01	1.00					
7. Misinformation (number)	0.05	0.09 **	0.04	0.04	0.03	0.07 *	1.00				
8. Misinformation (belief)	−0.22 **	−0.06 †	−0.10 **	0.03	−0.04	−0.14 **	−0.05	1.00			
9. Trust in government	0.29 **	0.05	0.25 **	0.05	−0.05	0.07 *	0.01	−0.04	1.00		
10. Trust in science	0.47 **	0.14 **	0.32 **	−0.01	0.02	0.23 **	0.09 *	−0.14 **	0.36 **	1.00	
11. Trust in medical professionals	0.39 **	0.13 **	0.28 **	0.03	−0.02	0.22 **	0.08 *	−0.16 **	0.28 **	0.61 **	1.00

Note. *: $p \leq 0.05$; **: $p \leq 0.01$; †: $0.06 < p < 0.10$.

As can be noted, intention to vaccinate was positively and significantly correlated with perceived risk, pro-sociality, fear of COVID-19, use of preventive behaviors, trust in government, trust in science, and trust in medical professionals. Thus, acceptance of COVID-19 vaccines was higher in those participants who perceived more risk, were more prosocial, had more fear of the virus, used preventive behaviors more frequently, and were more trustful of government, scientists, and medical practitioners. In addition, intention to vaccinate was negatively and significantly associated with belief in misinformation; thus, participants who had higher levels of belief in COVID-19-related misinformation stated that they were less likely to vaccinate (as compared to participants who had lower levels of belief in misinformation). To determine the psychological predictors of the intention to vaccinate, we ran a hierarchical regression analysis, using the simultaneous method (see Table 5).

Table 5. Simultaneous regression predicting the intention to vaccinate.

Steps	Predictors	β	*t*
Step 1	Gender		
	Males	- [a]	-
	Females	−0.08	−2.56 **
	Age		
	18–30 years	- [a]	-
	31–40 years	0.02	0.59
	41–50 years	0.04	1.27
	51–60 years	0.09	2.41 *
	>61 years	0.17	4.99 **
	Education		
	High school or less	- [a]	-
	Bachelor's degree	−0.00	−0.12
	Master's degree	0.01	0.54
	Postgraduate	0.06	2.05 *
	Marital status		
	Single	- [a]	-
	Married	−0.14	−3.23 ***
	Divorced/widowed	−0.09	−2.60 **
	Living condition		
	Alone	- [a]	-
	Family/Partner	0.01	0.24
	Friends/Housemates	0.01	0.27
	Region		
	Central Italy	- [a]	-
	North Italy	0.00	0.02
	South Italy	−0.02	−0.78
	Area		
	Red	- [a]	-
	Orange	−0.02	−0.58
	White/Yellow	−0.04	−1.17
Step 2	Psychological predictors		
	Perceived risk	0.05	1.64
	Pro-sociality	0.02	0.73
	Fear of COVID-19	0.11	3.73 ***

Table 5. *Cont.*

Steps	Predictors	β	*t*
	Direct experience	−0.00	−0.04
	Use of preventive behaviors	0.06	2.08 *
	Misinformation (number)	−0.00	−0.09
	Misinformation (belief)	−0.16	−5.55 ***
	Trust in government	0.11	3.86 ***
	Trust in Science	0.29	7.91 ***
	Trust in medical professionals	0.14	4.07 ***

Note. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; [a]: reference category.

Demographic factors were entered in the first step as a series of dummy variables, to control for their influence. The overall model was significant ($F(26, 942) = 17.14$, $p < 0.001$). Demographic variables explained 4.7% of the variance in the intention to vaccinate ($F(16, 952) = 2.95$, $p < 0.001$); specifically, vaccination rates increased for participants who were older than 50 years and had a postgraduate degree, but decreased for participants who were females, married, or divorced/widowed. Psychological variables explained 27.4% of the variance ($F(10, 942) = 38.02$, $p < 0.001$): vaccination rates increased for participants who had fear of COVID-19, used more preventive measures, and were trustful of science, government, and medical professionals, whereas it decreased for participants who had high levels of belief in misinformation.

