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An investigation of autonomic arousal and attentional mechanisms in children with ADHD and Autism

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Abstract

The present doctoral project was aimed at investigating the impact of Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD) on measures of physiological arousal, alerting/vigilance, attention orienting and executive functions. 106 children between 7 and 15 years of age (31 typically developing; 24 ADHD-only; 18 ASD-only; 33 ADHD&ASD) performed a battery of eye-tracking and EEG experimental paradigms, while parent-reported measures were used to evaluate the severity of symptoms of ASD, ADHD and other psychiatric conditions.

Children with clinical diagnoses of ADHD and ASD showed condition-specific signs of dysregulated physiological arousal and vigilance, with ADHD more likely to be associated with difficulties in up-regulating and maintaining an optimal level of vigilance to the environment, and ASD more associated with over-reactivity to sensory information and difficulties in down-regulating autonomic arousal in line with contextual demands. We also demonstrated that executive function and cognitive control mechanisms are likely to be less effective in children with comorbid ADHD+ASD, with negative effects on performance accuracy. In the discussion of this dissertation, some suggestions for clinical practice and future research studies, besides a description of the implications of the findings on the everyday life of people with ADHD and/or ASD, are provided.

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Several people contributed to the realisation of this doctoral thesis and should be thanked for it.

Sara, I can never thank you enough for your continued support during these years and your precious ability to get me back on the right track in the most difficult and stressful moments. Thanks for being on my side every day!

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Contribution of the author

Dr Maddie Groom (MG) and Prof Chris Hollis (CH) were the supervisors of Alessio Bellato (AB) doctoral project, while MG, CH and Dr Danielle Ropar (DR) supervised Iti Arora (IA) doctoral project.

I (AB) had a primary role in preparing documents to be submitted to obtain ethical approval for the main study; designing the three experimental paradigms and analysing the measures used to investigate the hypotheses of my doctoral project; obtaining informed consent and collecting general information about the child from parents, providing instructions to parents and teachers about the questionnaires, carrying out the cognitive assessment and the testing session with the children; scoring questionnaires and analysing interviews for creating the final database of clinical and demographic data.

Iti Arora was similarly responsible for designing the tasks she used to investigate the hypotheses of her doctoral project, obtaining informed consent and collecting general information from the parents, providing instructions to parents and teachers about the questionnaires, carrying out the cognitive assessment and the testing session. She was also responsible for carrying out the ADOS assessment, and we worked together in scoring questionnaires and analysing interviews for creating the final database of clinical and demographic data.

Dr Puja Kochhar (PK) was responsible, together with CH, for the assessment of the clinical measures, the confirmation of clinical diagnoses of ADHD and/or ASD, and assisting AB and IA in the categorisation of participants in the groups used for the main analyses.

I shall acknowledge that valuable moments of discussion with my principal supervisors, Dr Maddie Groom and Prof Chris Hollis; Iti Arora; colleagues within the University department; and experts at national and international conferences, helped me to develop and refine the main hypotheses of the dissertation. Therefore, although this dissertation is indirectly fruit of a teamwork, I confirm it is my own work, it is original, it contains my own ideas, and any idea or thought belonging to someone else is appropriately acknowledged.

During my PhD, I have also been involved in teaching activities, including delivering of lectures and small-groups seminars, demonstrations and experiential workshops; supervising undergraduate and post-graduate students during their final projects, including training them on lab procedures, discussing data analyses pipelines and giving oral and written feedback on performance; delivering one-on-one personal help sessions and academic tutoring; assistance on technical issues during practical workshops and large groups teaching; marking and giving feedback to students' assignments. These experiences have helped my professional development and indirectly positively affected the writing of this dissertation.

"The future belongs to those who believe

in the beauty of their dreams"

Eleanor Roosevelt

To everyone who believes in the beauty of their dreams

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List of Abbreviations

ACC. Anterior Cingulate Cortex

ADHD. Attention Deficit Hyperactivity Disorder

ADOS. Autism Diagnostic Observation Scale

AI. Anterior Insula

ANCOVA. Analysis of Covariance

ANOVA. Analysis of Variance

ANS. Autonomic Nervous System

ASD. Autism Spectrum Disorder

BF. Bayesian Factor

BH. Benjamini-Hochberg

BPM. Beats Per Minute

CD. Conduct Disorder

CNS. Central Nervous System

CRS. Conners' Rating Scales

CSI. Cardiac Sympathetic Index

CVI. Cardiac Vagal Index

DA. Dopamine

DAWBA. Development and Well-Being Assessment

DMN. Default Mode Network

DSM. Diagnostic and Statistical Manual of Mental Disorders

ECG. Electro-cardiogram

EDA. Electrodermal Activity

EEG. Electro-Encephalography

ERP. Event-Related Potential

FDR. False Discovery Rate

fMRI. Functional Magnetic Resonance Imaging

FSIQ. Full Scale IQ

GABA. Gamma-Aminobutyric Acid

HR. Heart Rate

HRV. Heart Rate Variability

IBI. Inter-Beats Interval

IQ. Intelligence Quotient

LC. Locus Coeruleus

LC-NE. Locus Coeruleus-Norepinephrine system

mPFC. Medial Pre-Frontal Cortex

MPH. Methylphenidate

NDDs. Neurodevelopmental Disorders

NE. Norepinephrine

NICE. National Institute for Health and Care Excellence

NS-SCRs. Non-specific Skin Conductance Responses

ODD. Oppositional Defiant Disorder

OFC. Orbitofrontal Cortex

PCC. Posterior Cingulate Cortex

PFC. Pre-Frontal Cortex

PIQ. Performance IQ

PNS. Parasympathetic Nervous System

POP task. Preparing to Overcome Prepotency task

PS. Pupil Size

RMSSD. Root Mean Square of Successive Differences

RRBs. Restricted and Repetitive Behaviours

RTV. Reaction-Times Variability

SC. Superior Colliculus

SCL. Skin Conductance Level

SCQ. Social Communication Questionnaire

SCR. Skin Conductance Responses

SD. Standard Deviation

SDQ. Strengths and Difficulties Questionnaire

SNS. Sympathetic Nervous System

SRTs. Saccadic Reaction Times

VBA. Microsoft Office Visual Basic for Applications

VIQ. Verbal IQ

vmPFC. Ventromedial Pre-Frontal Cortex

WASI. Wechsler Abbreviated Scale of Intelligence

Chapter 1. Investigating attention and arousal regulation mechanisms in Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD)

1.1. An introduction to Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD)

1.1.1. Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is a neurodevelopmental psychiatric condition characterized by 'a persistent pattern of inattention and/or hyperactivity and impulsivity that interferes with functioning or development' (American Psychiatric Association, 2013). It has an estimated worldwide prevalence of about 5% in children (Polanczyk et al., 2014) and 2.5% in adults (Simon et al., 2009). An increased percentage of males diagnosed with ADHD, compared to females, has been frequently reported (American Psychiatric Association, 2013). This gender imbalance in ADHD diagnoses is likely to reflect a referral bias, indicating that a reduced percentage of females with ADHD in the general population are referred to clinical services, compared to males with the same symptoms (Nøvik et al., 2006; Rucklidge, 2008, 2010). A diagnosis of ADHD has negative consequences on the quality of life, including repercussions on psychological and social wellbeing, and academic achievement, with an indirect negative impact on family life (Danckaerts et al., 2010).

A multifactorial aetiology, involving genetic and environmental factors, seems to underlie the atypical development of many brain structural and functional networks, and the consequent onset of symptoms of ADHD (see Faraone et al., 2015, for a review). Genetic factors are very likely to play a relevant role, as demonstrated by findings of high heritability of ADHD throughout the entire lifespan (70-80%; Faraone & Mick, 2010) and increased prevalence of ADHD-like symptoms in first-degree relatives of people with ADHD (Thapar et al., 2013). Although a recent international collaboration between different research consortiums was able to find, for the first time, a specific

series of genome regions which have been directly associated with ADHD (Demontis et al., 2018), the heritability of traits of inattention and hyperactivity has been demonstrated not sufficient, per se, to trigger the onset of clinical symptoms of ADHD. Environmental factors, such as pre- and perinatal risk factors (e.g., preterm birth, low birth weight, maternal smoking during pregnancy, and exposure to toxic elements) might therefore exaggerate the effects of genetic risk factors and, overall, increase the risk of ADHD onset. However, while a single factor (either genetic or environmental) might increase the vulnerability risk for ADHD, clinically relevant symptoms are likely to appear only when multiple genetic and environmental factors additively interact to augment the presence of ADHD traits above a certain threshold (Faraone et al., 2015).

Evidence showing that ADHD is likely to arise from multiple factors may explain the vast heterogeneity of symptoms of ADHD, which has been conceptualised as three different presentations (labelled *subtypes* in the previous versions of the DSM; American Psychiatric Association, 2013): (a) *predominantly inattentive*, (b) *predominantly hyperactive* and (c) *combined*. Although this classification seems to explain the different profiles of primary symptoms of ADHD, it does not fully consider sub-threshold or co-occurring symptoms of other conditions, meaning that the clinical heterogeneity of ADHD is even greater than this diagnostic classification suggests. Therefore, a more complete approach for describing ADHD symptomatology would be to consider the clinical manifestation of ADHD as the extreme end of a continuous spectrum of traits, including but not limited to inattention and hyperactivity, which are inter-connected and manifest at different levels within the same individual, and may vary throughout different developmental stages (Heidbreder, 2015).

The diagnosis of ADHD mainly derives from the clinical observation of a child's behaviour and familial history, with the contribution of standardised rating scales, such

as the Conners' Rating Scales (CRS; Conners, 2008), usually completed by parents and teachers, which are all considered by the clinicians as a broad and comprehensive inventory of the child's behavioural patterns in the domestic and school environment. The clinical diagnosis of ADHD, in fact, requires that symptoms of the condition are persistent (they have been continuously present for at least 6 months), pervasive (they are present across different life situations, such as at home and at school) and have an impact on a child's functioning (American Psychiatric Association, 2013). Although research has been improving the diagnostic assessment and classification of ADHD, no objective, biological marker of ADHD has been found sufficiently reliable to assist clinicians during the evaluation of symptoms of ADHD (Mahone & Denckla, 2017). Furthermore, the diagnostic process may also be affected by the co-occurring presence of symptoms which can be attributable to other neurodevelopmental disorders (NDDs) (see paragraph 1.1.3), with evident consequent difficulties in choosing the best intervention plan and in foreseeing the impact of clinical symptoms on the global functioning of children with ADHD.

ADHD is usually managed through pharmacological and non-pharmacological interventions (National Institute for Health and Care Excellence, NICE; guideline NG87, 2018; https://www.nice.org.uk/guidance/ng87). Among the pharmacological treatments, stimulants such as methylphenidate (MPH) and amphetamines, and non-stimulants such as atomoxetine or guanfacine, are used for their efficacy in improving inattention and reducing hyperactive and impulsive behaviours (Faraone et al., 2015). Stimulants (both MPH and amphetamines) mainly act by blocking the reuptake of dopamine and norepinephrine in pre-frontal systems, therefore increasing the levels of these neurotransmitters in the synaptic space. Conversely, non-stimulants act by inhibiting the reuptake of norepinephrine, with atomoxetine selectively targeting

norepinephrine transporters and guanfacine targeting alpha-2-adrenergic receptors (Sharma & Couture, 2014). Due to slight differences in the action mechanisms, some people with ADHD might respond better to a specific medication than others (Sharma & Couture, 2014). When considering efficacy and tolerability, Cortese et al (2018) have shown that a first-choice short-term intervention for children and adolescents with ADHD is methylphenidate, while amphetamines should be preferred in adults. However, the medical treatment for ADHD, including the choice of stimulant or non-stimulant medication, and its dosage, should be planned by focusing on the individual's characteristics, and its effects on symptoms severity and other medical indices should be monitored regularly (NICE; guideline NG87, 2018).

Among non-pharmacological treatments, a combination of behavioural interventions, such as school-based behavioural intervention and parent training (Daley et al., 2014), and stimulant medication, is likely to be more beneficial than the single therapies (Catalá-López et al., 2017). Although the effects of neuro-cognitive interventions, including neurofeedback (Holtmann et al., 2014) or computerized training of visual attention (García-Baos et al., 2019) and working memory (Klingberg et al., 2005), have been widely investigated, the European ADHD Guidelines Group has found little evidence from Randomized Controlled Trials in support of the use of neurofeedback or cognitive training as interventions for individuals with ADHD (Cortese et al., 2015; 2016).

Among co-occurring psychiatric conditions usually reported in individuals with ADHD, conduct and oppositional-defiant disorders are frequent, together with mood and anxiety disorders; tic, language, learning and motor disorders; Autism Spectrum Disorder (ASD) and intellectual disability (Franke et al., 2018; Jensen & Steinhausen,

2015). While symptoms of ASD might be present at sub-clinical level in children with ADHD, therefore representing secondary symptoms of a primary diagnosis of ADHD, a comorbid diagnosis of ADHD and ASD reflects the presence of clinically significant symptoms of ADHD and ASD in the same patient. A double diagnosis of ADHD+ASD has been only allowed since the publication of DSM-5 (American Psychiatric Association 2013). Since then, studying the presence of clinically relevant symptoms of ASD in people with ADHD has received increasing interest within the scientific community, and researchers became more interested in disentangling the similarities and differences between the two conditions, and the frequent comorbidity (Rommelse et al., 2011). Anticipating that a main aim of this research study was to investigate the impact of a co-morbid diagnosis of ASD in children with ADHD, the main characteristics of ASD will be now briefly described.

1.1.2. Autism Spectrum Disorder (ASD)

Autism Spectrum Disorder (ASD) is a pervasive neurodevelopmental syndrome diagnosed in about 1% of children and adults (Lai et al., 2014). ASD is characterised by 'a persistent impairment in reciprocal social communication and social interaction, and restricted, repetitive patterns of behaviour, interests, or activities, which are present from early childhood and limit or impair everyday functioning' (American Psychiatric Association, 2013). Moreover, atypical sensory processing, including hyper- or hypo-responsivity to sensory information and difficulties in integrating sensory information coming from multiple modalities (Marco et al., 2011) is one of the symptomatic features of ASD (American Psychiatric Association, 2013). ASD is a very heterogeneous syndrome with different levels of symptom severity: the DSM-5 advices to determine

the clinical severity of symptoms by observing impairments in two main domains, namely social-communication deficits, and restricted/repetitive behaviours (RRBs; American Psychiatric Association, 2013).

The pathogenesis of ASD is not fully clear and it involves a combination of different risk factors. There is evidence of high heritability estimates (~64-91%; Tick et al., 2016), like previously demonstrated for ADHD, with interactions between genetic and environmental factors during late prenatal and early postnatal life likely to be at the basis of the etiological mechanisms of ASD (Rutter, 2013). Other similarities with ADHD are represented by the fact that the diagnostic evaluation of symptoms of ASD is primarily based on the clinical observation of behaviour, for example through standardised assessments such as the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2012); and that females with ASD are less likely to be referred to clinical services, even when showing the same symptomatologic profile as males (Baron-Cohen et al., 2011).

Although higher IQ, better social abilities and communication skills are likely to predict better outcomes of ASD, it has been shown that ASD has long-term consequences such as reduced independence in activities of daily living, poorer academic achievement, reduced rates of employment and poorer social relationships with peers (Howlin et al., 2004; 2013). In fact, while cognitive difficulties experienced earlier in development might improve throughout adolescence and young adulthood, symptoms of ASD, including social and communicative difficulties, and RRBs, are likely to remain stable and impact adaptive functioning (Simonoff et al., 2019). Interventions for ASD are usually based on behavioural approaches and they are usually aimed at increasing independence in everyday life, facilitating learning and stimulating cognitive abilities, besides improving social abilities and communication skills.

Medical interventions might be prescribed to treat comorbid symptoms, such as inattention or anxiety, or to reduce challenging and repetitive behaviours (Lai et al., 2014).

1.1.3. Symptoms of ASD in ADHD

Socio-emotional and communication difficulties might be present as secondary symptoms in people with ADHD, deriving from primary inattention, hyperactive and impulsive behaviours which tend to cause difficulties in social relationships and peer rejection, limiting the exposure to social situations and development of social skills (Leitner, 2014; Rommelse et al., 2011). However, social functioning is distinctly impaired in people with ADHD and with ASD, with ADHD more associated with externalising negative behaviours and less severe difficulties in experimental lab-based situations, and ASD more characterised by the absence of positive behaviours and difficulties in social cognition, as it is usually observed in laboratory settings (Mikami et al., 2019).

ASD symptomatology has been found positively associated with inattention and hyperactivity/impulsivity symptoms (Reiersen et al., 2007), even at subclinical level in the general population (Ronald et al., 2008). Moreover, first-degree relatives of patients with ADHD are at higher risk of having ASD, compared to individuals from the general population (Ronald et al., 2008). The commonalities between ADHD and ASD made some authors speculate that the two conditions might therefore be different phenotypical expressions of one overarching disorder, so that ADHD could be a milder expression of ASD-symptomatology (Rommelse et al., 2016). If this would be the case, individuals at elevated risk of developing either ADHD or ASD would be more likely

to also display clinically relevant symptoms of the other condition, while people with mild but clinically significant symptoms of ADHD or ASD might just have a single primary diagnosis and only subclinical traits of the other condition, if any.

Research on the aetiology of the comorbidity between ADHD and ASD has showed that the two conditions are likely to emerge from shared genetic and environmental factors, which are likely to interact and increase the susceptibility risk for the onset of behavioural traits of these conditions from early development (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019; Ghirardi et al., 2017; Rommelse et al., 2010). More specifically, inattention and reduced joint attention, high negative affect and emotionality, and deficits in effortful control, in early infancy, seem to be common pathways to both ADHD and ASD (Johnson et al., 2015; Visser et al., 2014).

While shared genetic and familial risk factors might influence early post-natal development and give rise to non-specific precursors of ADHD and ASD, the phenotypical expression of ADHD- and ASD-symptomatology is likely to diverge already during the second year of age. Around this time, ASD-specific symptoms seem in fact more associated with increased interest for non-social objects, high persistence, and increased perceptual sensitivity, distress, shyness, fear and sadness (see Visser et al., 2014 for a review). On the opposite, ADHD-specific symptoms have been found more associated with increased positive affect and extraversion, high anger and emotional reactivity, high distractibility, low attentional and inhibitory control (Visser et al., 2014). Different atypicalities in brain volume and cortical matter growth have also been reported (Dougherty et al., 2016). More specifically, brain overgrowth and increased volume (especially during childhood and adolescence) have been found in people with ASD, while decreased brain volume and cortical thinning is more prevalent

in individuals with ADHD. Moreover, deficits in executive functions are different in the two conditions, with ASD more associated with deficits in task shifting, while inhibition deficits are more likely to characterise ADHD (Visser et al., 2014).

The *additive model* of ADHD/ASD comorbidity suggests that while ADHD and ASD might emerge from shared or similar risk factors, the phenotypical expression of the conditions in the same individual would be an additive combination of the symptomatology and atypicalities reported in the two conditions, e.g., different executive functioning deficits (Banaschewski et al., 2007; Craig et al., 2016; Leitner, 2014; Tye et al., 2014). While this might be true for some domains, for other domains an *interactive model* of comorbidity would be more appropriate. According to the interactive model, in fact, people with ADHD+ASD are more likely to display an independent profile of impairments, resembling the atypicalities found in each disorder but at a greater severity than what found in the single conditions (Berenguer-Forner et al., 2015; Craig et al., 2015). The interactive model has been supported by studies showing that the simultaneous presence of clinical diagnoses of ADHD and ASD negatively affects patients' quality of life, impacting social, cognitive and adaptive functioning to a greater extent than a single diagnosis of ADHD (Leitner, 2014; van der Meer et al., 2012). Delayed language development (Berenguer-Forner et al., 2015) and lower IQ (Craig et al., 2015) have also been reported in children with co-occurring ADHD+ASD, when compared with children with ASD- or ADHD-only, leading to delayed diagnoses (up to 2 years later than children with a single condition), and direct or indirect negative effects on interventional outcomes (Kentrou et al., 2019). This may also be the case when non-clinical sub-threshold symptoms of ASD are present in a child with ADHD (Ronald et al., 2014). The co-occurring presence of ADHD and ASD seems to impact the outcome effects of medical treatments as well. For example, a

review by Davis and Kollins (2012) pointed out that traditionally used stimulant treatments for ADHD might have increased negative side effects (such as increased stereotypies and RRBs) and reduced positive outcomes in individuals with co-occurring ADHD+ASD, who might benefit more from different medications, such as non-stimulants.

Since ADHD and ASD are likely to be characterised by similarities in genetic and familial risk factors, investigating both convergences and differences in their behavioural, neuro-cognitive and physiological phenotypes, might prove helpful in clarifying the etiological pathways of these conditions, both when they emerge separately and when they co-occur (Kandel, 1998). Identifying transdiagnostic and condition-specific atypicalities associated with ADHD and ASD, and understanding at what level they are present in individuals with co-occurring ADHD+ASD, seems an important step towards improving the diagnostic classification of the most clinically complex cases, which might benefit from quicker and more specific diagnoses, and personalised interventions. Moreover, investigating if specific phenotypes are mainly associated with ADHD or ASD, in patients with comorbid ADHD+ASD, might help clinicians to identify the core areas of impairment which should be given priority for interventions. For example, if those with a comorbid diagnosis of ADHD+ASD showed a pattern of atypicalities separately associated with ADHD and ASD (additive model of comorbidity), using combined interventions separately designed for ADHD and ASD might be beneficial. Conversely, if more severe deficits are present in those with ADHD+ASD (in support of the interactive model of ADHD/ASD comorbidity), commonly used medications for ADHD, such as stimulants, might have negative consequences on the population of patients with comorbid ADHD+ASD.

1.1.4. General scope of the study

I used a battery of experimental paradigms to investigate indices of autonomic arousal and arousal regulation together with measures of attentional control and executive function in children with ADHD and/or ASD, to assess the impact of a comorbid diagnosis of ADHD+ASD on these mechanisms. I aimed to identify if behavioural, electrophysiological and physiological markers were ADHD- or ASD-specific, and which were common in both conditions, and test at which level these domains of impairment were present in children and adolescents with ADHD+ASD. In fact, it was investigated if a theoretical model of ADHD/ASD comorbidity (*additive* or *interactive*), or a combination of both (dependent on specific domains of impairment), could be supported by the empirical data. Previous theoretical frameworks and empirical research (discussed in more detail in paragraph 1.4) have suggested that difficulties in regulating autonomic arousal may contribute to higher level cognitive impairments in people with ADHD (Frazier et al., 2004; Mueller et al., 2017; Willcutt et al., 2005). Due to its crucial role in the regulation of basic and more complex attentional, cognitive and behavioural mechanisms, the autonomic nervous system will be now described, focusing on the locus coeruleus-norepinephrine system (LC-NE).

1.2. Attention and arousal regulation: why consider them when studying ADHD and ASD?

1.2.1. The role of the autonomic nervous system in regulating arousal and cognitive mechanisms

Arousal has been defined as the set of neural, behavioural and physiological mechanisms that characterise wakefulness and alertness in response to signals from the body and the environment (Lacey, 1967). These mechanisms, which affect the state of being alert, awake and attentive, are governed by interactions between the peripheral and the central nervous system (CNS). Being part of the peripheral nervous system, the autonomic nervous system (ANS) is responsible for the regulation of bodily functions (including heart rate, respiration, perspiration and pupil dilation) by controlling smooth muscle fibres, cardiac muscle fibres and glands.

While the sympathetic nervous system (SNS; one branch of the ANS) is activated in situations which necessitate fast allocation and mobilisation of energetic resources, eliciting 'fight or flight' responses, the parasympathetic nervous system (PNS) is responsible for 'rest and digest' responses aimed at preserving and maintaining energetic resources for longer periods of time. The norepinephrine-mediated mechanisms controlled by the SNS, including heart rate accelerations, pupil dilations and increased blood flow to vital organs, prepare the body for a rapid response. Conversely, the PNS contains cholinergic fibres and acts to conserve and restore energy by slowing down heart rate, constricting pupil dilation and slowing blood flow. Executive- and salience-processing cortical networks have been found more active during SNS-related activity, while the default mode network seems more involved with the PNS (Beissner et al., 2013). Although they seem to have antagonistic functions,

SNS and PNS act in a synergistic way to reach and maintain an optimal physiological state of arousal in line with environmental demands and internal states, characterised by adequate heart rate, respiratory behaviour, levels of glucose and oxygen in the blood, body temperature, perspiration and salivation.

The association between cognitive and attentional mechanisms, and functioning of the ANS, has been investigated for a long time. At the beginning of the 20th century, Yerkes and Dodson (1908) hypothesised an inverted U-shaped relationship between autonomic arousal and cognitive performance, proposing that either reduced or heightened arousal would negatively impact task-performance. More recently, the link between arousal and cognition has been further investigated through different studies, partly clarifying the parallel role of the ANS, brainstem structures and cortical systems in the regulation of behaviour, attentional and cognitive processes.