4. Discussion

In the present study, we investigated the intention to get vaccinated against COVID-19 in Italy in the period between March and May 2021. With respect to this first aim, we found that vaccine acceptance rates were substantially higher than those previously reported by Palamenghi et al. [4]. The overall percentage of participants who reported to be "very likely" and "absolutely likely" to vaccinate was 86.9% ($N = 850$). Likewise, the percentage of participants who were "very likely" and "absolutely likely" to recommend vaccination for their family members was 89.0% ($N = 870$). Similar estimates have been recently reported by Kerr et al. [6], who found that 85% of Italian respondents were likely to be vaccinated and 88% recommended vaccination to vulnerable friends or family members, and by Barello et al. [29], who estimated that 86% of Italian university students would choose to have a vaccination against COVID-19. This rapid increase in acceptance rates was expected, since the growing availability of COVID-19 vaccines has been accompanied by a widespread campaign of public health messaging specifically tailored to address people's negative attitudes towards vaccines. Our data are also consistent with the high number of doses administered so far in Italy: as reported in the Introduction Section, about 85% of the population over 12 years of age has been immunized with two doses and about 89% has received at least one dose. On the other hand, the relatively low rates of participants who declared to be hesitant about vaccination could be the result of the period in which our data were collected (in Italy, the overall number of infections was rapidly decreasing during the spring and fall of 2021 and this trend was primarily attributed by the authorities to the benefits of vaccination) and the characteristics of the sample that was recruited for the present study (most of our participants were young individuals aged between 18 and 30, with high educational levels, who might be particularly willing to get vaccination).

Our second aim was to determine the influence of individual differences in demographic variables on the intention to be vaccinated. We found that males and females did not differ in a direct comparison; however, in the following regression analysis, being female was associated with a reduced intention to get vaccinated against COVID-19. This is a common result which has been further confirmed by a recent meta-analysis [2] and may be attributed to the fact that females have typically high levels of mistrust about vaccine benefits and more negative concerns about future unforeseen side effects, which in turn are

important determinants of the willingness to be vaccinated [14]. In the present study, this tendency might have been exacerbated by the fact that females are more likely to use social media and were therefore overrepresented in our sample. Replicating the conclusions reached by Palamenghi et al. [4], we found that the middle-age group (41–50 years) was less likely to vaccinate, as compared to both the younger (18–30 years) and older (>61 years) groups. On the one hand, this outcome confirms the idea that elder people are aware of being more susceptible to the negative consequences of COVID-19 [30] and therefore more willing to vaccinate [31]. On the other hand, the present results echo previous data showing that Italian parents older than 35 years of age exhibited more hesitancy about the vaccination of their children and were less compliant with vaccination recommendations compared to parents younger than 35 years [32]. Surprisingly, statistical analyses revealed that the intention to be vaccinated was significantly higher in participants who were single than in those who were married or divorced/widowed. Furthermore, vaccine acceptance rates were not significantly higher in participants who lived with others (family members, partners, friends, or housemates) than in those who lived alone. This is apparently in contrast with available evidence indicating that, in both the United Kingdom and the United States, more respondents would accept a vaccine to protect family, friends, or at-risk groups than to protect themselves [13]. A potential explanation may be that, in the present study, marital status was confounded with age, such that participants who were single came predominantly from the youngest group (18–30 years, 88%), i.e., a group who, as stated below, exhibited high levels of willingness to be vaccinated; in contrast, participants who were married or divorced/widowed came predominantly from the 41–50 year (25.9% and 30.8%, respectively) and 51–60 year age groups (28.7% and 41.0%, respectively), i.e., two groups in which intention to get vaccinated was substantially below the mean level of the whole sample (see Table 3).

With respect to the third aim, determining the roles of several psychological variables in predicting the intention to be vaccinated against COVID-19, our results are largely consistent with previously published findings. More specifically, we found that trust in government, trust in science, and trust in medical professionals were among the strongest psychological predictors of the intention to be vaccinated. In agreement, people with high levels of trust in science have been shown to be more compliant with COVID-19 prevention guidelines [33–37] and more likely to get vaccinated against COVID-19 [4,6,38]. In the cross-national study by Kerr et al. [6], trust in medical doctors and nurses predicted vaccine acceptance in Italy, together with perceived infection risk and worry about the virus. Similarly, willingness to be vaccinated correlated with trust in scientific research in the study by Palamenghi et al. [4].