1.2.2. A link between arousal and attention: the locus coeruleus-norepinephrine (LC-NE) system

The locus coeruleus (LC) is a small group of norepinephrinergic neurons, situated in the pons, which has a role in arousal, sleep-wakefulness regulation and higher cognitive mechanisms, including attention allocation and information processing (Bast et al., 2018; Sara & Bouret, 2012; Aston-Jones & Waterhouse, 2000). The LC, in fact, has bidirectional connections with pre-frontal regions (anterior cingulate cortex, ACC; orbitofrontal cortex, OFC; and ventromedial prefrontal cortex, vmPFC), insula, hypothalamus and amygdala, besides receiving peripheral autonomic signals from the vagal nerve through the nucleus of the solitary tract (Critchley & Garfinkel, 2018) (Figure 1).
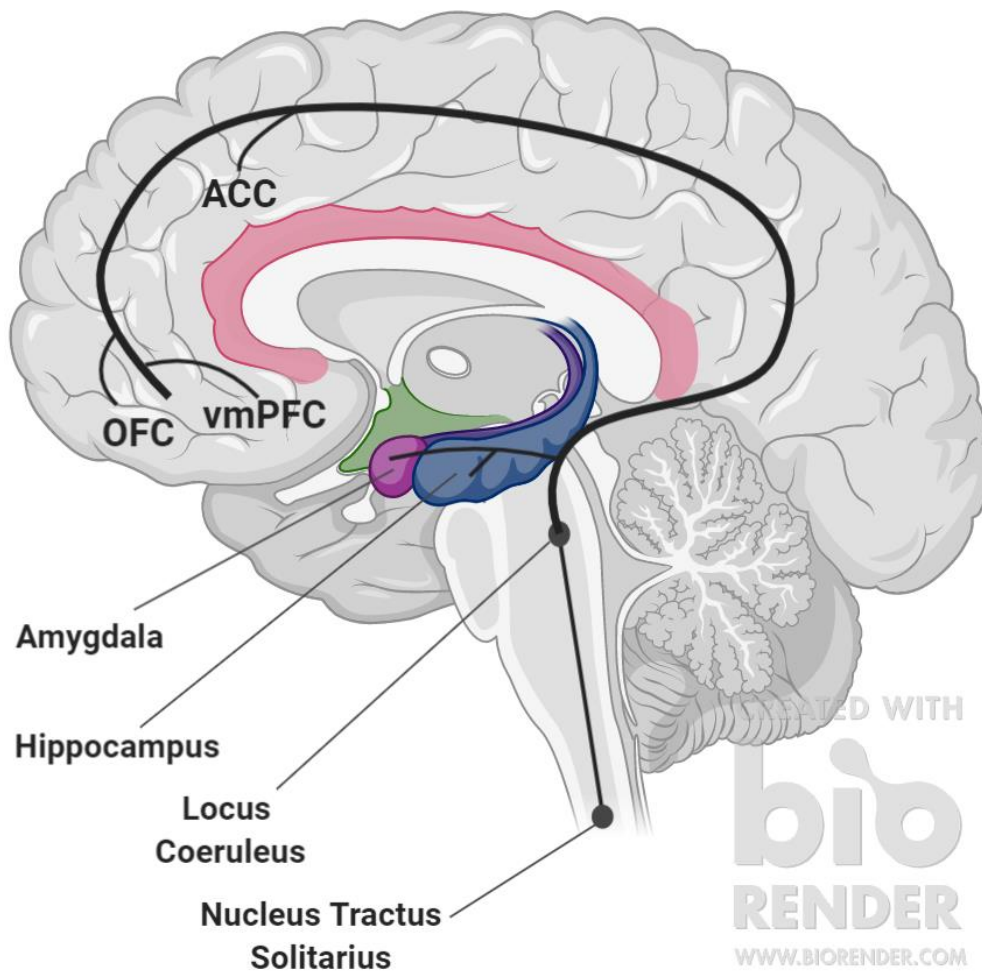
Figure 1. Visual representation of the LC-NE system in the human brain (created on https://biorender.com/). ACC: anterior cingulate cortex; OFC: orbito-frontal cortex; vmPFC: ventro-medial prefrontal cortex

The LC is the only structure controlling the release of norepinephrine (NE) in the cortex and, by modulating the availability of dopamine (DA), glutamate and gamma-aminobutyric acid (GABA) at specific sites (Mather et al., 2016), it has an indirect but relevant role in influencing sensory perception, attention, memory and executive functions (Sara & Bouret, 2012). More specifically, the LC has a primary role in the regulation of the diurnal sleep-wakefulness cycle, showing increased synchronised neural firing during waking, a reduction of neural activity during

drowsiness and sleepiness, and almost absent neuronal activity during the deepest stages of sleep (Aston-Jones & Bloom, 1981). Fluctuations in baseline *tonic* activity of the LC are slow and are accompanied by fluctuations in cortical arousal (Howells et al., 2012). During wakefulness and alertness, LC neurons fire at low-frequencies (usually in the range 1-3 Hz; less than 2 Hz during quiet waking and around 2-3 Hz during active wakefulness, see Figure 2), constantly releasing NE to the cortex and therefore facilitating exploratory behaviours reflecting a general state of alertness and the search for rewarding stimuli in the environment.
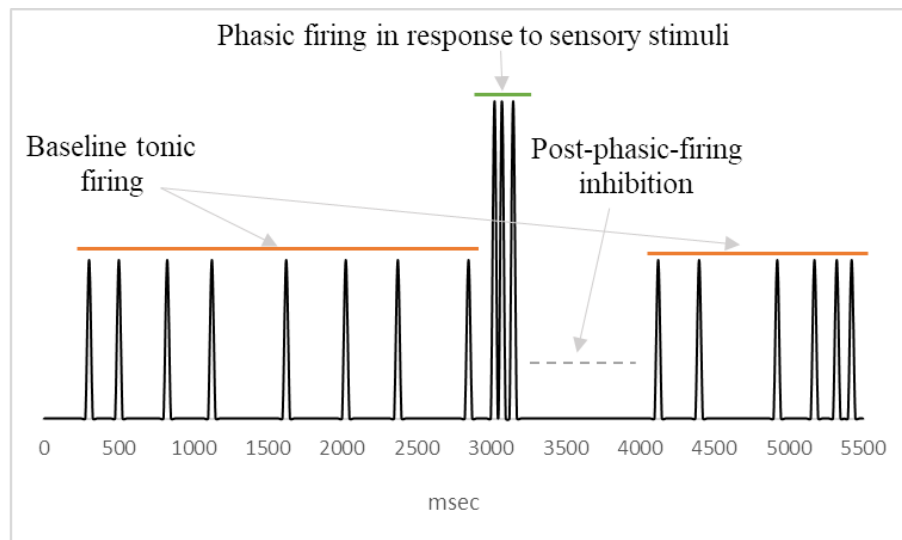
Figure 2. Simplified visual representation of LC neurons' baseline tonic firing (in orange), phasic firing in reaction to an incoming sensory stimulus (in green) and inhibition after phasic discharge (dashed line).

The LC is also part of a wider attentional system that is responsible for driving the orienting of attention towards sensory input from the environment. More specifically, a subcortical pathway comprised of LC, superior colliculi, thalamus,

ventral striatum and amygdala, interacts with the anterior cingulate cortex (ACC) and anterior insula (AI) to rapidly evaluate the salience of incoming sensory information, even before it reaches perceptual awareness (i.e., in the first 150 msec from stimulus onset; Pissiota et al., 2003, Joshi et al., 2016). During this tiny temporal window, the activation of subcortical and brainstem structures is modulated by the bottom-up characteristics of sensory stimulus. In parallel, the ACC, the AI and the ventral striatum are responsible for quick detection of any salient features of sensory information and any associated rewards. If the sensory stimulus is recognised as salient or interesting to be further processed, the LC receives top-down input from the ACC and the AI, and its neurons display an intense burst of activity at higher frequencies (10-20 Hz), which causes an immediate phasic release of NE and changes in autonomic activity, such as heart rate accelerations, pupil dilations and changes in electro-dermal activity (Sara & Bouret, 2012).

These autonomic reactions parallel the activation of fronto-parietal attentional systems and have a concurrent role in orienting attention towards the sensory stimuli that have been evaluated as salient and have triggered a phasic response of LC neurons (Orekhova & Stroganova, 2014). Phasic discharges of LC neurons are very short (usually lasting 200-300 msec) and are followed by a temporary inhibition of synchronised firing (300-700 msec), before neurons start firing again at 1-3 Hz (Sara & Bouret, 2012; see Figure 2). If a sensory stimulus is neutral (for example, without any positive or negative valence) or if it has not been previously associated with a reward/reinforcement, the phasic response of the LC rapidly habituates, i.e., the response is maximum over the first presentation of the sensory stimulus but gradually decreases to consecutive presentations of the same stimulus. However, as discussed in more detail in the next paragraphs, if the sensory stimulus has salient or task-relevant

characteristics, it is relevant for carrying out a specific activity, or it is associated with a reward, LC neurons display a more consolidated phasic response which tends to disintegrate less quickly (Sara & Bouret, 2012).

Phasic responses of LC neurons are partly dependent on tonic baseline activity of the LC (Berridge & Waterhouse, 2003). More specifically, during states characterised by behavioural drowsiness and reduced vigilance, tonic activity of the LC is reduced, and sensory stimuli might not be able to trigger a sufficient phasic response of LC neurons, causing reduced allocation of attentional resources to the environment. Similarly, in situations when tonic activity is increased, such as during excessive alertness or physiological stress, phasic responsivity of LC neurons is less specific for relevant stimuli, since the baseline threshold of activity is already high and phasic reactivity of LC is triggered by any stimulus in the environment (Howells et al., 2012). This partially resembles the Yerkes and Dodson' law (1908) which proposed that either too increased or reduced levels of autonomic arousal would affect task-directed behaviours.

1.2.3. The relationship between the LC-NE and pre-frontal systems: the adaptive-gain theory

While the LC has a role in modulating cortical arousal to maintain general wakefulness/alertness and facilitate processing of sensory information, frontal systems retro-actively influence LC activity and reactivity based on context-related information (Sara & Bouret, 2012). The interaction between the LC-NE and frontal systems is therefore likely to be crucial in cognitive control, i.e., the ability of regulating behaviour and attention mechanisms according to environmental demands.

Norepinephrine, together with dopamine, has a neuro-modulatory effect on the PFC, and acts by increasing the *signal-to-noise* ratio of neural activity in the frontal systems, therefore facilitating transient reorganisation and strengthening of functional connectivity in systems responsible for cognitive, attentional and executive functions ('adaptive gain theory'; Aston-Jones et al., 2000; Aston-Jones & Cohen, 2005). During active wakefulness, the LC should be sufficiently reactive to any environmental sensory stimulation, facilitating the *exploration* of those stimuli that may be salient or rewarding. During cognitive or attentional tasks, instead, the LC shall specifically respond to task-relevant information, so that it could be further processed and *exploited,* while task-irrelevant or distracting information should be ignored or filtered.

Aston-Jones & Cohen (2005) proposed that the LC functions in two main modes which have different characteristics of baseline activity and stimulus-locked reactivity. Understanding how the switch between these two modes happens, might be crucial to understand how LC activity affects cortical systems and is retro-actively affected by changes in contextual and situational demands. According to these authors, the *tonic mode* is characterised by the predominance of increased tonic discharge of LC neurons (i.e., firing at 1-3 Hz) and reduced phasic bursts (10-20 Hz; Figure 3). This modality of functioning of the LC facilitates exploration of the environment and searching for new stimuli or rewards to be exploited, and it is characterised by active wakefulness, increased distractibility, restlessness and sensory over-responsivity (Berridge & Waterhouse, 2003). The *phasic mode* is instead characterised by the predominance of sustained phasic discharge of LC neurons, which facilitates the processing of specific stimuli (usually those which are more salient or associated with the aims of task).
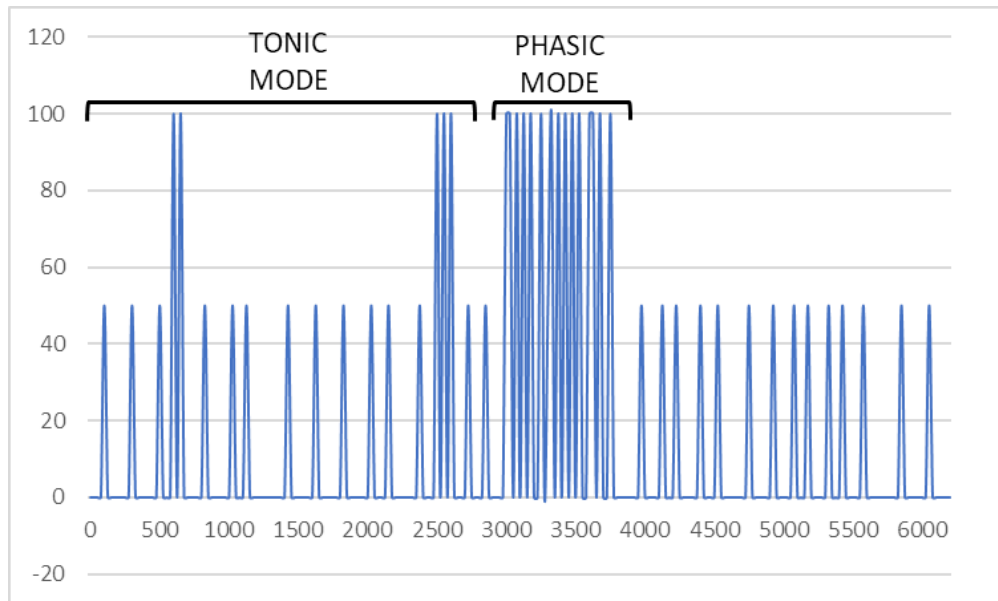
Figure 3. Simplified visual representation of the 'tonic' and 'phasic' modality of

functioning of the LC, as proposed by Aston-Jones & Cohen (2005).

The switch between the tonic and the phasic modes, i.e., between exploration of

the environment (*tonic mode)* and exploitation of resources (*phasic mode)*, should be

dynamic to facilitate efficient identification and processing of relevant and rewarding

sensory information from the surrounding environment, and to extract its crucial

characteristics, hidden significance and learning content without exacerbating the costs

associated with this process. Among the brain structures involved in the transitions

between tonic and phasic LC modes, the orbito-frontal cortex (OFC) and the anterior

cingulate cortex (ACC) are likely to be primarily involved in rapidly evaluating costs

and benefits associated to maintaining or withdrawing from a specific mode. While the

OFC processes inputs from sensory systems and is more active in response to rewards,

due to its connections with the amygdala and ventral striatum, the ACC is primarily

activated during the evaluation of costs (supported by connections with somatosensory

and limbic systems, including insula, ventral striatum and amygdala) (Devinsky et al.,

1995). From another perspective, the OFC is mostly activated by anticipation and delivery of rewards, and the value of the reward is usually proportional to the increase of neural activity in this brain area, which gradually decreases when reward has been obtained and exploited (Critchley & Rolls, 1996; Wallis & Miller, 2003). The ACC, conversely, mostly reacts to aversive and negative sensory stimulation, such as pain (Peyron et al., 2000), but it is also activated by errors in performance, increased task difficulty (for example, during conflict processing and decision-making), loss of rewards and negative feedback (Aston-Jones & Cohen, 2005).

The OFC and the ACC are the pre-frontal regions with most projections to the LC (Chandler et al., 2014), therefore they are likely to be involved in influencing LC activity and in regulating the switch between the tonic and the phasic modes. For example, when an individual is involved in a cognitive task, the LC is maintained in the phasic mode, probably through top-down modulation by the OFC and the ACC. Processing of task-related information in this situation is in fact rewarding and overcomes the costs associated with the use of attentional and cognitive resources. However, as soon as allocating attentional effort to the task is not rewarding anymore, which is behaviourally represented by temporary inattention, distractibility, sleepiness and worsening of performance (increased number of errors or slower response), the evaluation of benefits and costs moves to a different level, which has been theorised through a computational model by Aston-Jones and Cohen (2005).

Although the theories about LC functioning would predict that, at this point, the tonic mode shall become predominant to facilitate disengagement from the task and exploration of environmental resources, this only happens when the task utility is low both at short-term (within seconds) and long-term (minutes), for example when the task is not engaging or it has not brought any benefits to the individual so far. Similarly,

situations characterised by high short term utility, including quick achievement of rewards, but low long-term utility, are likely to promote the predominance of the tonic mode, since it is not beneficial to invest attentional and cognitive resources in an activity that will probably result disadvantageous on the long-term (Cohen et al., 2007). The switch from the phasic to the tonic mode is behaviourally characterised by increased motor movements and exploratory behaviours, and it has been shown to be anticipated by decreased release of NE from the LC to pre-frontal systems (Brennan & Arnsten, 2008).

On the other hand, when long-term task utility is high, and people are aware of the costs but are also conscious of the benefits resulting from maintaining attentional and cognitive resources directed to the task, top-down strategies of arousal regulation are activated to maintain arousal at the optimal level, especially when worsening of behavioural performance or attentional lapses are detected. Therefore, when performance monitoring results in the detection of errors, slowing of responses and disengagement from the task, input signals from ACC and OFC to LC trigger a sudden release of norepinephrine with the aim of up-regulating arousal, restoring the phasic mode and consequently increasing alertness and improving performance (Sara & Bouret, 2012).

Summarising, the LC is likely to be involved in basic mechanisms of regulation of arousal and vigilance, with an indirect impact on behaviour, attention and cognitive functions. More specifically, during processing of sensory information, the LC-NE system contributes to maintaining alertness to environmental sensory stimulation and to maintain attention directed towards sensory information to facilitate further processing. Activity of the LC is somehow top-down modulated by pre-frontal structures, including the ACC and the OFC, which are involved in rapid evaluation of

costs and benefits associated to a specific activity and in the transition between the tonic and the phasic modes of LC functioning. Atypical functioning of the LC-NE system, pre-frontal systems, or both, may therefore result in reduced ability to regulate arousal and alertness to contextual demands, with cascading consequences on attentional, cognitive and behavioural processes.

1.3. Studying the relation between arousal and attentional mechanisms in humans

1.3.1. Indirect measures of activity and reactivity of the autonomic nervous system

While direct measurement of ANS functioning, e.g., through single-unit recording, is widely used in animals, investigating autonomic arousal in humans can only be achieved through the analyses of peripheral indices of autonomic arousal, such as heart rate, pupil size and electro-dermal activity (Wass et al., 2015). Since electro-dermal activity was not measured in the present study, it will be presented only briefly, before describing in more detail pupil size and heart rate, which have been collected and analysed as measures of ANS functioning in this study.

Electrodermal activity (EDA) is a measurement of the changes in the dilations and constrictions of blood vessels, reflecting changes in activity of the ANS and consequent variations in skin electrical conductance (Wass et al., 2015). While skin conductance level (SCL) is a tonic measure and reflects slow changes in skin conductance over time, non-specific skin conductance responses (ns-SCRs; calculated as differences from the baseline SCL and not measured in response to task-related event or stimulus) and skin conductance responses (SCRs; changes in skin conductance, compared to the baseline SCL and associated to a specific event or stimulus) are indices of phasic reactivity of the ANS.

Usually measured through electrocardiogram (ECG), heart rate (HR) is primarily calculated as the average number of heart beats per minute (BPM). By analysing the time between cardiac beats, i.e., the inter-beats interval (IBI), it is possible to obtain a measure of the fluctuations in heart rate, namely Heart Rate Variability

(HRV). HRV is an index of the activation of both the sympathetic and parasympathetic branches of the ANS, so that increases in heart rate (accelerations) are triggered by the sympathetic branch when energetic resources should be quickly mobilised, while the parasympathetic branch facilitates heart rate decelerations during active processing of sensory information (Wass et al., 2015). In line with the theoretical approaches which suggest a direct association between regulation of arousal and cognitive-attentional regulatory mechanisms (Aston-Jones & Cohen, 2005; Yerkes & Dodson, 1908), it has been demonstrated that higher HRV is positively associated with sustained attention (Suess et al., 1994), behavioural inhibition (Porges, 2007; 2009) and emotional regulation (Gentzler et al., 2009). Furthermore, it has been suggested that heart rate may be directly influenced by the LC. More specifically, the LC-NE system has been found to have an excitatory effect on cardiac muscles, activating the sympathetic branch of the ANS (Wang et al., 2014) and inhibiting the effect of the PNS (Samuels & Szabadi, 2008). Different time-domain measures can be extracted from raw heart rate data, among which the Root Mean Square of Successive Differences (RMSSD) is a very reliable measure of parasympathetically-mediated HRV (Shaffer & Ginsberg, 2017). In addition to RMSSD, Toichi et al. (1997) proposed the use of two estimative indices of activity and tone of the sympathetic and parasympathetic branches of the ANS, namely the Cardiac Sympathetic Index (CSI) and the Cardiac Vagal Index (CVI) (which will be further discussed and explained in paragraph 2.2.6.2).

Pupil size (PS) is likely to be influenced by both the sympathetic and the parasympathetic branches of the ANS (Bast et al., 2018) and it represents an indirect index of the activation of the LC. In fact, although a direct anatomical pathway between the LC and the motor systems responsible for pupil dilations and constrictions has not been clearly identified (Nieuwenhuis et al., 2011), electrophysiological and imaging

studies have shown a direct correlation between activity of LC neurons and pupil dilations (Rajkowski, 1993; Murphy et al., 2014). The activation of the SNS, together with increased release of NE by the LC, is likely to trigger pupil dilations, while the activation of the PNS might be more likely to elicit pupil constrictions, for example in cognitive and mentally demanding activities. When the LC tonic mode is predominant, for example during exploration of the environment, baseline pupil size is increased and the variability of changes in pupil size is reduced (Gilzenrat et al., 2010). On the opposite, when the LC phasic mode is predominant, baseline pupil size might be reduced and the variability in pupil size dilations might be increased, reflecting increased phasic responsivity to task-relevant stimuli.

Changes in pupil size have also been found associated with mental effort (van der Wel & van Steenbergen, 2018). An increase in pupil size, for example, is likely to accompany increases in cognitive effort and sustained attention to the task. More importantly, it has been shown that disengagement from a task and sudden worsening of performance (Jepma & Nieuwenhuis, 2011) is likely to be preceded by increased baseline pupil diameter and reduced number of pupil dilations/constrictions (high-tonic/low-phasic mode). Moreover, it has been shown that baseline pupil size, measured before the presentation of a target visual stimulus, could predict accuracy and speed of the motor behaviours in response to the target stimuli (Gilzenrat et al., 2010; Murphy et al., 2011), partly supporting the presence of an inverted U-shaped relation between tonic arousal (indexed by pupil size) and motor behaviours. Baseline pupil size could therefore be used to track fluctuations in activity of LC neurons (Bast et al., 2018).

1.3.2. Using eye-tracking techniques to track alertness and orienting of attention

Eye-tracking is a non-invasive technique which is widely used to record ocular behaviour, including eye movements (i.e., saccades), fixations, blinks and pupil size. Through eye-tracking, it is possible to investigate the involvement of different neural systems in attentional and vigilance mechanisms. Eye-trackers generally work by directing an infra-red-light source towards the eyes, while a camera records the reflection on the cornea, allowing to track eye gaze behaviour. This technique has been found useful to test samples of children with different levels of functioning, including young children and children with neurodevelopmental conditions, where other types of methodologies, e.g., fMRI, would be more difficult to be used.

It has already been discussed (see paragraph 1.3.1) how pupil size is likely to reflect mechanisms of vigilance and attention, indirectly reflecting LC activity. Besides measuring pupil size, eye-tracking can also be used to investigate mechanisms of orienting of visual attention. The main outcome measures which are obtainable through eye-tracking recordings, in fact, are topographical and physical characteristic of eye gaze behaviour, including duration of fixations and latencies of eye gaze movements, and pupil size. Orienting of visual attention may be subdivided in three temporally consecutive components, i.e., 'disengagement', 'shifting' and 're-orienting' (Posner & Petersen, 1990), and may occur exogenously (as an eye movement triggered by the onset of a visual object) or endogenously (as a voluntary eye movement from one visual stimulus towards another). Specifically, the dorsal attentional network, including the anterior cingulate cortex (ACC), basal ganglia, temporoparietal junction (TPJ), Intra-Parietal Sulcus/Superior Parietal Lobe (IPS-SPL) and frontal eye fields (FEFs), is responsible for voluntary, endogenous disengagement of attention and programming of visual saccades (Corbetta & Shulman, 2002). The fronto-parietal ventral attentional

network, conversely, is responsible for exogenous disengagement and reflexive orienting of attention, where saccades are elicited by specific properties of visual stimuli (Godijn & Theeuwes, 2002). This system is comprised of right superior parietal cortex, temporal-parietal junction, vmPFC, anterior insula, pulvinar nucleus of the thalamus and superior colliculi (SC) (Corbetta & Shulman, 2002; Posner & Petersen, 1990; Petersen & Posner, 2012).

The SC is organised as a retinotopic map of the visual field, so that the onset of a visual stimulus in a specific location of the visual field elicits the activation of specific SC neurons, namely those associated to the retinotopic location where the stimulus has appeared. During fixation of a visual stimulus, for example, SC neurons associated with the foveal areas are activated, while firing of neurons in other areas of the retinotopic map is inhibited. When a second stimulus appear, a saccade toward that visual object is triggered by the activation of SC neurons associated with the retinotopic location of the new stimulus, but only when this activation overcomes a certain threshold (Godijn & Theeuwes, 2002). When considering this model within the theoretical frameworks of LC functioning, presented in paragraph 1.2, it could be speculated that the visual attentional span might be broader during exploration of the environment, and eye movements shall happen more frequently to facilitate orienting of visual attention to different locations of the visual field. In this situation, less effort should be paid to maintaining fixations, and quicker reflexive stimulus-driven saccades should be prioritised. Conversely, during exploitation of information, the visual attentional span should be narrow to facilitate focused attention on the sensory information that should be processed thoroughly.

Both reflexive and voluntary mechanisms of visual attention orienting are fundamental for efficiently processing the sensory characteristics of the surrounding

environment, and, when atypical, they have been found linked with atypical development of arousal regulation strategies (Harman et al., 1997; Posner & Rothbart, 1998). For example, before 3 or 4 months of age, the prolonged exposure to the same visual object is associated with increased physiological stress and negative emotional reactivity (Ruff & Rothbart, 1996). Orienting of attention away from a distressing stimulus is therefore used as a distress regulator by infants, before learning and implementing higher-level cognitive strategies of arousal regulation (Harman et al., 1997; Posner & Rothbart, 1998). Early malfunctions of attention orienting mechanisms have been considered as possible precursors of traits of ADHD (Shaw et al., 2014) and ASD (Zwaigenbaum & Penner, 2018). For example, it has been showed that less effective and dynamic orienting of attention, during the first year of age, is more associated with negative temperamental emotionality (Johnson et al., 1991; Rothbart et al., 1992), which has been associated with ADHD and ASD, later in the development (Visser et al., 2016).

1.3.3. Measuring indices of brain activity to investigate orienting of attention, sustained attention and executive functions

Electro-encephalography (EEG), a technique designed by Hans Berger in the first half of the 20th century to measure synchronised activity of localised groups of neurons through the recording of electrical signals on the scalp, has been widely used in cognitive neuroscience for studying brain functioning in relation to perceptual, cognitive and attentional mechanisms. A traditional approach to EEG data analysis is to investigate the temporal fluctuations (in range of milliseconds) of the activation of neural systems in response to specific events, i.e., Event-Related Potentials (ERPs; see

Sur and Sinha, 2009, for an overview). ERPs are very small changes in scalp electric voltage reflecting the synchronised activity of post-synaptic potentials produced by localised groups of cortical pyramidal neurons (Peterson et al., 1995). They are time-locked to a specific event, such as the onset of a sensory stimulus, and calculated as changes in electrical voltage compared to a baseline period that usually ranges between 100 or 200 msec before the stimulus' onset.

While early ERP components (< 100/150 msec after the event onset) are likely to reflect basic mechanisms of alertness and processing of physical features of sensory information, components detectable between 100/150 msec and 600/700 msec after the event onset are likely to represent higher-level cognitive and attentional mechanisms of information processing. Early ERP components (such as the N1 or the P1) indirectly reflect alertness and vigilance (Sur & Sinha, 2009) but are also influenced by top-down strategies that down-regulate the responsivity to distracting information in order to prioritise task-relevant information (Gaspelin & Luck, 2019). A later ERP component, the P3, has been hypothesised to reflect the activation of the LC-NE system in response to sensory stimulation. Nieuwenhuis et al. (2005; 2011), for example, have presented evidence of the involvement of the LC-NE system in the generation of the P3, therefore suggesting that analysing its amplitude and latency may be a useful method to indirectly track LC phasic responses.

Another approach to the analysis of EEG recordings is the spectral decomposition of the EEG signal, which involves calculating the distribution of power of the signal across different frequencies of interest, usually delta (0.5 – 4 Hz), theta (4 – 8 Hz), alpha (8 – 12 Hz) and beta (12 – 40 Hz). Power spectrum analysis can be carried out through stimulus-locked time-frequency analysis, i.e., analysing changes in the distribution of spectral power in relation to the presentation of a sensory stimulus

(similarly to ERPs), or over longer periods of time, when no specific event is experimentally manipulated, to observe spontaneous neuronal oscillatory behaviour.

When a person is not specifically involved in a task or activity and the environmental sensory stimulation is minimum, such as during breaks from an active task, the presence of alpha oscillations is likely to be linked with processing of internal information, such as memories or thoughts (Smallwood & Schooler, 2015). A group of brain regions, including the medial prefrontal cortex (mPFC), the posterior cingulate cortex (PCC) and inferior, medial and lateral parietal cortices, usually referred to as the Default Mode Network (DMN; Buckner et al., 2008), show maximal synchronised neuronal activation during these situations. However, voluntary suppression of the DMN by fronto-parietal executive systems is fundamental, during mentally demanding tasks, to efficiently sustain attention and perform well to the task (Liddle et al., 2011). Activity of the DMN and alpha have been found correlated in task- and resting-situations, so that when the individual is required to direct attentional resources to the processing of sensory stimuli, the presence of alpha oscillatory rhythms in visual cortical areas is associated with increased excitability of cortical regions that have a role in processing task-relevant information, i.e., fronto-parietal executive systems, and decreased activation of systems responsible for processing distracting information, including the DMN (Van Diepen et al., 2019). While the expectation of task-relevant information is characterised by increased alpha over occipital areas (*alpha synchronisation*) which should be associated with more effective filtering of distracting and irrelevant stimulus, alpha activity decreases after the onset of task-relevant stimuli (*alpha desynchronization*) and this is likely to be associated with increased orienting of attention and information processing (Klimesch et al., 2007).

Some studies tried to disentangle the relationships between brain activity and ANS functioning, converging on the idea that activity of the CNS and the ANS might change in parallel and mirror different states of arousal. It has been shown, for example, that brain activity (investigated by focusing on oscillations in different frequency range) paralleled fluctuations in cardiac activity during sleep (de Zambotti et al. 2018). Moreover, the decrease in vigilance before sleep onset was found associated with gradually reduced mean HR and SCL, which would reflect reduced activity of the ANS (Huang et al., 2018). During resting-state, reduced SCL was found associated with increased alpha power at eyes-closed, while a decrease in alpha power and an increase in SCL was reported after the eyes were opened (Barry et al., 2005; 2007; 2008). Overall, studying both oscillatory patterns and stimulus-locked changes in brain activity, might elucidate our knowledge about mechanisms of attention orienting, executive functioning and arousal.

1.4. Autonomic arousal, attentional, cognitive and behavioural mechanisms in individuals with ADHD and ASD

The developmental neuro-constructivist approach, introduced by Karmiloff-Smith (2009), theorises that human development is mainly influenced by interactions between specialising brain structures and systems, and the environment. The ability to regulate arousal, for example, develops rapidly during the first year of life and continues to improve until late adolescence (Calkins, 2007). Primitive self-regulation strategies, which are used to reach and maintain an optimal physiological state, can already be seen in 2/3-month-old infants, who are able to self-calm using a pacifier or when hugged by parents (Berger et al., 2007). However, the typical development of these regulatory mechanisms, which are fundamental for the emergence of higher-level strategies of behaviour regulation and to efficiently carry out everyday activities, depends on the development of structural and functional interactions between three main brain systems: a) the brainstem, b) the limbic system and c) cortical systems (Geva & Feldman, 2008; 2017). The vertical-integrative model by Geva & Feldman (2008) suggests that atypical pre-natal structural development of brainstem systems might lead to short- and long-term consequences on development, including:

- physiological dysregulation and atypical sensory processing in the first weeks of life;

- physiological and emotional distress in the first year of life;

- atypical maturation of fronto-limbic systems;

- development of maladaptive strategies of regulation and control of behavioural, attentional and socio-cognitive mechanisms.

If the LC-NE system is functionally atypical from the earliest stages of life in infants and children later developing ADHD and ASD, this might give rise to early inattention (which has been shown to be an early pathway to both conditions; Johnson et al., 2015; Visser et al., 2014), dysregulated autonomic arousal, reduced reactivity or over-responsivity to sensory stimulation. This may in turn affect the development of structural and functional connections between brainstem and frontal systems, with consequent less efficient modulation of arousal and attentional mechanisms by frontal systems, such as the PFC. Maladaptive strategies of arousal regulation, including motor hyper-activity, restlessness and fidgetiness, reduced exploratory behaviours and stereotypies, might therefore emerge as a consequence.

Summarising, atypical functioning of the LC-NE system from the very beginning of life might contribute to the emergence of non-specific precursors of ADHD and ASD, even before the onset of clinical symptoms (Geva & Feldman 2008; Geva et al., 2017: Keehn et al., 2013; Visser et al., 2016). While these theories are interesting and would merit further discussion, imaging of the LC or investigation of its neural activity has been proven difficult, especially in younger people (Liu et al., 2017). Different techniques, including measurement of heart rate, pupil size, eye-tracking and EEG (see paragraph 1.3), can be used to assess and track activity in systems directly or indirectly involved in arousal and attention regulation, and cognitive control, and might help to clarify the basic mechanisms underlying these processes in children with ADHD and/or ASD. A summary of results from previous studies that investigated these mechanisms in children with ADHD-only, ASD-only and co-occurring ADHD+ASD, will be now presented, before discussing the specific research questions of the study.

1.4.1. Autonomic arousal, vigilance and alertness

Signs of dysregulated arousal have been found in individuals with ADHD, including sleep disorders (Hvolby, 2015), emotional dysregulation (Faraone et al., 2019) and problems regulating appetite (Hanc & Cortese, 2018). These atypicalities are likely to be present even before the emergence of clinical symptoms of ADHD: infants later diagnosed with ADHD have in fact been found to show sleep problems (Vélez-Galarraga et al., 2016), increased negative emotional reactivity (Isaksson et al., 2012) and reduced exploratory behaviours (Auerbach et al., 2004; 2008).

Different theoretical models, including Geissler et al. (2014), Kuntsi and Klein (2012) and Sergeant (2000), proposed that reduced alertness and vigilance, paralleled by insufficient allocation of attentional resources to the environment, are core deficits of ADHD symptomatology and may partly underlie higher-level behavioural and cognitive deficits. It has been speculated that LC neurons might fire at slightly lower frequencies in people with ADHD, causing chronically reduced tonic release of norepinephrine and cascading negative effects on the LC phasic response (Aston-Jones et al., 2000; 2007; Howells et al., 2012). More specifically, if the tonic firing of LC is insufficiently efficient in modulating the release of norepinephrine to different neural systems, exploration of sensory information might be reduced, causing states of inattention and reduced vigilance. Hyperactivity and restlessness might therefore be strategies that help people with ADHD to compensate for under-reactive alertness and vigilance systems. If these models were proved true, the fact that the tonic mode is prevalent and LC tonically fires at lower frequencies than expected in children with ADHD, might explain the presence of distractibility in this clinical population. The LC might in fact respond non-specifically to both task-relevant and task-irrelevant stimuli, since a lower threshold of sensory stimulation would be necessary to elicit phasic

activation of the LC-NE system and consequent attention orienting to all these stimuli, irrespectively of their relevance to the ongoing activity. The theoretical models presented throughout the present chapter, seem to suggest that people with ADHD might suffer from chronic difficulties in regulating arousal, so that reduced vigilance and inattention might characterise difficulties in exploration and exploitation of information in specific situations, such as during slow-paced or less engaging activities, and hyperactivity and restlessness might be strategies to up-regulate arousal and self-regulate behaviour, but they can also be present in situations where exploration of the surrounding environment is exaggerated, causing distractibility. Difficulties in focusing and sustaining attention, in ADHD, might therefore derive from reduced vigilance and drowsiness, but also from distractibility.

We evaluated the evidence of hypo- or hyper-arousal in ADHD through a systematic review of the literature on functioning of the ANS in ADHD (Bellato et al., 2020). Overall, we found some evidence of ANS dysfunction in individuals with ADHD, more often in the direction of hypo-arousal than hyper-arousal, especially at rest and during cognitive tasks that required sustained attention and response regulation. More specifically, atypical heart rate (HR), electro-dermal activity and pupillometry measures have been found both at baseline and during resting-state, but also in relation to active cognitive tasks, indicating difficulties in regulating arousal to the demands of the context. For example, reduced EDA during resting-state was a relatively consistent finding in our review. Clear differences on measures of ANS functioning, between individuals with ADHD and typically developing controls, have not been reported by studies which used salient stimuli, such as rewards or socio-emotional information, in their experimental paradigms. It might therefore be that people with ADHD benefit from the presence of salient or rewarding sensory stimuli, which help them to regulate

arousal. While methylphenidate (MPH), for example, has been shown to augment the neural activation of fronto-parietal cortical systems (Zimmer, 2017) and to facilitate the deactivation of the DMN in people with ADHD during cognitive tasks (Liddle et al., 2011), similar effects have been found for motivational incentives and salient task-related stimuli (Groom et al., 2013; Liddle et al., 2011). Together, salient and rewarding stimuli, and stimulant medication, may have a positive effect on ANS mechanisms and autonomic arousal, but this has not been tested thoroughly. However, it has been demonstrated that medication for ADHD is likely to have some effects on cardiac measures (Hennissen et al., 2017). We analysed the effects of methylphenidate on measures of ANS functioning, as reported by studies included in our review (Bellato et al., 2020), and found that this medication might have an effect in up-regulating autonomic arousal in people with ADHD, supporting the theoretical models proposing hypo-arousal and reduced vigilance as core atypicalities of ADHD.

Behavioural signs of reduced vigilance and alertness across multiple experimental paradigms have been reported in ADHD, including increased intra-individual reaction time variability (RTV; see Kofler et al., 2013, for a meta-analysis), especially during slow-paced and monotonous cognitive tasks (Metin et al., 2012). Although in some cases performance was not found impaired in ADHD, for example in tasks requiring less mental effort (Borger and van der Meere, 2000) and in presence of rewards or feedback (Groom et al., 2010; Groom et al., 2013; Liddle et al., 2011), these findings indicate that difficulties in maintaining an optimal level of vigilance are experienced by individuals with ADHD, and they are likely to impact higher-level information processing and decision-making. Reduced amplitude and delayed latency of the P3 in response to sensory stimuli have also been reported in ADHD (see Johnstone et al., 2013, for a review), often accompanied by atypicalities in early ERP

components, including reduced N1 and P2. Summarising, these findings indicate that people with ADHD are more likely to display physiological, behavioural and electrophysiological indices of hypo-arousal, reduced vigilance and difficulties in sustaining and regulation attention.

Indices of both autonomic hypo- and hyper-arousal have been reported in people with ASD (Keehn et al., 2013; Lydon et al., 2016), but more recent studies seem to converge towards suggesting the presence of states of hyper-arousal in ASD, which would be opposite to what has been found for ADHD. For example, increased heart rate variability, decreased activation of the PNS and reduced vagal tone (Klusek et al., 2015), atypical sensory processing (Robertson & Baron-Cohen, 2017), higher levels of cortisol (Corbett & Simon, 2014) as well as increased pupil diameter and increased skin conductance responses (Orekhova & Stroganova, 2014), have been reported in people with ASD. Although results are heterogeneous, findings of states of hyper-arousal and increased autonomic responsivity in ASD would contrast with evidence of hypo-arousal and reduced vigilance in ADHD.

Individuals with co-occurring ADHD+ASD have been found to display behavioural atypicalities indicating reduced vigilance (such as increased intra-individual RTV), but these were specifically associated with ADHD symptomatology, and not ASD (Adamo et al., 2019; Karalunas et al., 2014; Lundervold et al., 2016; Tye et al., 2016). A recent meta-analysis by Cui et al. (2016) showed that clear findings of reduced or heightened brain responsivity to sensory stimuli (specifically reflected in the P3) could not be found in ASD. Conversely, deficits in vigilance and attention allocation to sensory stimuli (i.e., reduced P3 amplitude) were found in children with

co-occurring ADHD+ASD (Tye et al., 2014), but these might be more likely associated with ADHD symptomatology than ASD.

Findings indicating that alpha oscillatory activity is reduced at rest in ASD and, similarly, in ADHD (see Newson and Thiagarajan, 2018, for a review), suggest that this may be a common and shared atypicality. Some other studies found that people with ADHD- and ASD-only showed reduced alpha desynchronization (i.e., increased alpha power) in response to task-relevant stimuli (Keehn et al., 2017; Lenartowicz et al., 2018) and this predicted worse task performance (longer RTs and reduced task accuracy). Few studies investigated alpha oscillations in people with co-occurring ADHD+ASD: Shephard et al. (2018), for example, found an atypical resting-state neurophysiological profile in the comorbid group, which supported the additive model of ADHD/ASD comorbidity.

In summary, while ADHD symptomatology is more likely to be associated with the presence of behavioural and physiological indices of reduced alertness and vigilance, ASD and ADHD might have an interactive effect on measures of autonomic arousal. In fact, since quite opposite findings have been reported in the single conditions, it should be tested if empirical data support the additive or the interactive model of ADHD/ASD comorbidity. Therefore, in the first investigation of the present study, I aimed to:

   a) Verify that indices of atypically reduced vigilance and alertness are found in children with ADHD and children with co-occurring ADHD+ASD, and are mostly related with ADHD symptomatology;

b) Verify that signs of reduced autonomic arousal are found in children with ADHD-only:

c) Test if children with comorbid ADHD+ASD show an additive profile of atypical measures of ANS functioning (both indices of hypo-arousal, as children with ADHD-only, and hyper-arousal, like children with ASD-only) or interactive effects (presumably, a *compensation* of indices of ANS activity, when compared to children with ADHD- and ASD-only).

1.4.2. Visual attention

While research about visual attention mechanisms in ASD is wide and converges towards the presence of specific deficits, this research area has not widely been explored in ADHD literature. ASD has been found associated with specific atypicalities in orienting of visual attention, from the earliest stages of development (Elsabbagh et al., 2013), such as more fragmented saccadic pathways and slower initiation of eye movements (Keehn et al., 2013), less accurate and slower orienting of attention towards visual stimuli presented in the peripheral visual fields (Townsend et al., 2001; Wainwright & Bryson, 2002). Moreover, difficulties in controlling visual attention, such as slower disengagement and re-orienting of attention, have been widely reported in ASD (Elsabbagh et al., 2013; Keehn et al., 2013; Sacrey et al., 2014) and have been proposed to be related to reduced activation of the ventral attentional network and cerebellar cortical regions (Keehn et al., 2016).

A meta-analysis by Huang-Pollock and Nigg (2003) concluded that visuo-spatial mechanisms of attention orienting seem not dysfunctional, per se, in ADHD. However, reduced activity in higher-level neural systems involved in visual attention

have been found in people with this condition (Amso & Scerif, 2015; Cortese et al., 2012; Hart et al., 2013), making some authors to speculate that atypicalities in visual attention might instead derive from general difficulties in regulating vigilance and alertness (as reported in paragraph 1.4.1), and difficulties in saccade preparation and attentional control (Ortega et al., 2013). In ADHD, if exploratory behaviours are prevalent during attentional tasks, this should elicit a broader attentional span, reduced focused attention on task-relevant stimuli (as proposed by Varela Casal et al., 2019) and possibly faster eye movements. In ASD, instead, hyper-sensitivity and hyper-reactivity might elicit distractibility with quick but less accurate eye movements or, on the opposite, less effective top-down control over oculomotor mechanisms might give rise to slower eye movements.

Few studies investigated visual attention mechanisms in people with co-occurring ADHD+ASD, and found general difficulties in attentional orienting (Lundervold et al., 2016) and atypical orienting of attention to human faces (Groom et al., 2017; Sinzig et al, 2008). However, it would be interested to investigate if children with ASD- and ADHD-only could be differentiated based on measures of reflexive and voluntary visual attention orienting, and at what level any atypicalities reported in the single disorders are present in the comorbid group. The second investigation of the present study is therefore aimed to:

a. Confirm that atypicalities in basic mechanisms of visual attention orienting are more associated with the presence of ASD-symptomatology, and not ADHD;

b. Verify if the co-occurring presence of ADHD and ASD have an interactive effect (giving rise to a separate profile of atypicalities in visual attention in children with ADHD+ASD, when compared to the single disorders) or if the

additive model would be more supported by our data (i.e., children with comorbid ADHD+ASD would display the same atypicalities found in children with ADHD- and ASD-only).

1.4.3. Executive functions

Findings from neuroimaging studies indicate that atypical functioning of fronto-striatal and fronto-parietal systems are similarly present in people with ADHD (Cortese et al., 2012; Rubia, 2018) and ASD (Delmonte et al., 2013). More specifically, reduced cortical surface in frontal, cingulate, and temporal regions involved in executive function, have been reported in ADHD (Hoogman et al., 2019). Interestingly, a review of neuroimaging studies by Rommelse and colleagues (2017) concluded that the co-occurring presence of ADHD and ASD, compared to the presence of just one condition, seems to have a more impactful effect on the structural and functional development of frontal systems (including ACC and PFC), with more severe negative outcomes in the development of executive functions, conflict monitoring and cognitive control abilities.

When investigated separately, ADHD and ASD have been found associated with different atypicalities in executive functions. While ADHD seems more characterised by deficits in sustained attention, performance monitoring and response inhibition, deficits in executive function in ASD are instead more characterised by atypicalities in flexibility, conflict monitoring, task switching and planning (Geurts et al., 2014; Panerai et al., 2016). However, the debate about this research topic is still open, especially because it is not clear if executive function deficits reported in ADHD and ASD, when taken separately, are the same atypicalities which are found in children with co-occurring ADHD+ASD. In fact, it may be that increased structural and functional

atypicalities in higher-level neural systems, separately related to ADHD and ASD symptomatology, cause exacerbated executive function deficits in people with comorbid ADHD+ASD, compared to those with ADHD- or ASD-only (Rommelse et al., 2017). The third investigation of the present study is therefore aimed to:

a) Investigate if electrophysiological and behavioural measures of executive functions and cognitive control were differently associated with ADHD and ASD-symptomatology;

b) Investigate if executive function deficits in individuals with comorbid ADHD+ASD are better explained by the additive model of ADHD/ASD comorbidity (similar atypicalities like ADHD- and ASD-only, but not at a different level) or the interactive model (separate profile with more exacerbated executive function deficits).

1.5. Aims of the study

A battery of experimental paradigms (presented in detail in paragraph 2.2.3) was designed to collect empirical data and investigate mechanisms of autonomic arousal, vigilance, alertness, visual attention and executive functions, in a sample of children and adolescents with ADHD and/or ASD. I analysed the association between indices of autonomic hypo-arousal, reduced vigilance and alertness, and ADHD symptomatology, and tested if the additive or the interactive model of ADHD/ASD comorbidity was more supported by the data (paragraph 3.1). Secondly, I investigated if visual attention orienting mechanisms were differentially affected by ADHD and ASD, with more impairments associated with ASD- than ADHD-symptomatology (paragraph 3.2). Lastly, I analysed behavioural and electrophysiological indices of executive functions and cognitive control and their association with ADHD and ASD-symptomatology, investigating at what level these atypicalities were present in children with co-occurring ADHD+ASD, and which model (additive or interactive) was better supported by the data (paragraph 3.3).

Chapter 2.   The SAAND study

2.1.   Study characteristics, sample, recruitment and ethical approval

The SAAND study (Studying Attention and Arousal in children and adolescents with Neurodevelopmental Disorders) is a research study conducted at the University of Nottingham (UK) by Dr Maddie Groom (Chief Investigator), Dr Danielle Ropar, Prof Chris Hollis, Dr Puja Kochhar, Iti Arora and Alessio Bellato. The study was approved by the National Research Ethics Committee (REC reference 17/EM/0193) and the Health Research Authority (HRA; IRAS study ID 220158; date of approval: 16th August 2017; amendments: February and August 2018) (https://www.hra.nhs.uk/planning-and-improving-research/application-summaries/research-summaries/saand-project-attention-and-arousal-in-neurodevelopmental-disorders/).

Funding was obtained by the following sources:

- University of Nottingham (Vice Chancellor's Scholarship for Research Excellence) funded Alessio Bellato (AB) doctoral stipend
- The Waterloo Foundation – Child Development Fund [grant number 980-365] funded part of the research costs for the study, attendance to conferences for dissemination of results and open access fees for publications
- The Baily Thomas Charitable Fund funded Iti Arora (IA) doctoral stipend and part of the research costs of the study
- The National Institute for Health Research Nottingham Biomedical Research Centre Mental Health & Technology Theme [grant number BRC-1215-20003] funded attendance to conferences for dissemination of results and open access fees for publications.

2.1.1. Recruitment and sample size

Children between 7 and 15 years of age, their parents and teachers, were recruited in the study between September 2017 and March 2019. Informed written consent was obtained from the parent/legal guardian of each child, together with the child's written assent to take part to the study, while the teachers gave informed written consent after the testing session with the child but before filling the questionnaires regarding the child's behaviour. All personal data have been stored in accordance with the Data Protection Act 2018. The recruitment of children took place by contacting local support groups for families of children with a diagnosis of ADHD. Moreover, child psychiatrists and paediatricians in secondary & tertiary NHS services (Community Paediatric Clinics and Child & Adolescent Mental Health Services) in Nottinghamshire and Derbyshire identified potential participants and provided them with information about the study. In order to recruit control participants, head teachers at local primary and secondary schools were asked to identify pupils between 7 and 15 years of age and send a letter to their parent/legal guardians informing them about the SAAND study. The School of Psychology (University of Nottingham) has been collecting a participant database which consists of typically developing children who have agreed to be contacted about new studies. The managers of this database were contacted, and they agreed to contact families on behalf of the research team, providing information about the SAAND study. If any parent or child was interested in taking part in the study, they could contact the research team to request further information, and an information sheet, together with the informed consent form, were sent to them. Information about the study were also shared on the online social networks Facebook and Twitter, and in a blog published on the Association of Child and Adolescent Mental Health website (www.acamh.org).

A power calculation was conducted before the start of the study (November 2016) to determine the appropriate sample size of participants. Considering that the study involved a battery of different experimental paradigms and all the experimental measures had not previously been investigated together and systematically in children with ADHD, ASD and comorbid ADHD+ASD, it was difficult to derive an appropriate effect size on which to base a power calculation. Based on previous research which implemented the same or similar experimental paradigms and on a-priori power calculations carried out in G*Power (Faul et al., 2007) for the main statistical analyses on the measures of interest (paragraph 2.3), it was determined that a sample size of at least 25 participants per group (ADHD, ASD, comorbid ASD&ADHD and control group of typically developing children; 100 participants in total) would be sufficient to detect medium effect sizes (considering 80% power, 0.05 significance level and 4 groups) on the main outcome of the studies. Therefore, to control for attrition and potential exclusions due to poor quality or incomplete collected data, we aimed to increase this by 20%, giving us a recruitment target of at least 120 children in total, 30 in each group.

2.1.2. Inclusion and exclusion criteria

For the aims of this study, children between 7 and 15 years of age, diagnosed with or under clinical assessment for ADHD and/or ASD, and children between 7 and 15 years of age from the local community, were recruited. Before including the children in the study, their parents/legal guardians had to give informed consent for the child, besides confirming they were happy to complete a set of self-reported questionnaires about their child's behaviour. Children under pharmacological treatment for ADHD

with stimulants, were asked to withdraw the medication for at least 24 hours before the testing session. They were not withdrawn from any other medications.

Participants were excluded from the present study (before starting data collection) if any known neurological problem that would likely influence brain functioning (such as epilepsy or Tourette's syndrome) was reported by their parent during the screening process. Children on non-stimulant medication (for example, atomoxetine) could not take part in the study, because it was not ethically appropriate to remove children from such medication for any period of time. Children were also excluded if they or their parent/legal guardians were unhappy with having stimulant medication being withdrawn for 24 hours prior the testing session, or if they did not speak fluent English. Children recruited as typically developing controls (i.e., whose parents did not report any formal diagnosis or concerns during the screening process) were not included in this study if the rating scales administered to parents suggested the possible presence of any symptoms of ADHD, ASD, Conduct Disorder, Oppositional Defiant Disorder, Tic disorder. Typically developing controls were also excluded if they were siblings of a child with a formal clinical diagnosis of one of these conditions.