Interestingly, we found that fear of COVID-19, but not perceived risk, was associated with increased vaccine uptake in the regression analysis, suggesting that the affective component of risk perception was more important than the cognitive component in predicting participants' behaviors during the pandemic [12]. Previous research examining the role of these two factors reported mixed findings. Studies conducted during the first wave typically found significant effects of perceived severity of COVID-19 on vaccination intent [10,32]. Gagneux-Brunon et al. [9], for example, showed that fear of COVID-19 and individual perceived risk were both positively correlated with vaccine acceptance in a sample of French healthcare workers (also see Detoc et al. [39]). On the other hand, Qiao, Tam, and Li [40] found that fear of COVID-19, but not perceived susceptibility to the infection, was associated with increased willingness to be vaccinated in a sample of college students in North Carolina (also see [41]). It seems likely that variables such as the period in which the surveys were conducted and the demographic characteristics of the recruited samples might explain these discrepancies. Specifically, our study was performed during the second wave of the COVID-19 infection and most of our respondents were young people, aged between 18 and 30. These two factors might have resulted in relatively low levels of perceived risk, which in turn contributed to the non-significant association with intention to get vaccinated.

While the effects of trust variables were positive, a variable which reduced participants' intention to be vaccinated in our study was susceptibility to misinformation. This is in line with the conclusions obtained by a randomized controlled trial conducted by Loomba et al. [13], who found that recent exposure to misinformation induced a decline in vaccination intent of 6.2 percentage points in the UK and 6.4 percentage points in the USA. Similar findings have been reported by Roozenbeek et al. [15], who showed that, across five different countries (UK, Ireland, USA, Spain, and Mexico), increased susceptibility to misinformation led to a significant decrease in people's willingness to get vaccinated against the virus and to recommend the vaccine to vulnerable friends and family.

Our findings have practical implications for developing interventions aimed at increasing the acceptance of COVID-19 vaccines. First, since trust in science and trust in medical professionals play a key role in predicting participants' willingness to be vaccinated, public health institutions should try to increase the feeling of cooperation between scientists and citizens [4]. The scientific community should create a dialogue aimed at educating and sensitizing common people towards the logic and the limits of scientific research [42]. The mission of all scientists involved in the battle against COVID-19 is not simply to explain the reasons that justify the adoption of restraining measures, but to help create an enduring debate in which public concerns about the safety and effectiveness of vaccines can be expressed and properly addressed. In Italy, where most of the adult population has already received two doses, the establishment of such a climate would be particularly helpful in increasing acceptance of the so-called booster dose—which is still low (66%), according to a recent poll [43]. Along the same lines, a successful COVID-19 vaccination campaign must actively fight against the spreading of misinformation, which seems to be especially fast on social media [44]. This issue is particularly important since previous studies have shown that even brief exposures to misinformation can result in long-lasting negative effects on intention to get vaccinated [45]. Social media such as Facebook and Twitter have already adopted algorithms and fact-checking platforms to ensure amplification of right and trustable sources, to direct users to reliable websites and to filter out fake news about COVID-19 [46]. In addition, experimental evidence suggests that people tend to endorse false claims about COVID-19 because they do not spend sufficient time evaluating content accuracy and that a simple reminder at the beginning of presentation is sufficient to boost the level of trust discernment in participants who subsequently share information on social media [47].

The present study has both strengths and limitations. The strengths are that we provided an updated picture of the vaccination intentions at the beginning of 2021, in a period in which the Italian government had just launched the immunization campaign, whereas previous studies were mostly conducted during the first lockdown phase between March and May 2020 [4]. Moreover, in line with previous international research [12], we assessed a wide array of predictors, covering the cognitive, emotional, experiential, and sociocultural implications of the current pandemic, whereas previous studies have been often focused on single aspects. Lastly, it is interesting that participants were recruited mainly using social media, considering that, as previously noted, the spreading of misinformation seems to be especially fast on these media. Regarding limitations, our sample was not representative of the general Italian population because participants were recruited through different social media and were therefore mostly young, between 18 and 30 years of age, with high education levels. Second, the method was cross-sectional and correlational in nature, which means that we could not determine whether demographic and psychological factors were causally related to intention to be vaccinated. Lastly, despite the large number of predictors included in our survey, not all relevant variables were considered, such as political ideology [48], personality traits [49,50], and general vaccine attitudes and beliefs [9,10]. Future studies should consider these variables to better understand the multifaceted process underlying people's intention to get vaccinated.

Author Contributions: Conceptualization, A.S., V.C., C.R.-A. and P.S.; methodology, A.S. and P.S.; formal analysis, P.S., M.C. and F.D.; investigation, A.S., V.C., C.R.-A. and M.C.; resources, V.C. and C.R.-A.; data curation, A.S., V.C. and C.R.-A.; writing—original draft preparation, A.S., V.C., C.R.-A. and P.S.; writing—review and editing, V.C., C.R.-A., F.D. and M.C.; supervision, V.C. and C.R.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of La Sapienza University of Rome (Protocol N.0000476—4 March 2021).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ipsos. Available online: https://www.ipsos.com/en/covid-19-vaccination-intent-has-soared-across-world (accessed on 11 April 2021).
2. Robinson, E.; Jones, A.; Lesser, I.; Daly, M. International estimates of intended uptake and refusal of COVID-19 vaccines: A rapid systematic review and meta-analysis of large nationally representative samples. *Vaccine* **2021**, *39*, 2024–2034. [CrossRef]
3. Our World in Data. 2021. Available online: https://ourworldindata.org/covid-vaccinations (accessed on 4 July 2021).
4. Palamenghi, L.; Barello, S.; Boccia, S.; Graffigna, G. Mistrust in biomedical research and vaccine hesitancy: The forefront challenge in the battle against COVID-19 in Italy. *Eur. J. Epidemiol.* **2020**, *35*, 785–788. [CrossRef] [PubMed]
5. Caserotti, M.; Girardi, P.; Rubaltelli, E.; Tasso, A.; Lotto, L.; Gavaruzzi, T. Associations of COVID-19 risk perception with vaccine hesitancy over time for Italian residents. *Soc. Sci. Med.* **2021**, *272*, 113688. [CrossRef] [PubMed]
6. Kerr, J.R.; Schneider, C.R.; Recchia, G.; Dryhurst, S.; Sahlin, U.; Dufouil, C.; Arwidson, P.; Freeman, A.L.; van der Linden, S. Correlates of intended COVID-19 vaccine acceptance across time and countries: Results from a series of cross-sectional surveys. *BMJ Open* **2021**, *11*, e048025. [CrossRef] [PubMed]
7. Pivetti, M.; Di Battista, S.; Paleari, F.G.; Hakoköngäs, E. Conspiracy beliefs and attitudes toward COVID-19 vaccinations. *J. Pac. Rim Psychol.* **2021**, *15*, 18344909211039893. [CrossRef]
8. Cadeddu, C.; Sapienza, M.; Castagna, C.; Regazzi, L.; Paladini, A.; Ricciardi, W.; Rosano, A. Vaccine Hesitancy and Trust in the Scientific Community in Italy: Comparative Analysis from Two Recent Surveys. *Vaccines* **2021**, *9*, 1206. [CrossRef]
9. Gagneux-Brunon, A.; Detoc, M.; Bruel, S.; Tardy, B.; Rozaire, O.; Frappe, P.; Botelho-Nevers, E. Intention to get vaccinations against COVID-19 in French healthcare workers during the first pandemic wave: A cross-sectional survey. *J. Hosp. Infect.* **2021**, *108*, 168–173. [CrossRef]
10. Sherman, S.M.; Smith, L.E.; Sim, J.; Amlôt, R.; Cutts, M.; Dasch, H.; Rubin, G.J.; Sevdalis, N. COVID-19 vaccination intention in the UK: Results from the COVID-19 vaccination acceptability study (CoVAccS), a nationally representative cross-sectional survey. *Hum. Vaccines Immunother.* **2020**, *17*, 1612–1621. [CrossRef]
11. Van der Linden, S. The social-psychological determinants of climate change risk perceptions: Towards a comprehensive model. *J. Environ. Psychol.* **2015**, *41*, 112–124. [CrossRef]
12. Dryhurst, S.; Schneider, C.R.; Kerr, J.; Freeman, A.L.J.; Recchia, G.; van der Bles, A.M.; Spiegelhalter, D.; van der Linden, S. Risk perceptions of COVID-19 around the world. *J. Risk Res.* **2020**, *23*, 994–1006. [CrossRef]
13. Loomba, S.; de Figueiredo, A.; Piatek, S.J.; de Graaf, K.; Larson, H.J. Measuring the impact of COVID-19 vaccine misinformation on vaccination intent in the UK and USA. *Nat. Hum. Behav.* **2021**, *5*, 337–348, Correction in *Nat. Hum. Behav.* **2021**, *5*, 960. [CrossRef]
14. Paul, E.; Steptoe, A.; Fancourt, D. Attitudes towards vaccines and intention to vaccinate against COVID-19: Implications for public health communications. *Lancet Reg. Health Eur.* **2021**, *1*, 100012. [CrossRef]
15. Roozenbeek, J.; Schneider, C.R.; Dryhurst, S.; Kerr, J.; Freeman, A.L.J.; Recchia, G.; van der Bles, A.M.; van der Linden, S. Susceptibility to misinformation about COVID-19 around the world. *R. Soc. Open Sci.* **2020**, *7*, 201199. [CrossRef]
16. Lazarus, J.V.; Wyka, K.; Rauh, L.; Rabin, K.; Ratzan, S.; Gostin, L.O.; Larson, H.J.; El-Mohandes, A. Hesitant or Not? The Association of Age, Gender, and Education with Potential Acceptance of a COVID-19 Vaccine: A Country-level Analysis. *J. Health Commun.* **2020**, *25*, 799–807. [CrossRef]
17. Frank, K.; Arim, R. Statistics Canada. Available online: https://www150.statcan.gc.ca/n1/pub/45-28-0001/2020001/article/00043-eng.htm (accessed on 11 April 2021).
18. Reiter, P.L.; Pennell, M.L.; Katz, M.L. Acceptability of a COVID-19 vaccine among adults in the United States: How many people would get vaccinated? *Vaccine* **2020**, *38*, 6500–6507. [CrossRef]