2.2. Experimental tasks, clinical assessment and outcome measures

2.2.1. Clinical assessment

Participants were categorised in one of the four experimental groups (typically developing controls; ASD-only; ADHD-only; comorbid ADHD+ASD), by analysing information collected from parents and teachers, and the direct assessment of clinical symptoms of ASD. A diagnosis of ASD and/or ADHD was confirmed using combined information from the clinical measures presented in this paragraph, including:

- Conners' Rating Scales (CRS-3)
- Social Communication Questionnaire (SCQ)
- Autism Diagnostic Observation Schedule (ADOS-2)
- Development and Well-Being Assessment (DAWBA)

2.2.1.1. ADHD symptoms: Conners' Rating Scales, Third Edition (CRS-3)

The evaluation of symptoms of ADHD was primarily derived from the CRS-3 (Conners, 2008), which were completed by children's parents and teachers, and gave, for each participant, a profile of different behavioural symptoms associated with ADHD symptomatology. Besides giving information about problems associated to inattentiveness and hyperactivity/impulsivity, the CRS provide a set of output measures about executive functioning, learning problems, aggression and relations with peers or family members. The manual of the CRS-3 suggests that a cut-off T-score of 65 on these scales is likely to differentiate individuals with behavioural problems associated with clinically significant ADHD symptomatology from those who show non-clinical levels of ADHD-like behaviours (Conners, 2008).

2.2.1.2. ASD symptoms: Social Communication Questionnaire (SCQ)

The SCQ (Berument et al., 1999; Rutter et al., 2003) is a commonly used screening measure of ASD symptomatology, which showed high sensitivity (96%) and specificity (80%) in discriminating between patients showing symptoms of ASD from individuals showing no signs of this condition (Chesnut et al., 2017). Specifically, a total score of 15 on SCQ has been suggested as the threshold to differentiate between people at-risk vs people not-at-risk of ASD (Rutter et al., 2003). Participating children's parent and teacher completed the SCQ: while parents completed the SCQ-Lifetime version, which identifies behavioural signs of ASD during early infancy and childhood, the SCQ-Current version was completed by teachers, who were asked to answer referring to the child's behaviour in the past 3 months.

2.2.1.3. Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)

The ADOS-2 (Lord et al., 2012) is a semi-structured, standardised tool used to indicate the presence of clinical symptoms of ASD in children and adolescents. This measure is widely used in academic and clinical practice, since it has been recognised a gold standard for the diagnostic evaluation of ASD, especially among children and adolescents (Kamp-Becker et al., 2018). It is comprised of different activities, involving play and verbal questioning, but also stimulating social interaction with the examiner. It provides an objective measure of social, communicative, play and stereotyped behaviours which are part of the ASD phenotype. Specifically, the coding of the entire assessment by trained researchers, provides a diagnostic label of ASD, ASD spectrum or 'no autism', according to different cut-offs which are dependent on the ADOS-module and age of participants, but also dimensional scales of different aspects of ASD symptomatology.

2.2.1.4. Development and Well-Being Assessment (DAWBA) and Strengths and Difficulties Questionnaire (SDQ)

The DAWBA (Goodman, Ford, Richards, Gatward, & Meltzer, 2000) is a battery of questionnaires and interviews which was completed by the children's parents and gave a computer-generated summary of prediction for different psychiatric conditions. Within the DAWBA assessment, parents filled the Strengths and Difficulties Questionnaire (Goodman, 2001), which gives a measure of children and adolescents' prosocial behaviours and psychopathology. Computer generated DAWBA diagnostic predictions and SDQ scores, were evaluated and confirmed or overturned by experienced clinical practitioners (CH and PK). The DAWBA has been shown to be effective in discriminating patients showing psychiatric or psychological symptoms from people who did not show any sign of these conditions, with high specificity (89%) and sensitivity (92%) in recognising the presence of clinical signs of psychopathology in children and adolescents (Goodman et al., 2000).

2.2.1.5. Wechsler Abbreviated Scale of Intelligence, Second Edition, (WASI-II)

The WASI-II (Wechsler, 2011) was used to obtain a complete and reliable measure of cognitive functioning across the sample of participating children. The WASI-II is a revision of the WASI, which has been reported to show high validity and reliability (McCrimmon et al., 2012). It includes 4 subtests, assessing verbal (Vocabulary and Similarities sub-tests) and perceptual reasoning (Block Design and Matrix Reasoning sub-test) abilities. Three output measures can be obtained, namely full-scale IQ (FSIQ), verbal comprehension index (VIQ) and perceptual reasoning index (PIQ).

2.2.1.6.   Child Sensory Profile 2

The Child Sensory profile, Second Edition (Dunn, 2014) is a standardized evaluation of sensory processing behaviours in childhood, and it was used to obtain a parent-based measure of children's sensory issues and atypicalities, which could not be noticeable in the experimental setting. Little et al. (2017) used this tool to assess sensory processing mechanisms in children with ASD and ADHD, showing that the Sensory Profile is a reliable and valid measure to compare different sensory processing behaviours of individuals with these conditions and typically developing controls. Information about four characteristics of information processing, associated with sensitivity to sensory stimulation and self-regulation strategies (Dunn, 1997), are the main dimensional outcomes derivable from this tool, as following: seeking, avoiding, sensitivity and registration. Furthermore, information about sensory processing mechanisms is collected from parents regarding child's auditory, visual, touch, and oral sensory modalities, besides patterns of movement and body positioning in the space.

2.2.1.7.   Socio-economic status

A short semi-structured interview, indicated as the first choice by the UK government for both official statistics and academic research, i.e., the National Statistics Socio-economic Classification, NS-SEC (Rose et al., 2005), was carried out during the collection of general information about the children from their parent, with the aim of evaluating the children's family socio-economic status.

2.2.2. Sample characteristics

Overall, a total number of 133 children were recruited for the present study (see Figure 4 for a detailed flowchart). However, 17 participants were excluded after the testing session, for one or more of the following reasons:

a. Some exclusion criteria were fulfilled after the beginning of the testing session (parents reported a genetic condition not disclosed during the screening process, the assessment showed the presence of significant clinical symptoms in typically developing controls, etc.) (9 participants excluded)

b. Assignment of the child to a clinical group was not possible, due to missing information from parents who did not complete the entire set of questionnaires and interviews (4 participants excluded)

c. The testing session could not be started, due to refusal by the participant after giving oral and written consent (4 participants excluded)

In addition, 10 participants were siblings of participants who later were assigned to one of the clinical groups and therefore they could not be assigned to the group of typically developing children, due to shared genetic susceptibility for ADHD or ASD.

Potential participants, whose parents contacted the
research group for information about the SAAND study
(n = 284)

Participants who
gave consent to start
screening process
(n = 173)

Participants not included
(n = 40)
Screening not completed by parents (n = 13)
Participants who met exclusion criteria (n = 27)

Participants who
attended the testing
session
(n = 133)

Participants excluded after the
testing session (n = 27)
Exclusion criteria fulfilled after the testing session
(n = 9)
Assignment of the child to a clinical group not
possible (n =4)
Testing session did not start (n = 4)
Siblings of participants later assigned to a clinical
group (n = 10)

Final sample of
participants
(n = 106)

Figure 4. Flowchart describing the recruitment of participants for the SAAND study

Overall, data were analysed from a sample of 106 children and adolescents
between 7 and 15 years of age (Age: mean = 10.81 years [10 years and 10 months]; SD
= 2.06 years; 70 males, 36 females). All participants had normal or corrected vision and
15 of them (14.2 %) wore glasses during the testing session. Participants were
categorised in four groups based on their profile of clinical symptoms. Thirty-one
children did not present with any clinically relevant symptoms of a psychiatric or
psychological condition; therefore, they have been assigned to the control group of
typically developing participants. Among the remaining 75 children who presented
clinically significant symptoms of ADHD and/or ASD, 24 were assigned a diagnosis of

ADHD (but not ASD), 18 were assigned a diagnosis of ASD (but not ADHD), while 33 met criteria for both conditions and, for this reason, they have been assigned to the comorbid group (ADHD+ASD). Analyses of between-groups differences on clinical and demographic measures were carried out through one-way ANOVAs for each of the main measures of interest (see Table 1). If significant effects emerged, follow-up analyses were carried out to conduct further investigations.

Table 2 summarises, for each group, the number of participants displaying comorbid symptoms of anxiety, depression and conduct disorder/oppositional defiant disorder, as evaluated through the parent-report DAWBA. As reported in Table 1 and in more detail below, the four groups were similar in age ($F_{3,100} = 0.139$; $p = 0.936$), but differed on the main clinical measures collected during the assessment, including the WASI Full IQ score ($F_{3,100} = 5.056$; $p = 0.003$), the SCQ-parent total score ($F_{3,100} = 50.375$; $p < 0.001$), the CRS-3-parent global index ($F_{3,100} = 171.223$; $p < 0.001$), the CRS-3-parent inattention index ($F_{3,100} = 108.083$; $p < 0.001$) and the CRS-3-parent hyperactivity/impulsivity index ($F_{3,100} = 115.563$; $p < 0.001$).

Table 1. Main socio-demographic and clinical characteristics of the sample.

	Sample	Typically Developing (TD)	ADHD-only	ASD-only	ADHD+ ASD	Group differences
N	106	31	24	18	33	--
Males/Females	70/36	18/13	16/8	11/7	25/8	--
Gender ratio (F: M)	1: 1.9	1: 1.4	1: 2	1: 1.6	1: 3.1	--
Age (years) [SD]	10.81 [2.06]	10.89 [2.45]	10.57 [2.25]	10.91 [2.09]	10.86 [1.51]	None
WASI – FSIQ [SD]	107.95 [16.21]	116.26 [13.09]	108.12 [11.65]	104.61 [15.64]	101.85 [19.02]	ADHD+ASD < TD
WASI – VIQ [SD]	107.83 [16.32]	115.00 [12.51]	110.52 [10.69]	103.39 [18.49]	101.44 [18.81]	TD > ASD and ADHD+ASD; ADHD > ADHD+ASD

	Sample	Typically Developing (TD)	ADHD-only	ASD-only	ADHD+ASD	Group differences
WASI – PIQ [SD]	106.34 [16.43]	113.94 [14.06]	103.91 [14.42]	105.78 [15.43]	101.03 [18.36]	TD > ADHD and ADHD+ASD
SCQ – Total score [SD]	14.94 [9.29]	5.10 [7.64]	15.29 [6.83]	19.11 [5.98]	21.06 [6.16]	TD < ADHD, ASD and ADHD+ASD; ADHD+ASD>ADHD
CRS-3 – ADHD Global Index [SD]	75.10 [18.81]	47.97 [8.36]	87.96 [4.18]	79.44 [12.59]	87.21 [5.26]	TD < ASD < ADHD and ADHD+ASD
CRS-3 – ADHD Inattention Index [SD]	72.75 [18.28]	47.62 [7.40]	85.04 [9.53]	76.28 [13.11]	83.97 [7.02]	TD < ASD < ADHD and ADHD+ASD
CRS-3 – ADHD Hyperactivity-Impulsivity Index [SD]	73.47 [18.59]	48.17 [7.99]	86.63 [6.15]	74.33 [13.52]	85.67 [8.31]	TD < ASD < ADHD and ADHD+ASD

Group means for Age; WASI FSIQ, VIQ and PIQ; SCQ total score; CRS-3 Global Index, Inattention Index and Hyperactivity-Impulsivity Index; are reported for the full sample and for each group, with standard deviations in parentheses. SCQ and CRS scores are derived from parent-report questionnaires. The final column summarises the results of pairwise comparisons (see text for full results)

Table 2. Number of participants, per group, showing symptoms of a comorbid condition, as derived from the parent-report DAWBA.

	Typically Developing (TD)	ADHD-only	ASD-only	ADHD+ASD
Total number of subjects included in each group	31	24	18	33
Anxiety	0	6 (25%)	10 (55.5%)	15 (45.5%)
Depression	0	1 (4%)	3 (16.7%)	6 (18.2%)
CD/ODD	0	17 (71%)	11 (61.1%)	22 (66.7%)

Compared to typically developing controls, Full Scale IQ (FSIQ) was reduced in children with ADHD+ASD (Mean difference = 14.41; $p < 0.001$; Benjamini-Hochberg [BH]-corrected) and in children with ASD (Mean difference = 11.65; $p = 0.036$; BH-corrected) (see Table 1 for mean scores and SD of the measures considered in these analyses, for each group). The three clinical groups did not differ on FSIQ ($p >$

0.2; BH-corrected). There was also a main effect of group on verbal (VIQ: $F_{3,100} =$ 4.756; p = 0.004) and performance scores (PIQ: $F_{3,100}$ = 3.778; p = 0.013). More specifically, typically developing children had higher VIQ than children with ASD-only (mean difference = 11.61; p = 0.039; BH-corrected) and comorbid ADHD+ASD (mean difference = 13.56; p = 0.006; BH-corrected), while VIQ was also marginally increased in children with ADHD-only compared to children with ADHD+ASD (mean difference = 9.08; p = 0.068; BH-corrected). I investigated this result by carrying out a Bayesian ANOVA on VIQ and found (weak) evidence in support of this finding (BF10 = 1.603). Moreover, PIQ was significantly increased in typically developing children compared to children with ADHD+ASD (mean difference = 12.90; p = 0.012; BH-corrected), and marginally significantly increased in TD children compared to children with ADHD-only (mean difference = 10.02; p = 0.069; BH-corrected). There was moderate evidence in support of this last marginally significant result, when investigating it through Bayesian statistics (BF10 = 3.842).

Typically developing children had a lower SCQ total score, compared to children with ADHD-only (Mean difference = 11.43), ASD-only (Mean difference = 15.25) and ADHD+ASD (Mean difference = 17.20) (all p < 0.001; BH-corrected). Moreover, SCQ total score was reduced in children with ADHD-only, compared to children with ADHD+ASD (Mean difference = 5.77; p < 0.001; BH-corrected) and children with ASD-only (Mean difference = 3.82; p = 0.042; BH-corrected), while there were no differences between children with ASD-only and children with ADHD+ASD on this measure (Mean difference= 1.95; p = 0.250; BH-corrected).

The three clinical groups had higher CRS-3 global index scores, compared to typically developing controls (ADHD: Mean difference = 39.99; ASD: Mean difference = 31.48; ADHD+ASD: Mean difference = 39.25; all p < 0.001; BH-corrected). Children

with ADHD+ASD were similar to children with ADHD-only (Mean difference = 0.75; p > 0.719; BH-corrected) in showing the highest scores, while children with ASD-only had lower scores than both children with ADHD+ASD (Mean difference = 7.77; p = 0.001; BH-corrected) and children with ADHD-only (Mean difference = 8.51; p = 0.001; BH-corrected).

Similar findings emerged for the CRS-3 inattention and hyperactivity/impulsivity indices, as following. Typically developing controls had the lowest scores on the CRS-3 inattention index, compared to children with ADHD-only (Mean difference = 37.42; p < 0.001; BH-corrected), ASD (Mean difference = 28.66; p < 0.001; BH-corrected) and ADHD+ASD (Mean difference = 36.35; p < 0.001; BH-corrected). Children with ADHD-only, like children with ADHD+ASD (Mean difference = 1.07; p > 0.658; BH-corrected) displayed the highest CRS-3 inattention scores, followed by children with ASD-only who showed lower scores than both children with ADHD+ASD (Mean difference = 7.69; p = 0.005; BH-corrected) and those with ADHD-only (Mean difference = 8.76; p = 0.003; BH-corrected).

Our analyses also showed that children with ADHD-only were like children with ADHD+ASD (Mean difference = 0.96; p = 0.69; BH-corrected) in showing the highest CRS-3 hyperactivity and impulsivity scores, higher than children with ASD-only (ADHD-only: Mean difference = 12.29; ADHD+ASD: Mean difference = 11.33; all p < 0.001; BH-corrected). Moreover, typically developing controls had reduced scores of hyperactivity/impulsivity than each of the clinical groups (ADHD-only: Mean difference = 38.45; ASD-only: Mean difference = 26.16; ADHD+ASD: Mean difference = 37.49; all p < 0.001; BH-corrected).

Summarising, while the presence of ASD (in children with ASD-only and comorbid ADHD+ASD) was associated with reduced Full Scale IQ (FSIQ) and Verbal

IQ (VIQ), compared to typically developing children, the presence of ADHD (in children with ADHD-only and ADHD+ASD) was associated with reduced Performance IQ (PIQ) compared to TD children. Moreover, there was a marginal trend showing that children with ADHD-only had increased VIQ than children with comorbid ADHD+ASD (probably due to the presence of ASD in the comorbid group, which were associated with reduced verbal abilities). Compared to the three clinical groups (ASD-only, ADHD-only and ADHD+ASD), typically developing children had lower SCQ total score, besides lower CRS-3 total scores, inattention and hyperactivity/impulsivity scores. While SCQ total scores were reduced in children with ADHD-only compared to children with ADHD+ASD, children with ADHD (ADHD-only and ADHD+ASD) had higher scores on CRS-3 global index, inattention index and hyperactivity/impulsivity index, when compared to children with ASD-only.

2.2.3. Experimental paradigms

The testing session was subdivided in two main batteries of experimental tasks, i.e., eye-tracking and EEG (see Table 3 for a description of their approximate duration). The clinical assessment with the child took place between the two main batteries of tasks. Parents filled the questionnaires when the child was carrying out the eye-tracking and the EEG battery. Appropriate breaks were granted to children and their parents, due to the length of the entire testing session, and their duration was decided together with the child and their parents. The experimental tasks on which this doctoral dissertation is focused, were the gap-overlap task, the auditory oddball task and the POP task, while data from the habituation task and the free viewing probabilistic task were designed to be investigated by Iti Arora in her doctoral dissertation.

Table 3. Description of the testing session

Battery	Approximate battery duration (including setup and breaks)	Task	Approximate task duration (minutes)
Eye-tracking	45 minutes	Gap-overlap task	15 min
		Habituation task	3 min
		Free-viewing - probabilistic task	20 min
EEG	1 hour and 45 minutes	Auditory oddball task	20 min
		POP task	25 min
Clinical assessment	2 hours	WASI (all children)	45 min
		ADOS (only children screened positive for ASD and/or ADHD)	60 min

2.2.3.1.   Gap-overlap task

A *gap-overlap* task, namely a simplified version of Posner's cueing task, has been used to investigate reflexive and voluntary processes of visual attention disengagement and orienting (Saslow, 1967). During this experimental paradigm, participants were asked to fixate a central visual object and carry out an eye movement (i.e., a saccade) towards any peripheral stimulus that appeared in the left or right visual field, laterally aligned with the central stimulus. The central stimulus was a colour-filled circle with a white cross in the middle, positioned at the centre of a uniform dark grey background. To encourage participants to fixate on this stimulus, it expanded and contracted at regular intervals (expanding for the first 500 msec, contracting for other 500 msec, and so on) until the participant had continuously fixated it for 1000 msec. At that point, the peripheral stimulus appeared either to the left or to the right side of the central stimulus, for a variable duration of 500- to 1500-msec before a blank screen was presented and a new trial started. The task was comprised of 12 blocks of 7 trials each, divided by 6-seconds-long video breaks, leading to a total of 84 task trials. The order of presentation of trials was randomised. The task was pilot tested at the Summer Scientist Week, an event organised by the School of Psychology (University of Nottingham) in August 2017. More specifically, eye-tracking data from 70 children, including their qualitative feedback about the task, was used to adapt the paradigm for the present study.

Three main variables (Condition, Stimulus and Modality) were manipulated as below, paying attention to balance the presentations of peripheral objects over left and right areas of the screen:

- Condition (baseline, overlap);
- Stimulus (social, non-social);

- Modality (static/unimodal and dynamic/multimodal);

- Target-stimulus Visual Field (left, right).

Conditions: baseline and overlap

The first variable manipulated in this experimental task was the temporal offset between the central and lateral stimuli in the baseline and overlap conditions (see Figure 5). In the *baseline* condition, the peripheral visual stimulus appeared immediately after the disappearance of the central object, while in the *overlap* condition the central visual stimulus did not disappear from the screen after the presentation of the peripheral object (thus, there was a temporal overlap of both stimuli presented on the screen). The presentation of visual stimuli in the baseline condition elicit a quick reflexive orienting response, with eye movement latencies in the range 100-200 msec (Fischer & Ramsperger, 1984, Bekkering et al., 1996), reflecting the involvement of the ventral attentional network. Conversely, in the overlap condition the dorsal attentional network is involved in facilitating the voluntary disengagement of attention from the central object and, then, in initiating a saccade towards the peripheral visual object. This results in longer eye movements' latencies during the overlap condition, usually in the range of 200-250 msec, or even longer.

Stimulus type: social and non-social

By manipulating the modality of presentation and the social nature of the peripheral stimuli, I aimed to investigate the effects of perceptual salience of the stimuli on attention disengagement and orienting. The peripheral stimulus could be a social or non-social visual object. Human faces have been used as social stimuli, for their widely

demonstrated ability to elicit faster saccadic orienting responses, compared to non-social visual objects (Crouzet et al., 2010). To form a set of experimental stimuli, a number of faces were selected from the UvA-NEMO Smile Database (Dibeklioglu et al., 2012) and adapted for the aims of the task. After a qualitative comparison with other online databases, the UvA-NEMO Smile Database excelled for its qualitative features, including dynamicity (i.e., it includes videos which were usable both as a static picture and as a video), quality (videos were recorded at high resolution and in a controlled environment with an artificially illuminated background) and appropriateness for studies involving eye-tracking. More specifically, 12 different video stimuli were selected, so that they included people of different age and gender. Six non-social stimuli, i.e., three-dimensional shapes following different rotation patterns (see Figure 6 for examples) were artificially created with CINEMA 4D (Maxon Computer; https://www.maxon.net/en/products/cinema-4d/overview/).

Modality of presentation: static/unimodal and dynamic/multimodal

The peripheral visual objects were presented in either a single modality, comprising visual static presentation with no sound, or in two parallel modalities, comprising a visual dynamic presentation of central and peripheral visual stimuli and the parallel presentation of sounds for each of these. More specifically, while in the static condition the visual objects were presented as static pictures without any sounds, in the multi-modal condition custom sounds were presented together with the visual objects. Non-vocal social sounds (for example, laughing) and non-social artificial sounds were downloaded from an online database of sound effects (http://soundbible.com) and balanced in terms of duration and volume, to create

dynamic multi-modal stimuli. Some short creative commons cartoons, downloaded from www.google.co.uk, were used to create video breaks between blocks of trials.
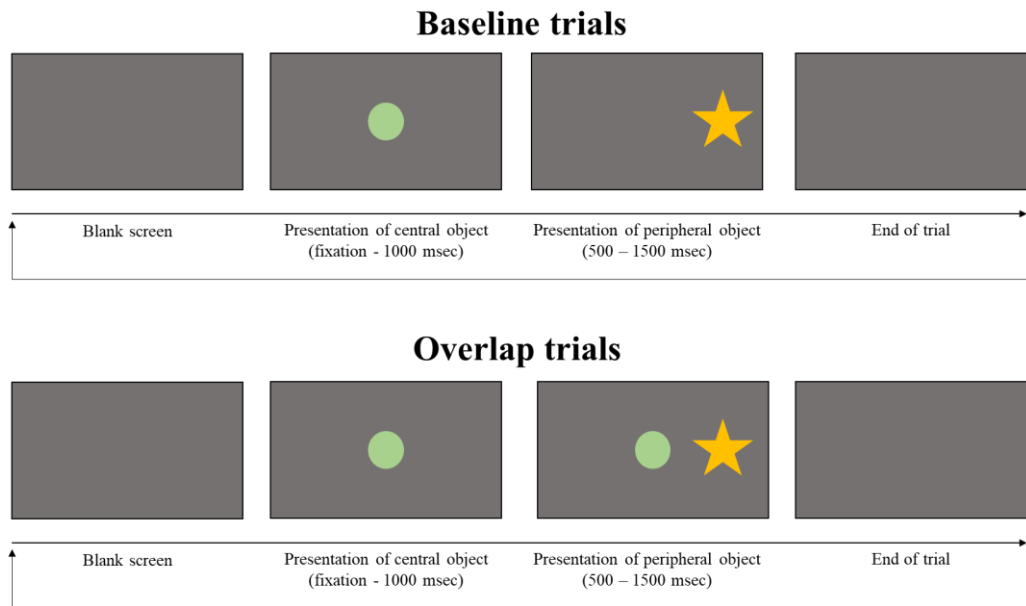
Figure 5. Gap-overlap task diagram
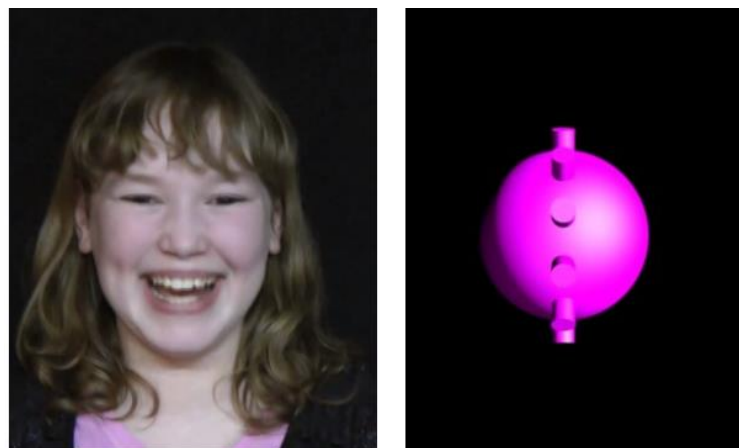
Figure 6. Example of social (left) and non-social (right) visual stimuli used in the gap-overlap task

2.2.3.2.   Passive auditory oddball task

A passive version of the *auditory oddball task* was designed to investigate neural mechanisms of automatic orienting of attention to auditory information (Johnstone et al., 2013). This paradigm involves the presentation of a series of repetitive stimuli ('standard') which are alternated by less frequent stimuli ('deviant') (Figure 7). Our version of the oddball task was passive: children were listening to but were not asked to actively pay attention to the sequence of sounds or to respond to the auditory stimuli following a fixed rule. During the presentation of auditory tones, they were watching a silent movie. In fact, engaging participants in a mentally undemanding task such as this, is recommended when investigating involuntary orienting of attention and discrimination between different auditory tones in a passive oddball task (Näätänen, 1990). The averaged neural response to these stimuli, i.e., an ERP, was analysed to extract the main components of interest (see paragraph 2.2.6.2).

Duncan et al. (2009) produced some guidelines for researchers about the design of auditory oddball tasks, in order to maximise the effects of the presentation of standard and deviant sensory stimuli. For example, attention should be paid to ensuring that standard and deviant sounds are different in terms of frequency, while volume, duration and inter-stimuli interval shall be kept constant. In line with these guidelines, the auditory tones used for the present task were created artificially, in order to better control their characteristics, i.e., volume, frequency and duration. Standard sounds were similar across the two blocks, and they were a simple 500 Hz sinusoidal tone created with the open-source and freeware software Audacity® (version 2.2.2; https://www.audacityteam.org). On the contrary, in our version of the auditory oddball task the nature of deviant tones was manipulated.