19. Wong, L.P.; Alias, H.; Wong, P.-F.; Lee, H.Y.; Abubakar, S. The use of the health belief model to assess predictors of intent to receive the COVID-19 vaccine and willingness to pay. *Hum. Vaccines Immunother.* **2020**, *16*, 2204–2214. [CrossRef]
20. Rosenstock, I.M.; Strecher, V.J.; Becker, M.H. Social Learning Theory and the Health Belief Model. *Health Educ. Q.* **1988**, *15*, 175–183. [CrossRef]
21. Floyd, D.L.; Prentice-Dunn, S.; Rogers, R.W. A Meta-Analysis of Research on Protection Motivation Theory. *J. Appl. Soc. Psychol.* **2000**, *30*, 407–429. [CrossRef]
22. Yahaghi, R.; Ahmadizade, S.; Fotuhi, R.; Taherkhani, E.; Ranjbaran, M.; Buchali, Z.; Jafari, R.; Zamani, N.; Shahbazkhania, A.; Simiari, H.; et al. Fear of COVID-19 and Perceived COVID-19 Infectability Supplement Theory of Planned Behavior to Explain Iranians' Intention to Get COVID-19 Vaccinated. *Vaccines* **2021**, *9*, 684. [CrossRef]
23. Jung, H.; Albarracín, D. Concerns for others increases the likelihood of vaccination against influenza and COVID-19 more in sparsely rather than densely populated areas. *Proc. Natl. Acad. Sci. USA* **2020**, *118*, e2007538118. [CrossRef]
24. Yu, Y.; Luo, S.; Mo, P.K.-H.; Wang, S.; Zhao, J.; Zhang, G.; Li, L.; Li, L.; Lau, J.T.-F. Prosociality and Social Responsibility Were Associated With Intention of COVID-19 Vaccination Among University Students in China. *Int. J. Health Policy Manag.* **2021**. [CrossRef] [PubMed]
25. Rosman, T.; Adler, K.; Barbian, L.; Blume, V.; Burczeck, B.; Cordes, V.; Derman, D.; Dertli, S.; Glas, H.; Heinen, V.; et al. Protect ya Grandma! The Effects of Students' Epistemic Beliefs and Prosocial Values on COVID-19 Vaccination Intentions. *Front. Psychol.* **2021**, *12*, 683987. [CrossRef] [PubMed]
26. Ahorsu, D.K.; Lin, C.-Y.; Imani, V.; Saffari, M.; Griffiths, M.D.; Pakpour, A.H. The Fear of COVID-19 Scale: Development and Initial Validation. *Int. J. Ment. Health Addict.* **2020**, 1–9. [CrossRef] [PubMed]
27. Soraci, P.; Ferrari, A.; Abbiati, F.A.; Del Fante, E.; De Pace, R.; Urso, A.; Griffiths, M.D. Validation and Psychometric Evaluation of the Italian Version of the Fear of COVID-19 Scale. *Int. J. Ment. Health Addict.* **2020**, *4*, 1–17. [CrossRef]
28. Lee, J.J.; Kang, K.-A.; Wang, M.P.; Zhao, S.Z.; Wong, J.Y.H.; O'Connor, S.; Yang, S.C.; Shin, S. Associations Between COVID-19 Misinformation Exposure and Belief With COVID-19 Knowledge and Preventive Behaviors: Cross-Sectional Online Study. *J. Med. Internet Res.* **2020**, *22*, e22205. [CrossRef]
29. Barello, S.; Nania, T.; Dellafiore, F.; Graffigna, G.; Caruso, R. 'Vaccine hesitancy' among university students in Italy during the COVID-19 pandemic. *Eur. J. Epidemiol.* **2020**, *35*, 781–783. [CrossRef]
30. Lazarus, J.V.; Ratzan, S.C.; Palayew, A.; Gostin, L.O.; Larson, H.J.; Rabin, K.; Kimball, S.; El-Mohandes, A. A global survey of potential acceptance of a COVID-19 vaccine. *Nat. Med.* **2021**, *27*, 225–228. [CrossRef]
31. Ruiz, J.B.; Bell, R.A. Predictors of intention to vaccinate against COVID-19: Results of a nationwide survey. *Vaccine* **2021**, *39*, 1080–1086. [CrossRef]
32. Giambi, C.; Fabiani, M.; D'Ancona, F.; Ferrara, L.; Fiacchini, D.; Gallo, T.; Martinelli, D.; Pascucci, M.G.; Prato, R.; Filia, A.; et al. Parental vaccine hesitancy in Italy—Results from a national survey. *Vaccine* **2018**, *36*, 779–787. [CrossRef]
33. Bicchieri, C.; Fatas, E.; Aldama, A.; Casas, A.; Deshpande, I.; Lauro, M.; Parilli, C.; Spohn, M.; Pereira, P.; Wen, R. In science we (should) trust: Expectations and compliance across nine countries during the COVID-19 pandemic. *PLoS ONE* **2021**, *16*, e0252892. [CrossRef]
34. Dohle, S.; Wingen, T.; Schreiber, M. Acceptance and adoption of protective measures during the COVID-19 pandemic: The role of trust in politics and trust in science. *Soc. Psychol. Bull.* **2020**, *15*, 1–23. [CrossRef]
35. Hromatko, I.; Tonković, M.; Vranic, A. Trust in Science, Perceived Vulnerability to Disease, and Adherence to Pharmacological and Non-pharmacological COVID-19 Recommendations. *Front. Psychol.* **2021**, *12*, 664554. [CrossRef]
36. Pagliaro, S.; Sacchi, S.; Pacilli, M.G.; Brambilla, M.; Lionetti, F.; Bettache, K.; Bianchi, M.; Biella, M.; Bonnot, V.; Boza, M.; et al. Trust predicts COVID-19 prescribed and discretionary behavioral intentions in 23 countries. *PLoS ONE* **2021**, *16*, e0248334. [CrossRef]
37. Plohl, N.; Musil, B. Modeling compliance with COVID-19 prevention guidelines: The critical role of trust in science. *Psychol. Health Med.* **2020**, *26*, 1–12. [CrossRef]
38. Viswanath, K.; Bekalu, M.; Dhawan, D.; Pinnamaneni, R.; Lang, J.; McLoud, R. Individual and social determinants of COVID-19 vaccine uptake. *BMC Public Health* **2021**, *21*, 818. [CrossRef]
39. Detoc, M.; Bruel, S.; Frappe, P.; Tardy, B.; Botelho-Nevers, E.; Gagneux-Brunon, A. Intention to participate in a COVID-19 vaccine clinical trial and to get vaccinated against COVID-19 in France during the pandemic. *Vaccine* **2020**, *38*, 7002–7006. [CrossRef]
40. Qiao, S.; Tam, C.C.; Li, X. Risk Exposures, Risk Perceptions, Negative Attitudes toward General Vaccination, and COVID-19 Vaccine Acceptance among College Students in south Carolina. *Am. J. Health Promot.* **2021**. [CrossRef]
41. Chu, H.; Liu, S. Integrating health behavior theories to predict American's intention to receive a COVID-19 vaccine. *Patient Educ. Couns.* **2021**, *104*, 1878–1886. [CrossRef]
42. Provenzi, L.; Barello, S. The Science of the Future: Establishing a Citizen-Scientist Collaborative Agenda After Covid-19. *Front. Public Health* **2020**, *8*, 282. [CrossRef]
43. Ipsos. Available online: https://www.ipsos.com/it-it/covid-terza-dose-vaccino-maggioranza-intervistati-riceverebbe-dose-richiamo (accessed on 29 December 2021).
44. Vosoughi, S.; Roy, D.; Aral, S. The spread of true and false news online. *Science* **2018**, *359*, 1146–1151. [CrossRef]
45. Pluviano, S.; Watt, C.; Della Sala, S. Misinformation lingers in memory: Failure of three pro-vaccination strategies. *PLoS ONE* **2017**, *12*, e0181640. [CrossRef]