Vowel-speech sounds have been found to elicit increased and faster electrophysiological indices of automatic discrimination and involuntary orienting of attention, compared to non-social sounds (Iino et al., 2018). Therefore, while in the non-social condition of the task the deviant stimulus was a 450 Hz sinusoidal tone created with Audacity®, in the social condition the deviant sound was a natural-sounding vowel created to resemble the English vowel /e/ (formant frequencies: F0 150, F1 530, F2 1840, F3 2480; Peterson & Barney, 1952). The social tone was created using the online Simplified Vowel Synthesis Interface (Timothy Bunnell, http://www.asel.udel.edu/speech/tutorials/synthesis/vowels.html), an online tool designed to synthesize English vowels through the Klatt synthesizer.

The deviant-to-standard ratio was 1:4, so that each of the two task blocks was formed of 640 standard tones (80 %) and 160 deviants (20 %). Each tone lasted 200 msec, with a 700-ms inter-stimulus interval, making the entire task lasting for about 26 minutes, with a 30-seconds-long resting period at the beginning of the task and between the two blocks. During this period, children kept watching the silent movie, but no auditory stimuli were reproduced. Moreover, participants were not explicitly instructed to rest. The alternation between standard and deviant tones, i.e., the number of standard tones in a row before presenting a deviant, was randomised during the task, so that at least 2 standard tones were presented before a deviant. The order of presentation of the blocks (i.e., social and non-social) was randomised across participants. Before starting the presentation of the auditory stimuli, participants were told that they would have listened to some sounds on the background, while they were watching a silent cartoon movie, and were told to not pay attention to the tones but to focus instead on the movie.
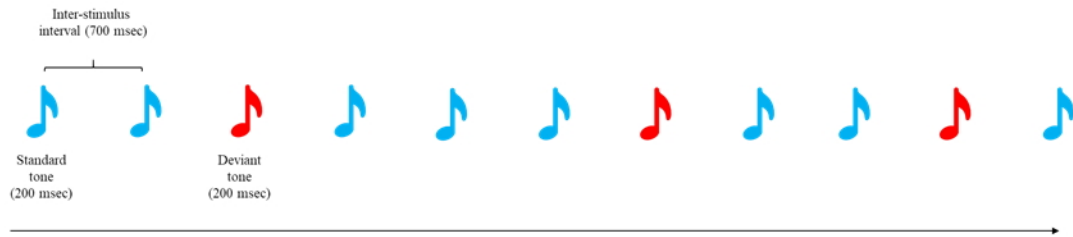
Figure 7. Passive auditory oddball task diagram

2.2.3.3. Preparing to Overcome Prepotency (POP) task

With the aim of measuring electrophysiological and behavioural measures of preparation and inhibition of motor responses, and sustained attention, I designed an adapted version of the Preparing to Overcome Prepotency (POP) task (Cho et al., 2006). This task was specifically chosen since it challenges the preparation and inhibition of motor responses in conditions with different cognitive demand. Participants, in fact, were instructed to press the left or right button on a response box as soon as possible after the appearance of a target, i.e., a left or right arrow. In half of the trials, the cue preceding the arrow was a green fixation cross, and this indicated that the motor response required after the onset of the target stimuli should be congruent with the arrow direction (pressing the right button in response to the right arrow; 'low-demand' trials). In the other half of trials, the cue was a red fixation cross, indicating that the behavioural response required after target presentation would be contralateral to the direction of the target arrow (pressing the left button, if a right arrow followed the red fixation cross; 'high-demand' trials) (see Figure 8).

Visual stimuli were presented in the centre of a computer screen with a dark grey background: the fixation cross (a text-stimulus '+') was presented for 1500 msec, followed by the arrow ('>' or '<') for 1500 msec. While there was no temporal interval

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between the offset of the fixation cross and the presentation of the arrow, there was an interval of 500 msec between the offset of the target stimuli and the start of a new trial (Figure 8). Therefore, the temporal window for the participant to carry out the motor response after the presentation of the target stimulus, was 2000 msec, spanning the arrow stimulus duration and the inter-trial interval. The POP task was comprised of 8 blocks of 36 trials each (288 trials in total). Before presenting the first block of the task, detailed instructions were given to the participants, who completed 20 practice trials. At the end of every block, a 50-seconds long break was followed by a 10-seconds-long visual countdown which indicated the re-starting of the task. Participants were told about the presence of the breaks, but they were not aware of the total duration of the task. The comfort of participants and their engagement with the experimental paradigm was monitored throughout the session. There was a short interval (around 30/60 seconds) between the end of the break after the 4th task block, and the beginning of the 5th task block, during which children's comfort was monitored.

Low-demand trials

| Fixation cross | Target | Manual response | Blank screen |
| (1500 msec) | (1500 msec) | | (500 msec) |

High-demand trials
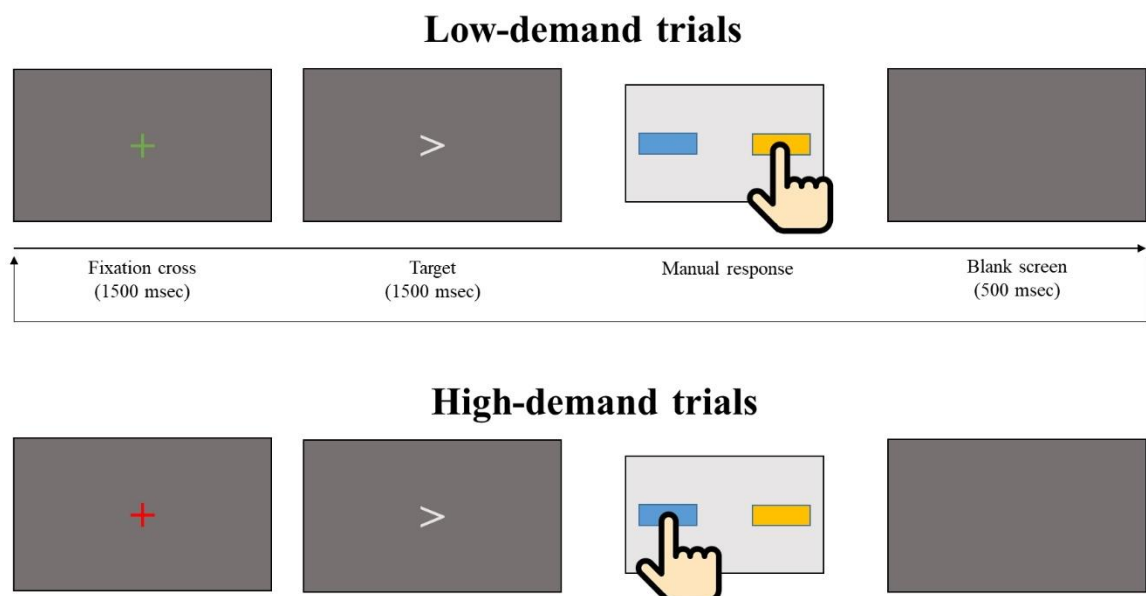
Figure 8. POP task diagram

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2.2.4. Apparatus

High-spec personal computers were used to design the experimental paradigms, collect and analyse the data, by using the following software or toolboxes:

- Eyelink® Experiment Builder (SR Research): design and delivery of the gap-overlap task

- Eyelink® Data Viewer (SR Research): preliminary analysis and exporting of raw eye-tracking data collected during the gap-overlap task

- Microsoft Office Visual Basic for Applications (VBA): pre-processing of eye-tracking data and extraction of eye-tracking outcome measures

- PsychoPy 2.5 (Peirce, 2007; 2009): design and delivery of the oddball and POP tasks

- Biosemi® ActiView: recording of EEG signal

- Brainstorm (Tadel et al., 2011; http://neuroimage.usc.edu/brainstorm): pre-processing of raw EEG and heart rate data

- IBM SPSS 26: statistical analyses

- JASP (the JASP Team, 2019) Version 0.11.1: statistical analyses

- G*Power (Faul et al., 2007): conduction of power analysis

2.2.5. Procedure

The eye-tracking (i.e., gap-overlap task) and EEG (i.e., oddball task and POP task) testing batteries were conducted on the same day or on two different days, if the child was too tired, or the parents expressed concerns about the length of the entire session. WASI and ADOS were carried out by the research team with the child, while parents completed all the other questionnaires, including SCQ, CRS-3, DAWBA, Sensory profile and NS-SEC. The teachers were contacted, upon written consent by the parents, after the testing session, and they were asked to fill the SCQ-Current and the CRS-3 questionnaires (teacher version).

Before the start of the gap-overlap task, a 9 points-of-gaze (POG) calibration was carried out, by presenting an attractive colourful stimulus in the centre and in other 8 areas of the screen. Participants' eye movements were recorded through an Eyelink® 1000 (SR Research) eye-tracking system. Eye movements from both eyes were recorded at 500 Hz through a 25-mm lens, without the use of any chinrest, from an average distance of 60 cm and with an estimated accuracy of $0.25°$ to $0.5°$. The gap-overlap task was delivered on a 21.5' LCD screen with 60 Hz refresh rate, placed behind the eye-tracking device. A dimmer switcher was utilised to keep the room luminance constant across the entire sample of participants and, in parallel, screen brightness was kept constant as well. The eye-tracking session, including calibration and gap-overlap task, lasted between 15 and 20 minutes.

A 64-channels Biosemi® head cap with an ABC layout, was used during the EEG session. The signal from the 64 electrodes was recorded at 512 Hz and saved on a personal computer hard drive, after being amplified through a Biosemi® ActiveTwo system. Four additional electrodes were placed around the participant's eyes, to record vertical and horizontal eye movements, and two were positioned on the earlobes as

reference. Raw heart rate signal was recorded from two free electrodes placed on participants' wrists. During the preparation to the EEG session, children were given the opportunity to watch age-appropriate videos on a tablet. After the setup was completed, participants were moved to another room, where all the electrodes were plugged into the system and a final check was carried out to ensure that the system was properly recording the EEG signal. The experimental tasks were delivered on a 21.5' LCD screen with 60 Hz screen with 60 Hz refresh rate, placed at an average distance of 60 cm from participants' eyes. Digital stimulus onset codes, including those associated to manual responses collected through a Cedrus button box during the POP task, were sent to the recording software through a parallel port. A set of speakers was used to reproduce sounds during the auditory oddball task. The EEG session, including the setup, the two experimental paradigms and the removal of electrodes and cap, lasted between 1.5 and 2 hours.

2.2.6. Overview of the outcome measures and pre-processing of raw data

2.2.6.1. Gap-overlap task

The outcome measures extracted from the raw eye-tracking data collected during the gap-overlap task were saccadic reaction times (SRTs) and pupil size (PS). SRTs have been calculated for each trial of the task, in order to obtain a measurement of eye movements' latencies, and have been operationalised as the time (in milliseconds) between the onset of a peripheral stimulus and the start of an eye movement (i.e., a saccade) from a fixated central object towards the peripheral object (Johnson et al., 1991). Baseline pupil size has been calculated by averaging, for each trial, the diameter of the pupil recorded in the temporal period between the onset of the central fixation stimulus and the start of the saccade towards the peripheral visual object. The slope of change in baseline pupil size (over time, i.e., from the beginning to the end of the task block) was calculated for each of the two blocks of the gap-overlap task and used for the analyses.

SRTs and PS were extracted from the raw data through Microsoft Office VBA scripts and they were further analysed only if the participant had at least 50% valid trials. The following exclusion criteria were used to discard invalid trials: 1) anticipation, i.e., the saccade towards the peripheral stimulus location occurred before the onset of the stimulus itself; 2) absence of a saccade towards the peripheral stimulus, or in the opposite direction compared to the peripheral stimulus; 3) SRTs shorter than 80 msec, which are likely to characterise eye motor reflexes, instead of eye movements (Hess et al., 1946); 4) data loss due to technical problems.

The intra-individual variability of SRTs, which is likely to reflect fluctuations in vigilance and attention to the task, was also calculated. Increased intra-individual

variability of SRTs, in fact, is likely to reflect the presence of a less consistent and more dysregulated performance with augmented presence of attentional lapses and inattention. The standard deviation of SRTs (SD-SRTs) was calculated in order to get a measure of SRTs variability.

2.2.6.2.   Auditory oddball task

The main outcome measures of the oddball task were the ERP components P3a and MMN. The P3a, a subcomponent of the P300, is a positive peak occurring between about 250 and 400 msec after stimulus onset, with maximal distribution over fronto-central electrodes. It has been suggested that the P3a is likely to reflect vigilance, sensory information processing and automatic orienting of attention (Yamaguchi & Knight, 1991). The MMN, an index of involuntary detection of changes in auditory information, is a negative deflection of the ERP signal that is maximal over fronto-central regions and it is usually detected between 100 and 250 msec after the onset of a deviant stimulus. The following procedures have been carried out to pre-process the raw EEG data collected during the auditory oddball task and extract the P3a and the MMN.

The pre-processing of EEG signal was performed with Brainstorm (Tadel et al., 2011). Firstly, the signal was band-pass filtered (0.05 - 30 Hz) and visual inspection of the filtered signal was carried out to manually exclude bad temporal segments of data from further analysis. Following this, power spectral density (PSD; Welch, 1967) was used to obtain an estimation of the power spectrum of the EEG signal for each electrode and over the entire recordings, so that flat or extremely noisy channels could be identified and rejected. Independent Component Analysis (ICA; Herault et al., 1985)

was then carried out on the continuous EEG to identify and remove artifacts associated with eye movements, blinks, muscular activity and any other temporary alterations of electrical activity not reflecting brain activity. After this step, the EEG signal at each electrode was re-referenced to the average of the signal at all remaining channels, before epochs locked to the stimulus onset were imported for standard and deviant tones, for each block of the task (social and non-social). The imported epochs were 800 msec long, and included a 100 msec pre-stimulus window, which was used as a baseline to normalise the signal on the 700 msec post-stimulus temporal window. Only epochs with electrical activity in the range $\pm100\mu V$ were further processed, to obtain four average ERP waveforms, reflecting the stimulus-locked synchronised brain activity for the standard and deviant tones, in the social and non-social blocks.

Similarly to previous studies, including the study from which our passive auditory oddball task was adapted (Whitehouse & Bishop, 2008), the P3a was calculated for both standard and deviant tones, while the MMN was calculated by subtracting the waveform to standard tones from the waveform to the deviant, for each block (social; non-social) (Näätänen et al., 2007). The P3a was determined as the maximal positive peak at the FCz electrode (fronto-central), in the single-subject ERP waveform between 250 and 400 msec after stimulus onset. Conversely, the MMN was identified as the most negative peak in the time window 100-250 msec in the single-subject difference waveform, at the same electrode (FCz). Latency and amplitude of the P3a and the MMN were extracted for further analysis.

Heart rate was recorded during the passive auditory oddball task, and different parameters were extracted. Raw heart rate data was utilised to extract time-domain and non-linear measures of HRV, i.e., the Cardiac Sympathetic Index (CSI), the Cardiac

Vagal Index (CVI) and the Root Mean Square of Successive Differences (RMSSD). Among the various time-domain measures which can be extracted from heart rate, the RMSSD is in fact one of the most reliable measure of parasympathetically mediated HRV (see Shaffer & Ginsberg, 2017, for an overview), and was calculated as following:

a) Raw heart rate signal collected from one of the free electrodes placed on participants' wrists during the EEG session, was band-pass filtered (8-20 Hz) to reduce the baseline fluctuation of the cardiac signal and to minimise the impact of artifacts and high frequency noise (Fedotov, 2016).

b) Automatic detection of cardiac beats was carried out in Brainstorm (Tadel et al., 2011), followed by visual correction of potentially erroneous or missing peaks, before calculating the time differences (in msec) between each successive heartbeat, i.e., the inter-beat interval (IBI).

c) RMSSD was calculated, as following. First, the time differences between successive IBIs were squared and averaged, for the two blocks of the task and the 30-seconds-long resting blocks before the start of each block; then, the square root was calculated for each of these, to obtain the RMSSD (Shaffer & Ginsberg, 2017):

$$RMSSD = \sqrt{\frac{1}{n-1} \sum_{k=1}^{k=n-1} (I_{(k+1)} - I_{(k)})^2}$$

with k = 1, 2, 3, …, (n - 1); n = number of IBIs within the period; I = IBI in milliseconds

Besides using RMSSD, we embraced the approach proposed by Toichi et al. (1997) to extract the Cardiac Sympathetic Index (CSI) and the Cardiac Vagal Index

(CVI), two indices of HRV which are likely to mirror activity of the sympathetic and parasympathetic branches of the ANS, respectively. To calculate CSI and CVI, a Poincaré plot is created by plotting every peak-to-peak interval (I_{k+1}) against the preceding interval (I_k), with k = (n – 1) and n = each of the cardiac beats extracted from the HR signal. This results in a two-dimensional graphical ellipsoid-shaped cloud of points, as represented in Figure 9. Two main parameters of this ellipsoid graph, i.e., SD1 and SD2, can be mathematically extracted from the distribution of R-R-intervals in a specific time window. Considering the line of identity as the 45° oriented line representing the identity $I_k = I_{k+1}$, SD1 is a measure of the dispersion of the points perpendicularly to the line of identity (i.e., the width of the ellipse), while SD2 represents the dispersion of points along the identity line (i.e., the length of the ellipse) (see Figure 9). More specifically, the mathematical calculations of SD1 and SD2 were carried out using the following equations:

$$SD1 = SD(\frac{1}{\sqrt{2}}I_{(k)} - \frac{1}{\sqrt{2}}I_{(k+1)})$$

$$SD2 = SD(\frac{1}{\sqrt{2}}I_{(k)} + \frac{1}{\sqrt{2}}I_{(k+1)})$$

with k = 1, 2, 3, …, (n - 1); and n = number of cardiac beats within the period.

SD = standard deviation of the sample

By multiplying SD1 and SD2 by four, it is possible to obtain an estimation of the transverse length (T) and the longitudinal length (L) of the ellipse, which are further used to calculate the CSI and the CVI (Toichi et al., 1997), as following:

$$CSI = \frac{4 \times SD2}{4 \times SD1} = \frac{L}{T}$$

$$\text{CVI} = \log_{10}(L \times T)$$

Summarising, the ERP components P3a and MMN, besides the CSI, CVI and RMSSD, obtained from the analysis of HRV, were the outcome measures extracted from the passive auditory oddball task.
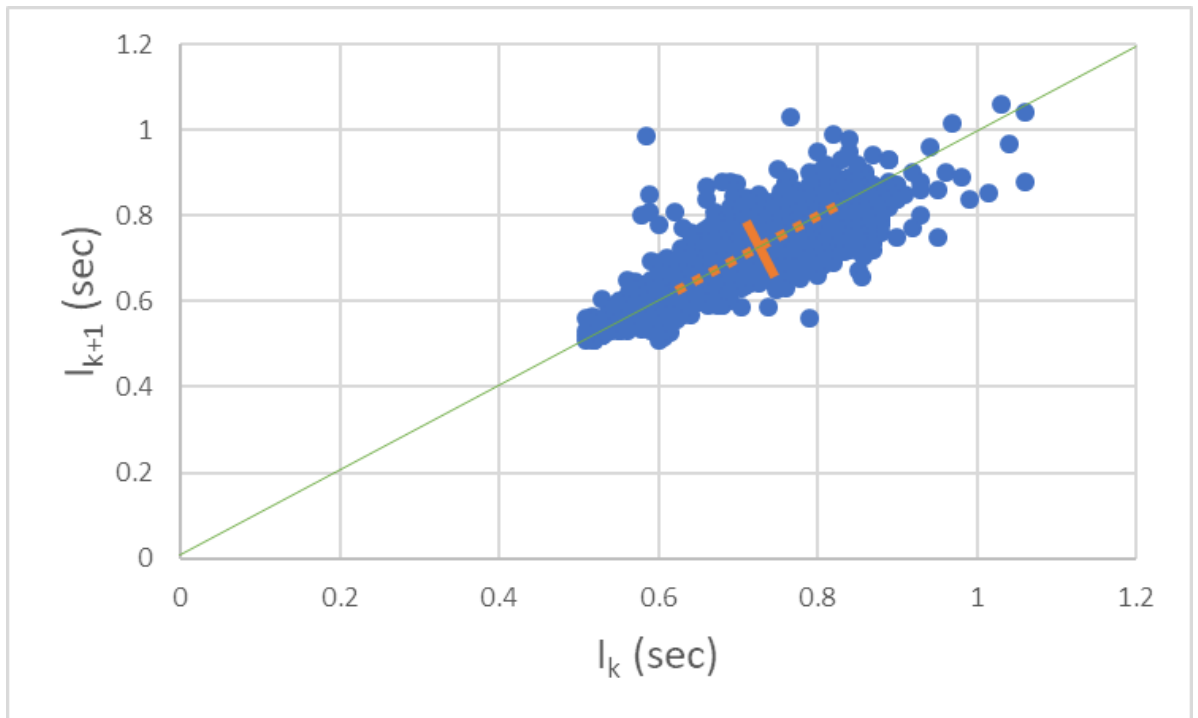
Figure 9. Example representation, based on collected HR data, of a Poincaré plot.
Green line: identity line. Straight orange line: SD1; Dotted orange line: SD2.

2.2.6.3.   POP task

Although none of the previous studies that adopted the POP task focussed on ERPs analysis, there is previous literature on tasks challenging similar cognitive processes, which directed the choice of the ERP components of interest for this task.

Since I was interested in analysing electrophysiological indices of cue-processing, I extracted the amplitude and latency of specific ERPs in response to the

presentation of the cue- and target-stimuli. More specifically, the P3 is a measure of information-processing, which is likely to be associated with consequent preparation of motor responses (Gratton et al., 1990, Hämmerer et al., 2010). In response to cue-stimuli, the parietal P3 has been found to be linked with activation of the dorsolateral pre-frontal cortex (DLPFC), and has been found increased during decision making and increased mental effort (MacDonald, et al., 2000; Rowe et al., 2000). I decided to focus on electrophysiological indices of conflict monitoring and suppression of a prepotent motor response, including the fronto-central N2 and the parietal P3 in response to target-stimuli (Hämmerer et al., 2010). Previously considered an index of response inhibition, the frontal N2 has been recently proposed to reflect conflict monitoring and it is associated with the activation of the ACC (Bekker et al., 2005, Nieuwenhuis et al., 2003). For example, N2 is usually larger following stimuli that anticipates a conflictual response, e.g., anti-saccades or No-Go responses (Hämmerer et al., 2010).

Similarly to what has been done for the oddball task, the pre-processing of EEG signal included band-pass filtering of the signal (0.05 - 30 Hz), excluding bad segments and using PSD to reject flat or extremely noisy channels, before carrying out ICA, removing artifacts and re-referencing the EEG signal to the average. Following from these, different procedures have been used to extract cue- and target-locked ERPs, and to extract spectral alpha power in different temporal windows. Firstly, in order to extract averaged ERPs, the EEG data was segmented into epochs locked to the cue and to the target stimuli, including a 200 msec pre-stimulus window, which was used for baseline correction, and a 1500 msec post-stimulus temporal window. Only epochs with electrical activity in the range ±100μV were further processed, to obtain single-subject ERP waveforms (cue- and target-locked, for low- and high-demand trials). The latency

and amplitude of the cue- and target-locked P3 was determined by extracting the maximal positive peak in EEG signal (at electrode Pz) between 250 and 400 msec after stimulus onset, while the most negative peak (at electrode FCz) in the time window 100-250 msec was identified to extract the latency and amplitude of the N2 in response to target stimuli.

Alpha activity during the POP task and, more specifically, during the breaks between the task blocks, before the onset of the cues (alpha synchronisation, reflecting filtering and gating of distracting information) and after the onset of the cue-stimuli (alpha desynchronization, mirroring the engagement and allocation of attentional resources to process the cue-stimuli) was analysed (Hwang et al., 2016). I also analysed alpha oscillations during the 50-seconds-long breaks of the POP task, which might be a non-specific index of cortical arousal and might be somehow associated with measures of autonomic arousal. To extract spectral alpha power, I segmented the artifacts-corrected and filtered EEG signal as following:

a) Consecutive 2-seconds long epochs were extracted from the 50-seconds long breaks between task blocks;

b) Pre-cue epochs (from 500 msec before the cue-onset until cue-onset) were extracted to investigate general allocation of attentional resources to the task;

c) Post-cue epochs (between cue- and target-onset; 1500 seconds long) were extracted to investigate alpha activity in low- and high-demand trials.

Each of these epochs was subjected to Fast Fourier Transformation (FFT) with 10% Hanning window, to compute spectral power in the delta (0.5-3.5 Hz), theta (3.5-8 Hz), alpha (8-12 Hz) and beta (12-22 Hz) frequency bands. Considering that absolute spectral power is likely to be partly associated with structural and physical

characteristics of the skull and cortex, I also calculated the relative measure of alpha power, therefore the power in the alpha frequency band as a proportion of overall power across all frequencies (0.5 – 22 Hz). Absolute and relative alpha power was extracted for midline frontal (Fz), central (Cz), parietal (Pz) and occipital (Oz) electrodes and was further subjected to natural log-normalisation (*ln*) before carrying out further statistical analysis.

Heart rate was collected during the POP task, as done in the oddball. The CSI, the CVI and the RMSSD were extracted for each of the eight task blocks and the breaks, following the procedures already described for the oddball task (paragraph 2.2.6.2). The overall number of correct responses was analysed as a measure of task accuracy, while the average of RTs for correct responses gave an index of performance speed. Although it does not allow to investigate specific components of RTV, unlike ex-gaussian analysis and the analysis of periodic patterns (Adamo et al., 2019), the standard deviation of RTs (SD-RTs) was calculated as an index of intra-individual variability of RTs (Kofler et al., 2013).

Table 4 includes a brief summary of the outcome measures investigated in the present study. Before presenting the main results (Chapters 3 and 4), I will now present the analyses plan and the specific investigations of this doctoral project.

Table 4. Summary of the main outcome measures investigated in the present study

Task	Measure		Function
Gap-overlap	Average SRTs		Latency of eye movements
	SD-SRTs		Intra-individual variability of SRTs
	Baseline pupil size	Slope	Overall change in PS throughout the task blocks
		Trial-by-trial	Categorisation of trials in small, medium and large baseline pupil size
Oddball	Heart Rate Variability	CSI	Activity of the SNS
		CVI	Activity of the PNS
		RMSSD	HRV/vagal tone
	P3a amplitude P3a latency		Automatic orienting of attention
	MMN amplitude MMN latency		Automatic discrimination between standard and deviant auditory stimuli
POP	Heart Rate Variability	CSI	Activity of the SNS
		CVI	Activity of the PNS
		RMSSD	HRV/vagal tone
	Average RTs (for correct trials)		Performance speed and accuracy
	Accuracy (% of correct trials)		
	SD-RTs		Intra-individual variability of RTs
	Cue-P3 amplitude Cue-P3 latency		Information processing
	Target-N2 amplitude Target-N2 latency		Conflict monitoring
	Target-P3 amplitude Target-P3 latency		Information processing
	Alpha power during the breaks		Resting-state alpha
	Pre-cue alpha power		Alpha synchronisation, efficiency of filtering and gating distracting information
	Post-cue alpha power		Alpha desynchronization, attention orienting

2.3.   Analysis plan

A full description of the statistical hypotheses and analysed used in this study are presented in Table 5 (page 102), and will be further discussed before presenting each set of results (paragraph 2.4, Chapters 3 and 4). The standardised residuals of the outcome measures have been analysed to verify the normality of their distributions and to identify any possible outliers. Since univariate and multivariate analysis of variance (ANOVA) and analysis of covariance (ANCOVA) have been shown to be robust to violations of normality and imbalances in sample sizes, and due to limitations of non-parametric tests (Blanca et al., 2017), these statistical analyses were used with both normally and not-normally distributed variables. Greenhouse-Geisser adjusted degrees of freedom are reported for those variables where sphericity was violated, which was evaluated through Mauchly's tests.

The effects of ADHD and ASD have been investigated by using two binomial between-subjects factors (i.e., ADHD-factor and ASD-factor; 0=no; 1=yes) reflecting the presence (or not) of a diagnosis of ADHD or ASD in an individual. In this way, we were able to compare children with and without ADHD (0: TD and ASD; 1: ADHD and ADHD+ASD) and children with or without ASD (0: TD and ADHD; 1: ASD and ADHD+ASD) to test specific effects related to one condition or the other. Moreover, we could investigate the impact of a comorbid clinical diagnosis of ADHD+ASD by analysing the interaction between ADHD- and ASD-factors.

2.3.1. Interpretation and follow-up of main effects and interactions

In order to follow-up main effects or interactions emerging from ANOVA and ANCOVA, a traditional approach in statistics is to analyse pairwise comparisons or post-hoc tests by adopting specific strategies to control for multiple comparisons and limit the risk of incurring in false positive and false negative results (Field, 2013). The R 'p.adjust' function was used to calculate adjusted p-values and verify the presence of between-groups differences when following-up significant interactions between ADHD and ASD factors. More specifically, p-values adjusted for multiple comparisons have been extracted, using the Benjamini-Hochberg (BH) method, which is based on the Bonferroni method but also controls for the false discovery rate (FDR), i.e., the proportion of false positives which may be present among the rejected hypotheses (Benjamini & Hochberg, 1995).

2.3.2. Covariates

Demographic and clinical measures, such as gender, age or IQ, are usually included in statistical models as covariates, since they may indirectly affect the main outcome measures. For example, if the investigated sample includes participants from a wide age range, age is usually added as a covariate. Since our sample included children in a broad age range (7 to 15 years) and in order to control for any possible effect of age on the main outcome measures, we decided to include age as a covariate. We also considered appropriate to add as covariates both verbal and performance IQ (since there were some group differences on IQ) and gender (since the four diagnostic groups exhibited different gender ratios; see Table 1, page 59).

2.3.3. Mixed frequentist/Bayesian approach

The traditional frequentist approach based on p-values results in interpretations based on specific set of rules. However, the tendency to consider an effect as 'present' (therefore real) or 'absent' by focusing on *p-values* only, has been recently challenged by the same researchers who have embraced this approach for decades (Wetzels et al., 2015). Different strategies, in fact, can improve the quality of the research outputs in testing their original hypotheses. For example, besides verifying if a p-value is under a certain threshold, to determine the presence of a difference between two or more groups on a specific measure, it would also be important to investigate the confidence intervals of the between-groups difference and the size of the effects (Dienes, 2014).

In support of more traditional interpretations of p-values, and to investigate marginally significant results and interactions, I decided to integrate Bayesian statistics in the analyses. Bayesian statistic is a data- and theory-driven approach that generally focuses on investigating the distribution of probability of two different hypotheses and analyses how much the observed data fit with each of them (Wetzels et al., 2015). The Bayes Factor (BF) is usually derived to represent how many times the observed data is likely to fit with an alternative hypothesis, compared to a null. For example, BF values between 0 and 0.33 indicates that data are more likely to support the null hypothesis, while BF values above 3 are likely to indicate that the alternative hypothesis is plausible and is supported by the observed data. Values between 0.33 and 3 are likely to indicate not enough evidence in support for either the null or the alternative hypotheses (Jeffreys et al., 1939/1961). The statistical analyses carried out on the outcome measures, to investigate the hypotheses of the present study, together with any follow-up analyses and the use of Bayesian statistics, will be discussed in Chapter 3 and Chapter 4.

2.4.　Primary and secondary investigations

I designed a battery of experimental paradigms to investigate three main research questions (see Table 5 for a summary):

- Question 1. Do indices of autonomic hypo-arousal, reduced vigilance and alertness, characterise children with ADHD, and how does the presence of a comorbid diagnosis of ADHD+ASD affect these measures?

- Question 2. Are atypicalities in visual attention orienting more associated with ASD-symptomatology, than ADHD, and what is the profile of children with co-occurring ADHD+ASD?

- Question 3. Are electrophysiological and behavioural measures of executive function and cognitive control more severely affected in children with co-occurring ADHD+ASD, compared to those with a single condition?

2.4.1. Question 1. Do indices of autonomic hypo-arousal, reduced vigilance and alertness, characterise children with ADHD, and how does the presence of a comorbid diagnosis of ADHD+ASD affect these measures?

Based on the findings from our systematic review (Bellato et al., 2020) and previous literature (see paragraph 1.4.1), we generally predicted to find signs of autonomic hypo-arousal, reduced vigilance and alertness in children with ADHD, while we expected to find indices of autonomic hyper-arousal in children with ASD. To test the theoretical models of ADHD/ASD comorbidity, we investigated if children with comorbid ADHD+ASD showed a) indices of atypical functioning of the ANS as a completely separate profile, compared to children with ADHD-only and ASD-only (*interactive model*) or b) a profile with atypicalities separately found in children with ADHD- and/or ASD-only (*additive model*). We therefore analysed measures of autonomic arousal and arousal regulation, vigilance and alertness, including CSI, CVI and RMSSD (during the oddball task and the POP task); latency and amplitude of the P3a and the MMN (oddball task); absolute and relative alpha power (POP task); slope of change in baseline pupil size and slope of change in SRTs (gap-overlap task); intra-individual variability of SRTs (gap-overlap task) and intra-individual variability of RTs (POP task).

CSI, CVI and RMSSD

In an fMRI study, Minzenberg et al. (2008) investigated how the LC-NE system might be involved in the POP task, and concluded that increased involvement and activation of the PFC was associated with sustained firing of LC neurons at higher frequencies (i.e., phasic mode), suggesting a parallel involvement of autonomic and

executive function systems during this task (Minzenberg et al., 2008). Considering these previous findings, we predicted that activity of the ANS (and more specifically, of the PNS branch) would be increased during the blocks of the task, compared to the breaks. We therefore expected to find reduced CSI, increased CVI and increased RMSSD during the task blocks, compared to the breaks, reflecting increased activation of the parasympathetic nervous system in this task.

While the POP task required active involvement of the participants, the oddball task was passive, and children were asked to watch a silent movie while the sequence of sounds was reproduced in the background. Due to the different nature of the task, we expected to find a different profile of HRV during the oddball task. More specifically, we expected that the presence of a continuous sensory stimulation (i.e., the sequence of sounds) might elicit increased activation of the LC-NE system, resulting in increased alertness and activation of the sympathetic branch of the ANS during the task blocks, compared to the resting blocks when sounds were not reproduced and children were just watching the silent movie. We therefore expected to find increased CSI during the task blocks, compared to the resting blocks. We also predicted that the progression of the task could lead to an increased involvement of the ANS in supporting exploitation of sensory information coming from the video and, indirectly, from the auditory stimulation. We therefore hypothesised to find increased CVI and RMSSD in the second part of the task, compared to the first block, which would indicate a time-related increased activation of the parasympathetic branch of the ANS to support information processing and sustained attention.

We expected to find generally reduced CSI in children with ADHD, while increased CSI was expected in children with ASD, in line with theoretical models and previous findings suggesting the presence of states of hypo-arousal in ADHD and

hyper-arousal in ASD. Since CSI is more likely to reflect the level of physiological arousal and general responsivity to sensory information, while CVI and RMSSD might also reflect top-down arousal and attention regulation mechanisms, we expected these to be atypical in both children with ADHD and ASD, although mirroring different underlying mechanisms. It may be, in fact, that the task-situation (active POP vs passive oddball task) might differently influence arousal and attention regulation in ADHD and ASD, and this will be further discussed when analysing the results.

Since opposite profiles were predicted for children with ADHD- and ASD-only, we investigated which model (additive or interactive) was more likely to explain the co-occurrence of atypicalities in HRV measures, found in children with ADHD- and ASD-only. If we found a separate profile of atypicalities in autonomic arousal and arousal regulation in children with comorbid ADHD+ASD, compared to those with single conditions, the interactive model would be supported. If, instead, different profiles were found for children with ADHD- and ASD-only, but in different task situations, children with comorbid ADHD+ASD would be more likely to show an additive profile with the same atypicalities reported in each condition.

P3a and MMN

Most of previous studies investigating electrophysiological markers of automatic attention orienting (P3a) and discrimination between sensory stimuli (MMN), focused on active versions of the oddball task, while a passive version was designed for the present study. A significantly increased P3a amplitude for deviant tones, compared to standard tones, is expected, as previously reported in literature as the 'oddball effect' (Duncan et al., 2009). Previous studies seem to indicate that

attention orienting might be impaired in ADHD, while stimulus discrimination might be more affected by ASD. In fact, signs of intact MMN but reduced and delayed P3a have been generally found in ADHD (Barry et al., 2003; Huttunen et al., 2007; Yang et al., 2005). Conversely, individuals with ASD have been found to show reduced MMN (Schwartz et al., 2018), while evidence of atypical P3a is not a consistent finding in ASD (Cui et al., 2016). However, some studies found indices of reduced automatic orienting of attention to speech-sounds (i.e., reduced P3a) in individuals with ASD (Whitehouse & Bishop, 2008). We therefore expected to find intact MMN and reduced/delayed P3a in children with ADHD, while we expected that children with ASD show an opposite profile, with intact P3a but reduced MMN amplitude. We predicted that children with comorbid ADHD+ASD would display an additive profile of deficits and would display reduced MMN amplitude, delayed P3a and reduced P3a amplitude.

Alpha oscillations

Alpha power has been proposed to reflect arousal, vigilance and engagement with a task or activity. While alpha oscillations during breaks from a mentally challenging task might be a non-specific index of cortical arousal, alpha oscillations during cognitive tasks are likely to be associated with other mechanisms. More specifically, when preceding the onset of a task-relevant stimulus, increased alpha activity (alpha synchronisation) might indicate efficient filtering of distracting information. Conversely, a decrease of alpha oscillatory activity after the presentation of task-relevant stimuli (alpha desynchronization) is likely to indicate information processing and orienting of attention. We therefore expected to find increased alpha during the POP task, compared to the breaks, and more specifically during the pre-cue

period (alpha synchronisation) compared to the post-cue temporal window (alpha desynchronization). We also predicted that increased alpha during the POP task would be associated with indices of reduced autonomic arousal, such as reduced CSI or increased CVI and RMSSD.

The presence of ADHD and ASD is likely to affect alpha oscillations. We therefore expected to find reduced alpha desynchronization (i.e., increased alpha) in children with ADHD, indicating weaker processing of cue-stimuli. We expected to find generally reduced alpha in ASD, probably more related to increased autonomic arousal. We then tested if children with comorbid ADHD+ASD showed an additive or interactive profile of atypicalities found in children with ADHD- and ASD-only.

Slope of changes in pupil size and SRTs

In line with the literature presented in paragraph 1.3.1, showing that task-related increases in baseline pre-stimulus pupil size are likely to reflect a switch towards the tonic exploratory mode, while a decrease of baseline pupil size is likely to reflect the LC functioning in the phasic mode and exploitation of task-related information, during the gap-overlap task we expected to find a time-related decrease in baseline pupil size in response to central visual objects, indicating a shift towards the exploitative LC mode as the task progresses. We therefore expected to find a negative slope of change in pupil size during the blocks of the gap-overlap task. Since the two blocks of the task were separated by a break, we investigated if the same pattern (i.e., negative slope of pupil size) was similarly present in both blocks. In parallel, we investigated if the negative slope of baseline pupil size change throughout the blocks was accompanied by any time-related changes in visual attentional performance and, more specifically, in SRTs.

We investigated if time-related changes in pupil size and SRTs were somehow affected by the presence of symptom of ADHD. In fact, based on the hypotheses that exploratory behaviours and reduced exploitation of task-related information might be predominant in ADHD, we expected that the time-related pupil size reduction would be more flattened in children with ADHD. In parallel, we also hypothesised that children with ADHD would show an overall worsening of performance over time, reflected in increased positive slope of change in SRTs during the gap-overlap task. Since we predicted that ASD should not affect these measures, we expected that children with comorbid ADHD+ASD would show a similar profile to children with ADHD-only.

Intra-individual reaction-times variability

We investigated intra-individual variability of SRTs (gap-overlap task) and of RTs (POP task), and we predicted to find increased SD-SRTs and SD-RTs in children with ADHD, in line with previous literature (see paragraph 1.3.1). Since previous research has demonstrated that RTV is not likely to be affected by ASD, we predicted that children with ASD-only would show a profile of RTV similar to typically developing controls, while children with comorbid ADHD+ASD would display increased RTV like children with ADHD-only.

2.4.2. Question 2. Are atypicalities in visual attention orienting more associated with ASD-symptomatology, than ADHD, and what is the profile of children with co-occurring ADHD+ASD?

The involvement of different neural mechanisms in the baseline and overlap conditions of the gap-overlap task, should result in reduced SRTs during the baseline condition, due to time-consuming processes of voluntary attentional disengagement and re-orienting (Kingstone & Klein, 1993; Reuter-Lorenz et al., 1991). This is usually referred to as the 'gap effect', indicating a facilitation to orient attention when attention orienting itself is exogenously driven by a sensory stimulus, compared to when endogenous mechanisms are involved. Social stimuli should also elicit shorter SRTs, compared to non-social stimuli, as widely reported in literature as a 'salience effect' of social stimuli that facilitate attention disengagement and orienting (Morand et al., 2010). Considered together, we expected to find a significant interaction between condition and stimulus, with longer SRTs to orient attention towards non-social stimuli, especially in the overlap condition. We also expected to find an effect of modality, with faster orienting of attention towards stimuli presented in the dynamic/multimodal condition, compared to the static/unimodal.

We expected to find signs of atypical visual attention orienting in children with ASD, especially in the overlap condition of the gap-overlap task. Despite scarcity of previous studies investigating visual attention in ADHD, we predicted that children with ADHD would show difficulties in voluntarily orient visual attention, probably associated to reduced functioning of fronto-parietal attentional systems responsible for voluntary disengagement and re-orienting of visual attention (see paragraph 1.4.3). While we expected that children with ASD would display slower orienting of attention in dynamic trials, due to difficulties in multi-sensory integration, and longer eye

movement latencies to orient visual attention to social stimuli, we did not expect children with ADHD to show atypicalities in these domains. We therefore expected that children with ADHD+ASD would display an additive profile of atypicalities that includes difficulties in voluntary orienting of attention, besides slower orienting of visual attention to social stimuli and in dynamic/multimodal trials.

2.4.3. Question 3. Are electrophysiological and behavioural measures of executive function and cognitive control more severely affected in children with co-occurring ADHD+ASD, compared to those with a single condition?

Electrophysiological and behavioural measures associated with cue-processing, response preparation and cognitive control, during the POP task, have been investigated. We predicted to find increased RTs in response to high- vs low-demand trials, reflecting the cognitive cost of inhibition of the prepotent response and the actuation of a motor response that is incongruent with the direction of the arrow-targets. We also expected that indices of performance speed and accuracy, especially in response to high-demand conditions, would be worsened by the presence of a diagnosis of ADHD or ASD and, at an even greater level, by the co-occurring presence of ADHD+ASD.

We expected that P3 amplitude in response to cue-stimuli (fixation cross) would be increased during high-demand trials, compared to low-demand. We also expected that target-stimuli (arrows) in the high-demand condition (i.e., when participants needed to inhibit the prepotent response in favour of the alternative) would elicit increased N2, index of conflict monitoring, and increased target-P3. We also predicted to find reduced cue-locked P3 in children with ADHD, with consequent reduced and delayed N2 and P3 in response to the arrow targets, in line with literature showing that these ERP components are likely to be affected in ADHD (Kaiser et al., 2020). We expected that children with ASD would display reduced N2 in response to the arrow targets, especially during high-demand trials, which was likely to be followed by reduced P3 (difficulties in conflict monitoring have in fact been reported in ASD by previous literature, see Panerai et al., 2016). We therefore predicted that children with comorbid ADHD+ASD would show reduced cue-P3 amplitude, like children with ADHD

(additive model), and a profile of even more reduced target-N2 and target-P3, compared

to children with ADHD- and ASD-only (interactive model).

2.4.4. Secondary investigations

Based on the theoretical models and rationale presented in Chapter 1, we aimed to test the presence of specific relations between measures of autonomic arousal, vigilance and alertness, visual attention, executive function and cognitive control, as following:

- Investigate the presence of any relations between measures of autonomic arousal, vigilance and alertness

- Investigate the presence of associations between indices of arousal and alertness, and visual attention mechanisms

- Investigate any relationships between indices of autonomic arousal, vigilance and alertness, and electrophysiological/behavioural measures of executive function and task performance

CSI and CVI have been proposed to be inversely related, so that higher CSI is usually an index of increased activation of the SNS and a predictor of reduced HRV, while CVI is likely to be an index of increased activation of the PNS and a predictor of higher HRV. In line with previous studies who supported this idea (e.g., see Bourdon et al., 2018; Oliveira, et al., 2019), we expected to find an inverse correlation between CSI and CVI, so that increased activity in one branch of the ANS (e.g., increased CSI) would be associated with reduced activity in the other branch (e.g., reduced CVI). We expected to find associations between HRV measures and alpha oscillations, so that increased alpha during the POP task might predict the presence of indices of reduced autonomic arousal, such as reduced CSI or increased CVI and RMSSD. Since the P3 is thought to specifically mirror activity of the LC-NE system, we investigated if higher P3a during the oddball task was associated with HRV measures collected during the same task.

We used a trial-by-trial approach to investigate the relation between baseline pupil size and SRTs during the gap-overlap task, to test if pupil size during fixation before an eye movement (index of tonic arousal and vigilance) could predict the latency of a saccade after the presentation of the peripheral stimulus, and if this differed in relation to the presence of ADHD and ASD. We also investigated the relations between measures of HRV (CSI, CVI and RMSSD) and executive functioning (performance speed and accuracy, cue-P3, target-N2 and target-P3).

Finally, a data-driven exploratory approach was used to analyse if clinical symptomatology was associated with specific profiles of autonomic arousal, alertness/vigilance and executive functioning measures. Since this question was predominantly addressed through an exploratory and descriptive approach, we did not have any predictions. However, we expected that children displaying more evident indices of dysregulated arousal (either hypo- or hyper-arousal) would show a profile characterised by more complex symptomatology (e.g., more severe comorbid symptoms, besides ADHD and ASD) and increasingly atypical electrophysiological and behavioural indices of attention and executive functioning.

Table 5. Summary of the hypotheses of the present study

Measure	Task	Task-related effects	ADHD	ASD	ADHD+ASD
CSI	POP task	Reduced during task vs breaks	Reduced	Increased	Interactive or additive effects?
CVI	POP task	Increased during task vs breaks	--	Reduced	Like ASD-only
RMSSD	POP task	Increased during task vs breaks	--	Reduced	Like ASD-only
CSI	Oddball task	Increased during task vs resting	Reduced	Increased	Interactive or additive effects?
CVI	Oddball task	Increased in block 2 vs block 1	--	Reduced	Like ASD-only
RMSSD	Oddball task	Increased in block 2 vs block 1	--	Reduced	Like ASD-only
P3a	Oddball task	Increased for deviant vs standard tones	Reduced amplitude Delayed latency	--	Like ADHD-only
MMN	Oddball task	Increased for social vs non-social stimuli	--	Reduced amplitude	Like ASD-only
Absolute/Relative alpha power (during breaks, pre-cue and post-cue temporal periods)	POP task	Increased during the POP task, compared to the breaks (pre-cue > post-cue)	Increased post-cue	Generally reduced	Interactive or additive effects?
Slope of change in baseline Pupil Size	Gap-overlap task	Within-block negative slope	Reduced negative slope	--	Like ADHD-only
Slope of change in SRTs	Gap-overlap task	Within-block positive slope	Increased positive slope	--	Like ADHD-only
Intra-individual variability of SRTs	Gap-overlap task		Increased RTV	--	Like ADHD-only

Measure	Task	Task-related effects	ADHD	ASD	ADHD+ASD
Intra-individual variability of RTs	POP task		Increased RTV	--	Like ADHD-only
SRTs	Gap-overlap task	Faster SRTs in baseline vs overlap trials; Faster SRTs for social vs non-social stimuli; Faster SRTs for social vs non-social stimuli, in overlap trials; Faster SRTs for dynamic vs static trials	Slower SRTs in overlap trials;	Slower SRTs in overlap trials; Slower SRTs to orient to social stimuli, especially in overlap trials; Slower SRTs in dynamic trials.	Additive effects
RTs (correct response)	POP task	Increased for high- vs low-demands trials	Slower RTs, especially in high-demands trials	Slower RTs, especially in high-demands trials	Interactive effect (slower RTs than ADHD-only and ASD-only)
% of correct responses	POP task		Reduced, especially in high-demand trials	Reduced, especially in high-demand trials	Interactive effect (reduced % than ADHD-only and ASD-only)
Cue-P3	POP task	Increased amplitude for high- vs low-demands trials	Reduced amplitude, especially during high-demand trials	--	Like ADHD-only
Target-N2	POP task	Increased amplitude for high- vs low-demands trials	Reduced amplitude Delayed latency	Reduced amplitude	Interactive effect (reduced amplitude than ADHD-only and ASD-only)
Target-P3	POP task	Increased amplitude for high- vs low-demands trials	Reduced amplitude Delayed latency	Reduced amplitude	

'--' indicates that no effect of ADHD or ASD was predicted for that specific measure

Chapter 3.   Results and discussion - Primary investigations

3.1. Question 1. Do indices of autonomic hypo-arousal, reduced vigilance and alertness, characterise children with ADHD, and how does the presence of a comorbid diagnosis of ADHD+ASD affect these measures?

The first research investigation was aimed at testing the presence of signs of autonomic hypo-arousal, reduced vigilance and alertness in children and adolescents with ADHD and with comorbid ADHD+ASD. In order to answer this first research question, I investigated changes in pupil size and in saccadic reaction times (SRTs) during the gap-overlap task, heart rate variability (HRV) during the oddball task and the POP task, and alpha power during the POP task, besides focusing on intra-individual variability of SRTs during the gap-overlap task and intra-individual variability of reaction times (RTs) during the POP task. Seven participants were not included in the analysis for the measures obtained in the gap-overlap task (see Table 6), because they did not carry out this task but completed the EEG session (n=3) or because they were excluded for not having a sufficient number of valid trials (n=4). Twenty-one participants were excluded from the final analyses for the oddball task, while twenty-three were excluded from the analyses of measures collected during the POP task, because they did not complete these experimental paradigms.

Table 6. Number of participants excluded from the final analyses, per group and for each task, and final sample size for each task

	TD	ADHD	ASD	ADHD+ASD	Final sample size
Gap-overlap task	2	3	0	2	99
Auditory oddball task	8	4	1	8	85
POP task	7	5	3	8	83

3.1.1. CSI, CVI and RMSSD

3.1.1.1. Mean HR

Although mean HR was not a primary outcome measure of the study, I analysed it before investigating CSI, CVI and RMSSD. In fact, the average number of beats per minute (BPM), in a certain period of time, has demonstrated to be somehow related with HRV measures (Shaffer & Ginsberg, 2017). For example, HRV seems reduced when HR is faster, while lower HR is associated with more variable fluctuations in heart rate, i.e., increased HRV. When comparing different groups on HR, higher HR has been usually interpreted as a sign of hyper-arousal, and lower HR as an index of hypo-arousal (Bellato et al., 2020).