46. Brindha, M.D.; Jayaseelan, R.; Kadeswara, S. Social Media Reigned by Information or Misinformation About COVID-19: A Phenomenological Study. *Alochana Chakra J.* **2020**, *9*, 585–602. [CrossRef]
47. Pennycook, G.; McPhetres, J.; Zhang, Y.; Lu, J.G.; Rand, D.G. Fighting COVID-19 Misinformation on Social Media: Experimental Evidence for a Scalable Accuracy-Nudge Intervention. *Psychol. Sci.* **2020**, *31*, 770–780. [CrossRef] [PubMed]
48. Calvillo, D.P.; Ross, B.J.; Garcia, R.J.B.; Smelter, T.J.; Rutchick, A.M. Political Ideology Predicts Perceptions of the Threat of COVID-19 (and Susceptibility to Fake News About It). *Soc. Psychol. Pers. Sci.* **2020**, *11*, 1119–1128. [CrossRef]
49. Hampshire, A.; Hellyer, P.J.; Soreq, E.; Mehta, M.A.; Ioannidis, K.; Trender, W.; Grant, J.E.; Chamberlain, S.R. Associations between dimensions of behaviour, personality traits, and mental-health during the COVID-19 pandemic in the United Kingdom. *Nat. Commun.* **2021**, *12*, 4111. [CrossRef]
50. Zajenkowski, M.; Jonason, P.K.; Leniarska, M.; Kozakiewicz, Z. Who complies with the restrictions to reduce the spread of COVID-19?: Personality and perceptions of the COVID-19 situation. *Pers. Individ. Differ.* **2020**, *166*, 110199. [CrossRef]

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland
Tel. +41 61 683 77 34
Fax +41 61 302 89 18
www.mdpi.com

Journal of Personalized Medicine Editorial Office
E-mail: jpm@mdpi.com
www.mdpi.com/journal/jpm

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland

Tel: +41 61 683 77 34

www.mdpi.com

ISBN 978-3-0365-4541-7