A repeated measures ANOVA on average HR was carried out with *Task* (2-levels; oddball and POP task), as within-subjects factor, and *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ. There was a significant main effect of Task ($F_{1,57} = 6.349$; p = 0.015; $\eta_p^2 = 0.100$), indicating that HR was increased during the POP task (Mean HR = 87.65 BPM; S.E. = 1.32) compared to the oddball task (Mean HR = 84.63 BPM; S.E. = 1.35). However, we also found a significant main effect of ASD ($F_{1,57} = 5.625$; p = 0.021; $\eta_p^2 = 0.090$) and a marginally significant effect of ADHD ($F_{1,57} = 3.596$; p = 0.063; $\eta_p^2 = 0.059$) on HR. Altogether, as shown in Figure 10, during both tasks children with ADHD-only had lower average HR, compared to children with ASD-only and children with ADHD+ASD.
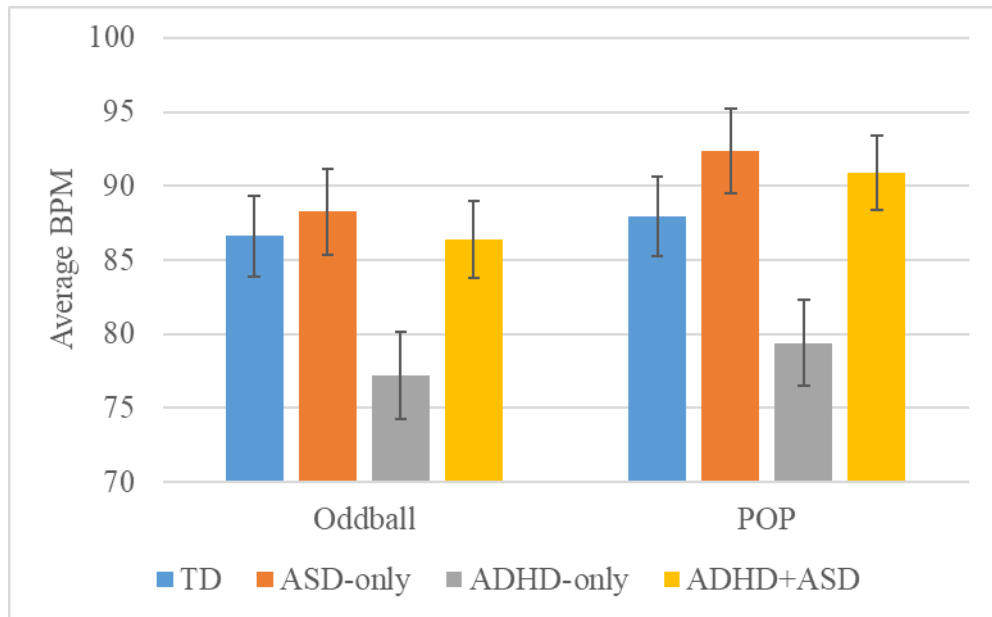
Figure 10. Average BPM in the oddball and POP tasks, across the four experimental groups. Error bars indicate the standard error (S.E.) of the mean

3.1.1.2. POP task

Three separate repeated measures ANOVA have been carried out on CSI, CVI and RMSSD measures collected during the POP task, with *Activity* (2-levels; task blocks and breaks), *Time* (2-levels; 1st part and 2nd part) and *Block* (4-levels; block 1 to 4) as within-subjects factors, and *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

CSI was increased during the breaks, compared to the blocks of the task (main effect of Activity: $F_{1,48} = 35.834$; $p < 0.001$; $\eta_p^2 = 0.427$; mean difference = 0.387; Figure 11), while there was no effect of Block or Time on CSI. There was a marginally significant effect of Activity on CVI ($F_{1,52} = 3.529$; $p = 0.066$; $\eta_p^2 = 0.064$; mean difference = 0.032), so that CVI was marginally increased during the breaks compared

to the task blocks, but the presence of this difference was not supported by follow-up Bayesian statistics analysis (BF10 = 0.974; anecdotal evidence for the absence of the effect). No other significant effects were found on CVI and RMSSD during the POP task.
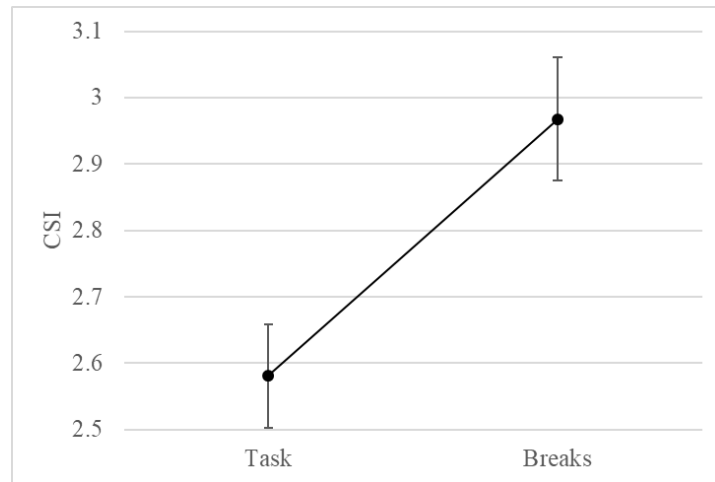
Figure 11. Comparison of CSI during the POP task and the breaks. Error bars indicate the standard error (S.E.) of the mean

A significant interaction Activity * Block * ASD ($F_{3,144}$ = 2.962; p = 0.034; η_p^2 = 0.058) was found on CSI, showing that children with ASD (ASD-only and ADHD+ASD), had increased CSI during the 3rd task block (mean difference = 0.312; p = 0.054) and during the 4th break (mean difference = 0.383; p = 0.051; Figure 12), compared to those without ASD (TD and ADHD-only). A just marginally significant interaction ADHD * Time ($F_{1,48}$ = 3.766; p = 0.058; η_p^2 = 0.073) was followed up and showed that in children with ADHD (ADHD-only and ADHD+ASD) there was a significant increase in CSI from Block 1 to Block 2, which was not present in children without ADHD (TD and ASD-only) (Figure 13).
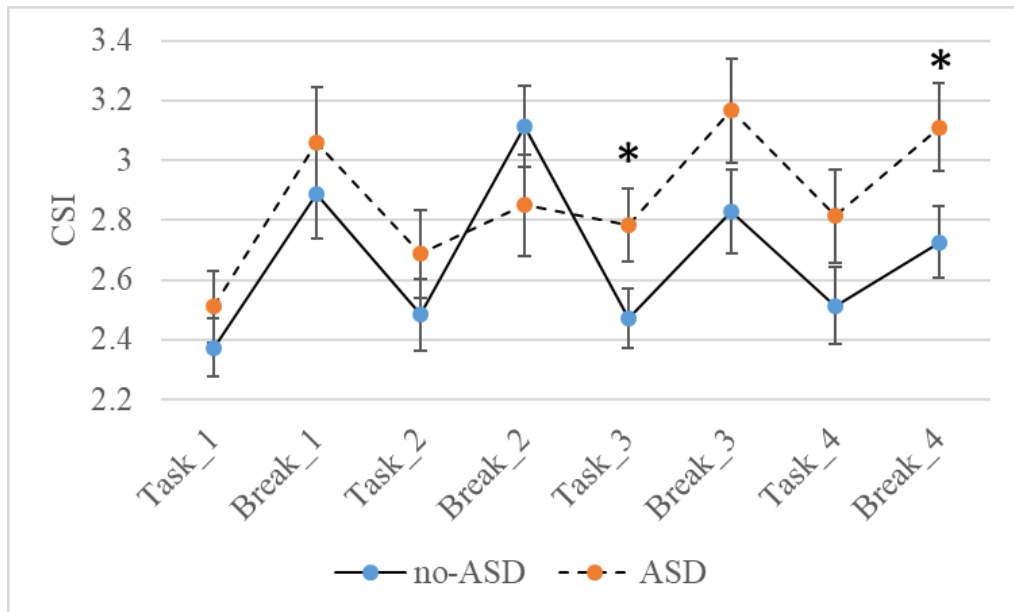
Figure 12. Comparison of CSI during the blocks and breaks of the POP task, in children with and without ASD. Error bars indicate the S.E. of the mean
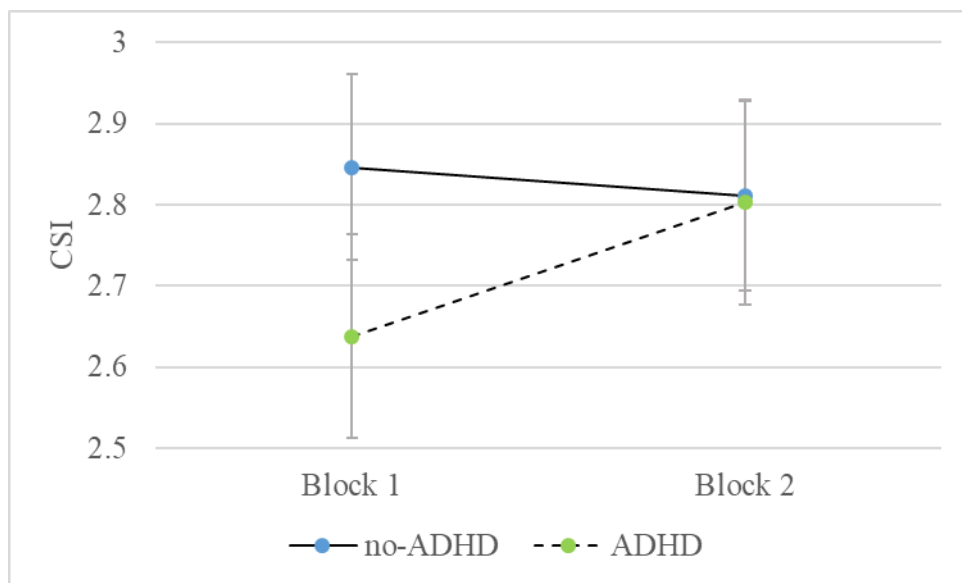
Figure 13. Comparison of CSI during the POP task, for blocks 1 and 2, in children with and without ADHD. Error bars indicate the S.E. of the mean

A significant main effect of ASD was found on CVI ($F_{1,52} = 4.895$; p = 0.031; $\eta_p^2 = 0.086$) and RMSSD ($F_{1,49} = 11.183$; p = 0.002; $\eta_p^2 = 0.186$). More specifically, children with ASD (ASD-only and ADHD+ASD) had reduced CVI (Table 7) and reduced RMSSD (Table 8) during the POP task, compared to children without ASD (TD and ADHD-only). Interestingly, there was an interaction Activity * ASD on RMSSD ($F_{1,49} = 5.622$; p = 0.022; $\eta_p^2 = 0.103$), indicating that while RMSSD was increased during the blocks of the task, compared to the breaks, in children without ASD (TD and ADHD-only; p = 0.009; $\eta_p^2 = 0.133$), this difference was not present in children with ASD (ASD-only and ADHD+ASD; p = 0.427; $\eta_p^2 = 0.013$) (Figure 14).

Table 7. Comparison of CVI values during the POP task, in children with and without ASD

	Mean	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ASD-no	4.804	0.061	4.682	4.925
ASD-yes	4.600	0.068	4.462	4.737
Difference	0.204	0.092	0.019	0.389

Table 8. Comparison of RMSSD values during the POP task, in children with and without ASD

	Mean	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ASD-no	60.623	3.705	53.177	68.068
ASD-yes	41.425	4.312	32.759	50.091
Difference	19.198	5.741	7.661	30.735
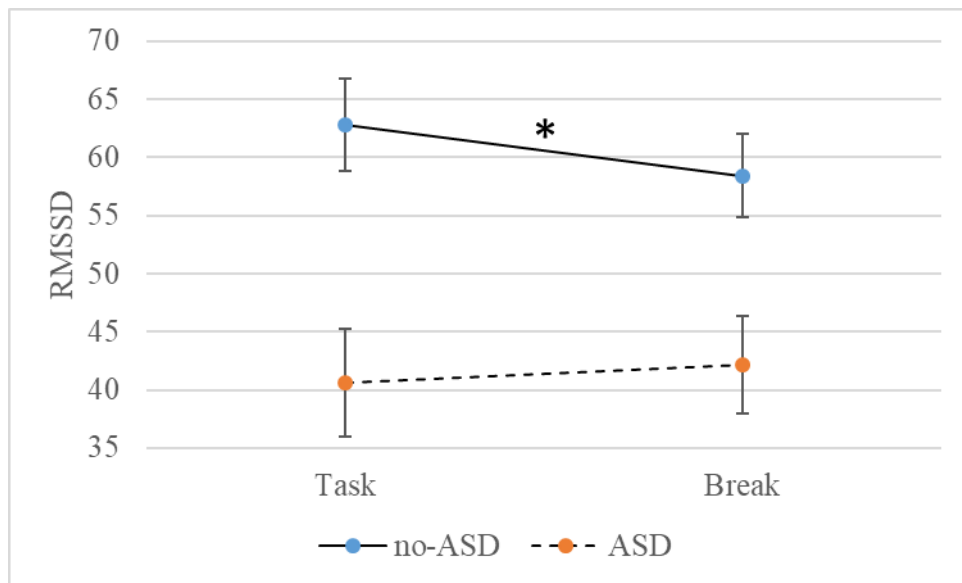
Figure 14. Comparison of RMSSD during the POP task and breaks, in chldren with and without ASD. Error bars indicate the S.E. of the mean

3.1.1.3.   Oddball task

We carried out three separate repeated measures ANOVA on CSI, CVI and RMSSD during the oddball task, with *Time* (2-levels; block 1 and block 2) and *Activity* (2-levels; resting blocks and oddball task blocks) as within-subjects factors, and *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

CSI was increased during the oddball task, compared to the 30-seconds resting blocks (effect of Activity: $F_{1,72} = 107.829$; $p < 0.001$; $\eta_p^2 = 0.600$; mean difference = 0.591), and it was increased in the second part of the task (Block 2) compared to the first (Block 1) (effect of Time: $F_{1,72} = 11.719$; $p = 0.001$; $\eta_p^2 = 0.140$; mean difference = 0.171) (see Figure 15). CVI was increased during the blocks of the task, compared to the resting blocks (effect of Activity: $F_{1,74} = 40.721$; $p < 0.001$; $\eta_p^2 = 0.355$; mean

difference = 0.119; see Figure 16), while the main effect of time on CVI was not significant ($F_{1,74}$ = 2.499; p = 0.118; η_p^2 = 0.033). No significant effect of Time ($F_{1,74}$ = 0.036; p = 0.850; η_p^2 < 0.001) or Activity ($F_{1,74}$ = 0.386; p = 0.536; η_p^2 = 0.005) was found on RMSSD.
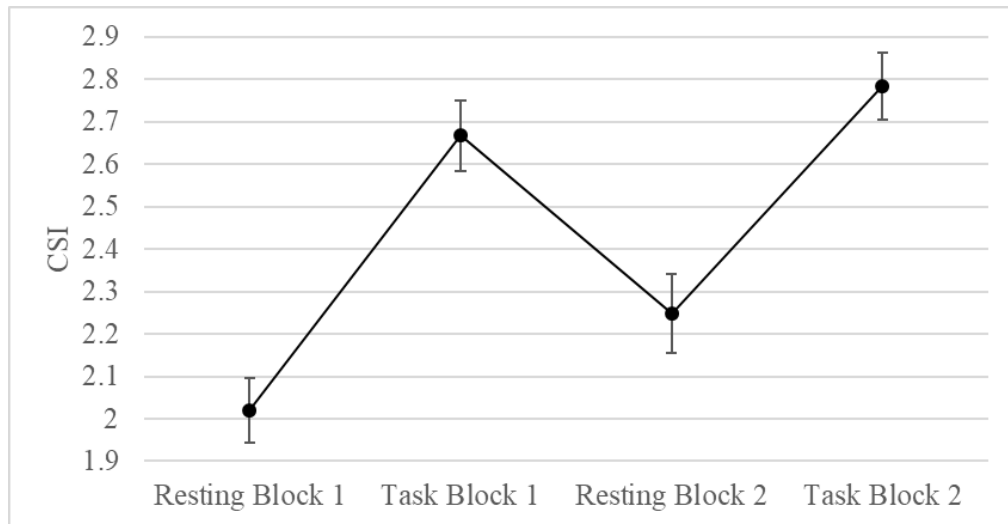
Figure 15. Comparison of CSI during resting and task blocks, for the first and second part of the oddball task. Error bars indicate the S.E. of the mean
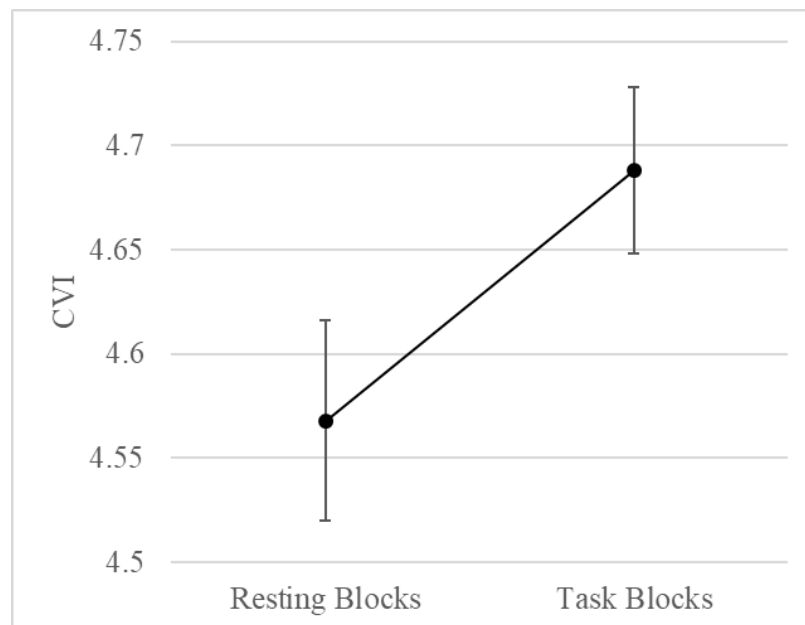
Figure 16. Comparison of CVI between resting and task blocks of the oddball task. Error bars indicate the S.E. of the mean

Besides finding a main effect of ADHD on CSI ($F_{1,72} = 4.786$; p = 0.032; $\eta_p^2 = 0.062$), showing that children with ADHD (ADHD-only and ADHD+ASD) had reduced CSI, compared to children without ADHD (TD and ASD-only; Table 9), we found a significant interaction between activity, ASD and ADHD factors ($F_{1,72} = 6.281$; p = 0.014; $\eta_p^2 = 0.080$). More specifically, during the 30-seconds-long resting blocks, children with ADHD-only had reduced CSI compared to typically developing controls (mean difference = 0.564; p = 0.033; BH-corrected), children with ASD-only (mean difference = 0.658; p = 0.018; BH-corrected) and children with ADHD+ASD (mean difference = 0.488; p = 0.036; BH-corrected) (Figure 17). We found a significant interaction Time * Activity * ASD on CVI ($F_{1,74} = 4.235$; p = 0.043; $\eta_p^2 = 0.054$), which showed that CVI was significantly reduced in children with ASD (ASD-only/ADHD+ASD), compared to children without ASD (TD/ADHD-only) during the first resting block (p = 0.033) and during the second block of the task (p = 0.038), and marginally significantly reduced during the first block of the oddball task (p = 0.058; BF10 = 0.819; anecdotal evidence against the presence of this effect) (Figure 18). There was a significant main effect of ASD on RMSSD during the oddball task ($F_{1,74} = 4.121$; p = 0.046; $\eta_p^2 = 0.053$) indicating that children with ASD (ASD-only/ADHD+ASD) had reduced RMSSD compared to children without ASD (TD/ADHD-only) during the oddball task (mean difference = 12.285) (Table 10).

Table 9. Comparison of CSI values during the oddball task, in children with and without ADHD

	Mean	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ADHD-no	2.588	0.103	2.384	2.793
ADHD-yes	2.271	0.098	2.076	2.466
Difference	0.317	0.145	0.028	0.607
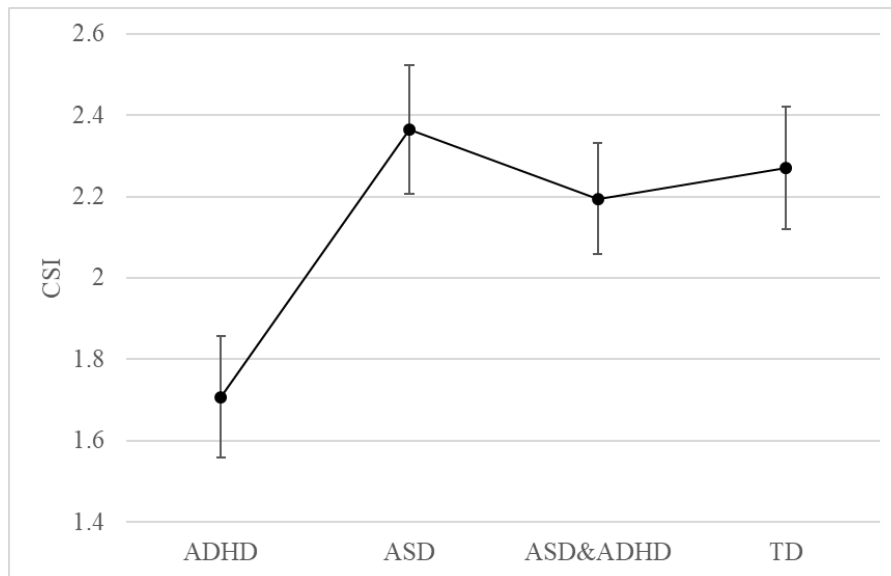
Figure 17. Comparison of CSI during the resting blocks (oddball task), in children with ADHD, ASD, ADHD+ASD, and typically developing (TD) children. Error bars indicate the S.E. of the mean
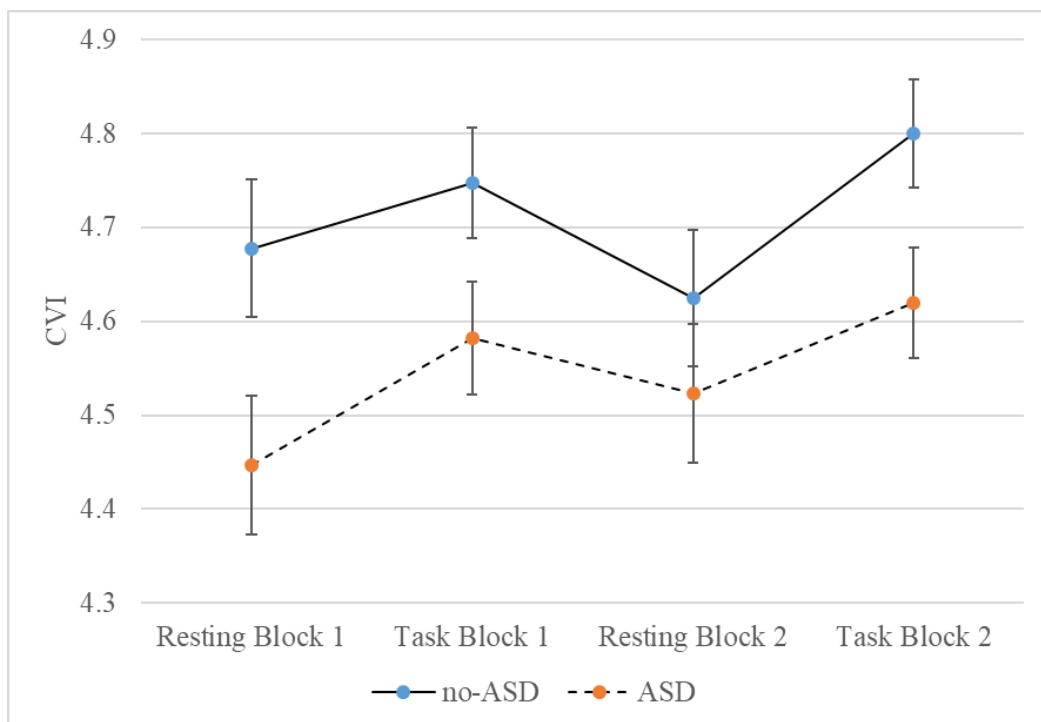

Figure 18. Comparison of CVI during the resting and task blocks of the oddball task, in children with and without ASD. Error bars indicate the S.E. of the mean

Table 10. Comparison of RMSSD values during the oddball task, in children with and

without ASD

	Mean	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ASD-no	59.550	4.104	51.372	67.728
ASD-yes	47.226	4.254	38.790	55.742
Difference	12.285	6.051	0.227	24.342

3.1.1.4.   Summary

Overall, these findings suggest that both the cardiac sympathetic (CSI) and the cardiac vagal (CVI) indices were increased during the blocks of the oddball task (i.e., during the presentation of auditory tones), compared to the 30-seconds resting blocks without sounds. During the POP task, CSI was instead increased during the 50-seconds breaks, compared to the blocks of the task. These findings partly confirmed our hypotheses and, as predicted, indicate that the task-to-breaks transition elicited an effect on heart rate, but this happened in a different way in the oddball and the POP tasks. More specifically, when passively listening to the auditory tones and watching the silent movie, children showed increased activation of the ANS (both for SNS and PNS mechanisms) compared to the resting blocks without sounds. This is likely to reflect the increased involvement of the ANS in facilitating the processing of sensory information (exploitation mode) (Aston-Jones & Cohen, 2005). During the POP task, activity of the ANS (more evidently, of the SNS) was reduced during the blocks of the task compared to the breaks, probably indicating reduced autonomic arousal during the active POP task. Increased activation and top-down control of frontal brain systems, involved in sustaining attention and maintaining a good level of performance during the POP task (Minzenberg et al., 2008), might have resulted in increased control over the ANS,

causing inhibition of the sympathetic branch and increased vagal control during the task.

Our findings about CSI and CVI seem to indicate a different involvement of the ANS in the passive auditory oddball task and in the more active POP task, suggesting that the task-to-break transition might trigger different changes in arousal based on the nature of the situation, i.e., if more passive and relaxed or more mentally challenging. It would be interesting to further investigate this and, more specifically, to verify in future studies if changes in CSI and CVI are directly associated with the amount of sensory stimulation and mental effort of the situation, and how the context affects indices of HRV in the task-to-rest transition. We carried out an exploratory analysis to investigate this (see Appendix A): we found that CSI was minimal during the breaks of the oddball task, followed by the blocks of the POP task, the blocks of the oddball task, and maximal during the breaks of the POP task. Similarly, CVI was minimal during the breaks of the oddball task, followed by the blocks of both oddball and POP task (where no differences were reported), and maximal during the breaks of the POP task. Although we had predicted that ANS activity would increase over time during the POP task, this was not confirmed by our data. However, our findings indicate that activity in the SNS increased throughout the oddball task, being higher in the second block compared to the first. This time-related increase of activity in the SNS partly reflects the effects of time on pupil size and SRTs reported in the gap-overlap task (see paragraph 3.1.4).

While we found evidence of reduced activity of the SNS during the oddball task in children with clinical symptoms of ADHD, reduced activity of the PNS was found in children with symptoms of ASD during some sections of the oddball task and during the entire POP task. Moreover, reduced HRV was found in children with ASD during both oddball and POP task and, interestingly, the modulation of HRV in the rest-to-task

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transition (increase of RMSSD from breaks to the POP task blocks) was not present in children with symptoms of ASD. In the last sections of the POP task, we also found increased activity of the SNS in children with symptoms of ASD. Children with comorbid ADHD+ASD displayed the same atypicalities found in children with 'pure' conditions, namely reduced CSI during the oddball task (like children with ADHD-only) and reduced CVI/RMMSD during the POP task (like children with ASD-only), supporting the *additive* theoretical model of ADHD/ASD comorbidity. Children with ADHD-only differed from both children with ASD-only and children with ADHD+ASD in showing reduced average HR. Moreover, they showed reduced activity of the SNS during the 30-seconds-long resting blocks of the oddball task, compared to children with co-occurring ADHD+ASD. This is likely to indicate that children with ADHD, but not with co-occurring ASD, might be particularly susceptible to low sensory stimulation and experience general hypo-arousal. Mentally challenging or more engaging situations, on the other side, may help children with ADHD to regulate autonomic arousal, in line with findings from our literature review (Bellato et al., 2020) and previous studies (see Groom et al., 2010, 2013; Liddle et al., 2011).

3.1.2. P3a and MMN – oddball task

The amplitude and latency of the P3a in response to auditory stimuli during the passive oddball task, were investigated through separate repeated measures ANOVA, where *Stimulus Type* (2-levels: standard and deviant) and *Time* (2-levels; block 1 and block 2) were the within-subjects factors, while *ADHD* and *ASD* (2-levels: yes/ no) were the between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

Fronto-central P3a amplitude was higher for deviant tones, compared to standard (effect of Stimulus Type: $F_{1,73}$ = 15.830; $p < 0.001$; η_p^2 = 0.178; mean difference = 0.287 µV) and this was not dependent on the type of deviant tone (social or non-social). We followed-up a marginally significant interaction Stimulus * Time ($F_{1,73}$ = 3.112; p = 0.082; η_p^2 = 0.041) which highlighted how the difference between standard and deviant was significant only during Block 1 ($p < 0.001$; η_p^2 = 0.156) and only marginally significant during Block 2 (p = 0.075; η_p^2 = 0.043; Figure 19).
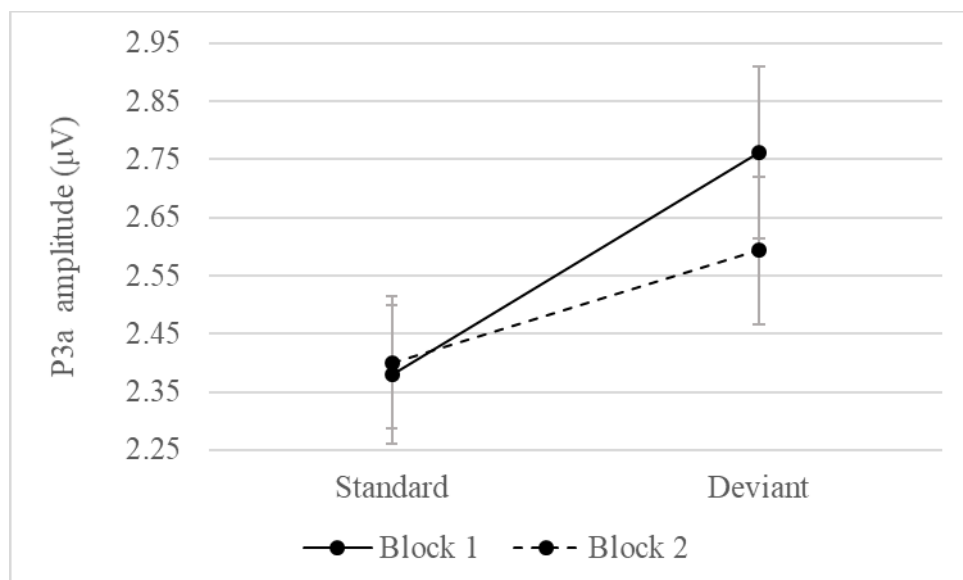
Figure 19. Comparison of P3a amplitude to standard and deviant stimuli, in block 1 and block 2 of the oddball task. Error bars indicate the S.E. of the mean

There was a significant main effect of ASD on P3a latency ($F_{1,74} = 6.086$; p = 0.016; $\eta_p^2 = 0.076$), indicating that P3a peaked earlier at fronto-central location in children with ASD (ASD-only and ADHD+ASD; mean latency = 333.278 msec; S.E. = 1.717) compared to children without ASD (TD and ADHD-only; mean latency = 339.367 msec; S.E. = 1.693) (Figure 20). The main effect of ASD on P3a amplitude was only marginally significant ($F_{1,73} = 2.870$; p = 0.094; $\eta_p^2 = 0.038$); however, when investigated through Bayesian statistics, anecdotal evidence against the presence of this effect was found (BF 10 = 0.798). No other main effects or interactions reached statistical significance (p < 0.05).
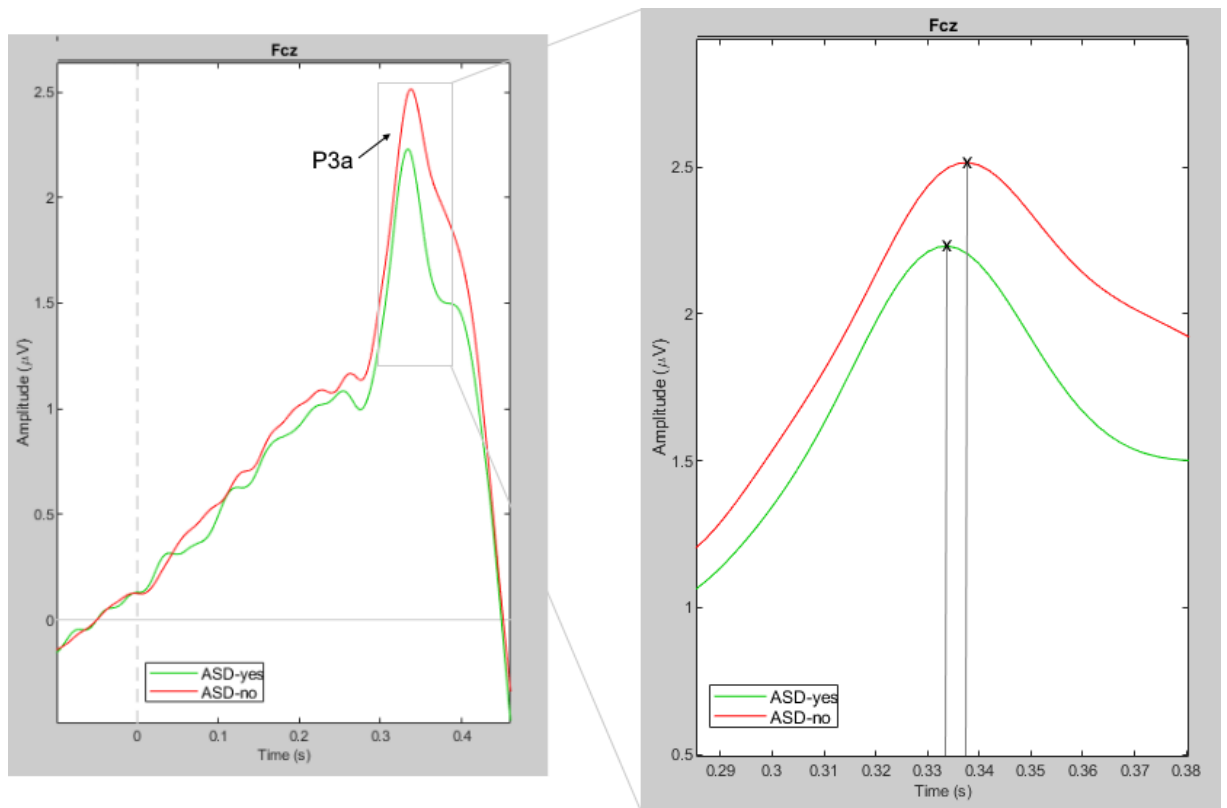
Figure 20. Visual representation of fronto-central P3a in response to auditory stimuli during the oddball task, compared between children with and without symptoms of ASD

We investigated amplitude and latency of the MMN through a repeated measures ANOVA, with *Time* (2-levels; block 1 and block 2) as the within-subjects factor, and *ADHD* and *ASD* (2-levels: yes/ no) as the between-subjects factors, controlling for the effects of age, gender, verbal and performance IQ. No significant main effect of time, ADHD and/or ASD was found on MMN latency and amplitude. However, we found a marginal effect of *Stimulus* (2-levels; social and non-social) on MMN amplitude ($F_{1,74} = 3.819$; $p = 0.054$; $\eta_p^2 = 0.049$), so that MMN negative amplitude was increased in the social condition (Table 11).

Table 11. Comparison of MMN amplitude to social and non-social deviant trials

	Mean (µV)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
Social	-0.761	0.097	-0.954	-0.567
Non-social	-0.515	0.076	-0.666	-0.363
Difference	0.246	0.126	-0.005	0.497

Overall, our findings indicate that the amplitude of the fronto-central P3a was higher for deviant tones, compared to standard, and this was not dependent on the type of deviant stimulus (social or non-social). This finding is in line with our hypotheses and previous studies, and suggests that the task design was appropriate and elicited the expected 'oddball effect'. The presentation of auditory stimuli that differed from the stream of standard tones, in fact, elicited an automatic increase in alerting and orienting of attention, as previously reported in literature (Yamaguchi & Knight, 1991). Since the difference in P3a amplitude between standard and deviant tones was higher during Block 1 than Block 2, we assume that children gradually habituated to the task. More specifically, the sequence of presentation of sounds had similar characteristics in the two blocks of the task, therefore children were already familiar with the structure of the

task when the second block started, probably giving rise to a slightly reduced 'oddball effect' in the second part of the task. We found that the amplitude of the MMN was increased in the social condition, compared to non-social, indicating that the discrimination between standard and deviant tones was higher if the deviant sound had social features, in line with previous findings (Iino et al., 2018). It could be that MMN amplitude was increased during the social condition because of the perceptual characteristics of the social deviants (more complex and with different formant frequencies, compared to the simpler non-social sounds). However, this version of the oddball task was not designed to have an intermediate condition between 'social' and 'non-social', so this could not be tested thoroughly.

The only significant result about the impact of clinical symptoms on electrophysiological measures of automatic orienting to auditory stimuli, was that the fronto-central P3a peaked earlier in children with ASD compared to children without ASD. It is interesting that a similar result (reduced latency of the parietal P3 in children with symptoms of ASD) was found when investigating the P3 during the POP task (see paragraph 3.3.2). This may therefore reflect generally increased alerting/vigilance and hyper-reactivity to sensory stimuli in children with ASD, which is in line with our results showing the presence of indices of hyper-arousal in ASD, and with previous literature discussing sensory processing atypicalities in this condition (see Robertson and Baron-Cohen, 2017).

3.1.3. Alpha power – POP task

We investigated absolute and relative alpha power, measured during the breaks of the POP task, in the 500-msec-long pre-cue temporal windows and in the 1500-msec-long post-cue/pre-target windows (for low- and high-demand trials). This has been done through a repeated measures ANOVA on alpha power, with *Condition* (4-levels; breaks, pre-cue, post-cue/low-demand, post-cue/ high-demand) and electrode *Position* (4-levels; Fz, Cz, Pz and Oz) as within-subjects factors, and *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

Absolute alpha power during the POP task was increased in the 500-msec-long pre-cue period, compared to the 1500-msec-long post-cue periods and the 50-seconds breaks from the task (effect of condition: $F_{3,62} = 38.761$; $p < 0.001$; $\eta_p^2 = 0.652$; pairwise comparisons: all $p < 0.001$; BH-corrected; Figure 21), while there was no difference between the two post-cue temporal windows (low- and high-demand; $p = 0.403$; BH-corrected). We also found a significant main effect of electrode position ($F_{3,62} = 25.316$; $p < 0.001$; $\eta_p^2 = 0.551$). More specifically, absolute alpha power was highest at Oz (Oz > Fz, Pz and Cz; all $p < 0.001$; BH-corrected; Figure 22) and lowest at Cz (alpha power on Cz < Fz, Pz and Oz; all $p < 0.001$; BH-corrected), while there were no differences between absolute alpha at Fz and Pz ($p = 0.258$; BH-corrected).

A significant effect of condition ($F_{3,61} = 4.009$; $p = 0.011$; $\eta_p^2 = 0.165$) and electrode position ($F_{3,61} = 26.056$; $p < 0.001$; $\eta_p^2 = 0.562$) was found on relative alpha power. More specifically, relative alpha was reduced in the 50-seconds breaks from the task, compared to both the 500-msec-long pre-cue period ($p = 0.018$; BH-corrected) and the 1500-msec-long post-cue periods (low-demand: $p = 0.012$; BH-corrected; high-demand: $p = 0.018$; BH-corrected), while it did not differ between the three task-related

conditions (all p = 0.748; BH-corrected; Figure 23). Relative alpha was maximal at Pz and Oz (no difference was found between these; p = 0.577; BH-corrected), compared to Cz and Fz (all p < 0.001; BH-corrected), while it was reduced at Fz compared to Cz (p = 0.013; BH-corrected; Figure 24). No significant main effect of ASD or ADHD was found on absolute and relative alpha power.
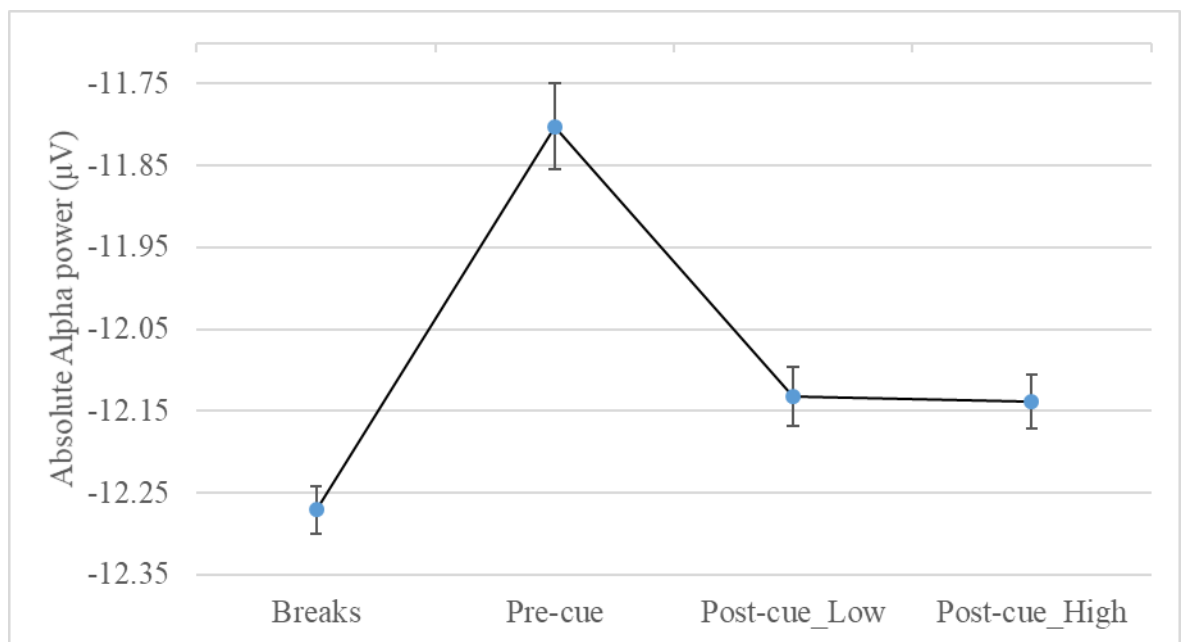
Figure 21. Absolute alpha power during breaks and POP task (pre- and post-cues).

Error bars indicate the S.E. of the mean
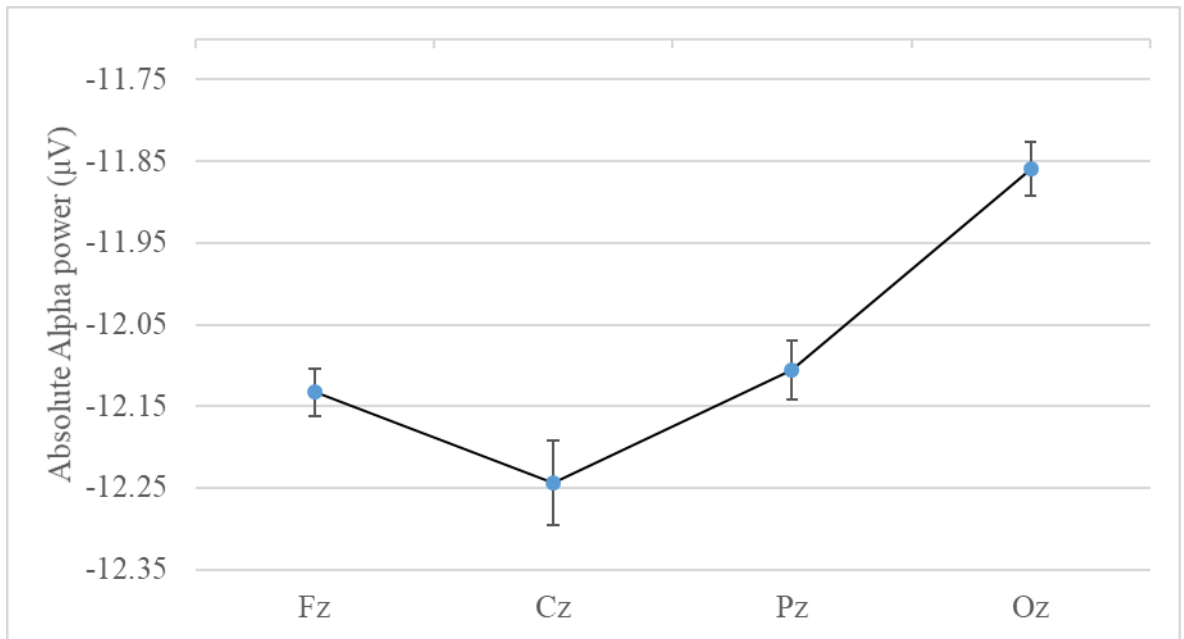
Figure 22. Absolute alpha power during the POP task, at different electrodes' position.

Error bars indicate the S.E. of the mean
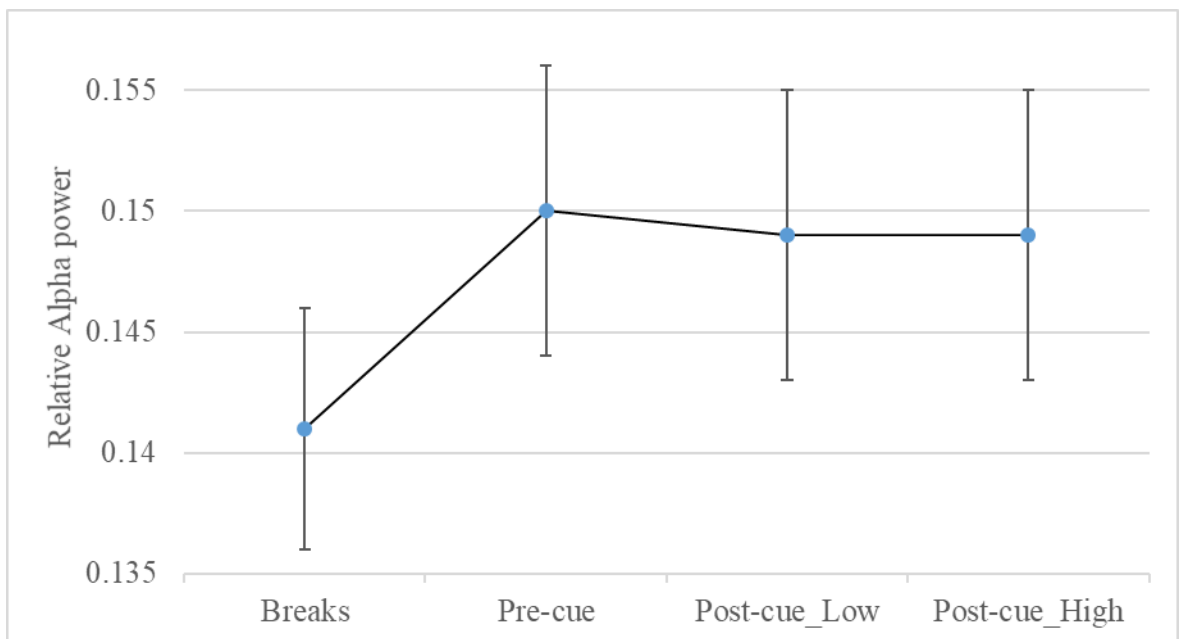
Figure 23. Relative alpha power during breaks and POP task (pre- and post-cues).

Error bars indicate the S.E. of the mean
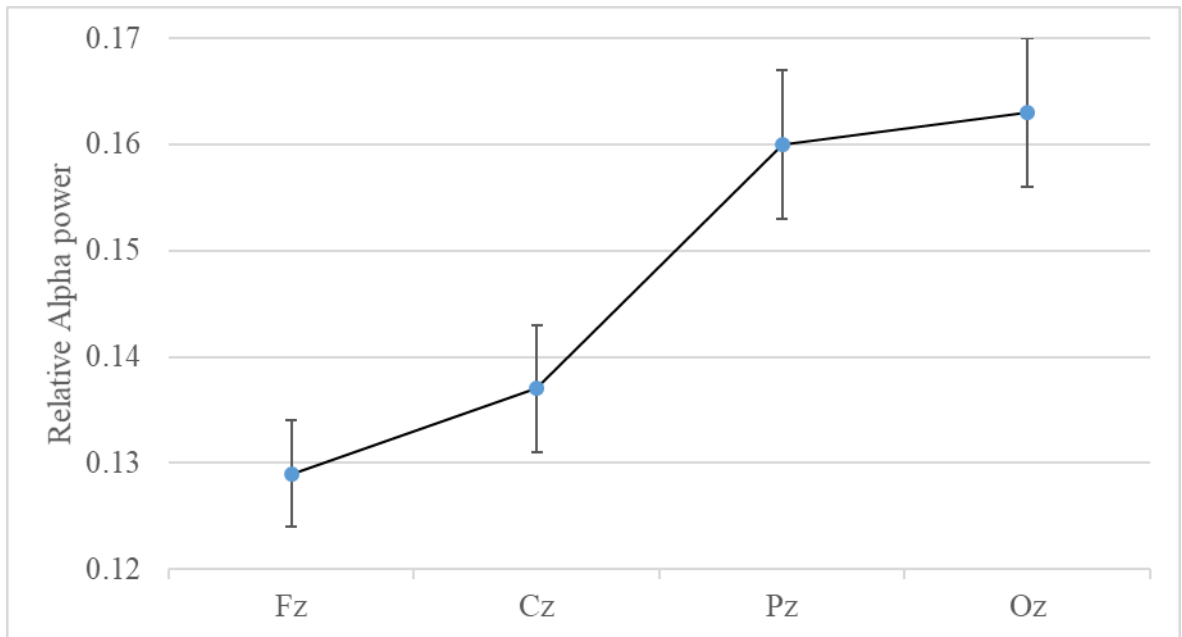
Figure 24. Relative alpha power during the POP task, at different electrodes' position.

Error bars indicate the S.E. of the mean

Our findings about alpha oscillations during the POP task showed that alpha power was reduced during the breaks and highest during the task, indicating an increase of spontaneous brain oscillations in the alpha band in the break-to-task transitions (in the opposite direction than ANS activity). Since alpha power has been proposed to reflect arousal, vigilance and engagement with a task, this finding is likely to indicate increased CNS activation in sustaining attention to the task, filtering task-unrelated information and prioritising the activation of cortical regions involved in processing task-relevant information (Van Diepen et al., 2019). Findings showing that alpha was increased before the onset of the cue-stimuli, than after the presentation, is in line with previous literature showing that expectation of task-relevant information elicit an increase in alpha oscillatory activity (alpha synchronisation), while alpha desynchronization after the presentation of informative stimuli is likely to indicate increased orienting of attention and information processing (Klimesch et al., 2007).

3.1.4. Pupil size and SRTs slope – gap-overlap task

In order to investigate if there were any changes in pupil size or SRTs associated with time, we carried out one-sample t-tests on the slope of change in pupil size and on the slope of changes in SRTs, for Block one and Block two of the gap-overlap task. We found that pupil size decreased throughout the first block of the task (mean slope = -1.77, SD = 4.91; $t_{(96)}$ = -3.55; p = 0.001), but not during the second block (mean slope = -0.71, SD = 4.21; $t_{(97)}$ = -1.66; p = 0.100). We also found that SRTs generally increased throughout both blocks of the task (block 1: mean slope = 0.88, SD = 2.96; $t_{(96)}$ = 2.93; p = 0.004; block 2: mean slope = 1.10, SD = 2.67; $t_{(97)}$ = 4.07; p < 0.001).

We investigated if there were any effect of ADHD and/or ASD on these measures, by carrying out two separate repeated measures analysis of variance (ANOVA) on pupil size and SRTs slope, with *ADHD* and *ASD* (2-levels: yes/ no) as the between-subjects factors and *time* as the within-subjects repeated measure (2 levels: block 1 and block 2). We controlled for the effects of age, gender, verbal and performance IQ. We found a significant effect of ADHD on the slope of pupil size change during the gap-overlap task ($F_{1,86}$ = 5.549; p = 0.021; η_p^2 = 0.061). More specifically, children with ADHD (ADHD-only/ADHD+ASD) showed a less negative and more flattened slope compared to children without ADHD (ASD-only/TD) (Table 12). The main effect of ADHD on SRTs slope was only marginally significant ($F_{1,86}$ = 3.081; p = 0.083; η_p^2 = 0.035), showing that the increase of SRTs over time was marginally steeper in children without ADHD, compared to children displaying symptoms of ADHD (Table 13). When investigating this effect through Bayesian statistics, we found weak evidence against the presence of this effect (BF10 = 0.932; anecdotal evidence).

Table 12. Comparison of the slope of changes in pupil size during the gap-overlap task, in children with and without ADHD

| | Mean | S.E. | 95% Confidence Interval | |
			Lower Bound	Upper Bound
ADHD-no	-1.964	0.506	-2.970	-0.959
ADHD-yes	-0.232	0.504	-1.234	0.770
Difference	1.732	0.735	0.270	3.194

Table 13. Comparison of the slope of changes in SRTs during the gap-overlap task, in children with and without ADHD

| | Mean | S.E. | 95% Confidence Interval | |
			Lower Bound	Upper Bound
ADHD-no	1.418	0.310	0.802	2.034
ADHD-yes	0.637	0.304	0.032	1.242
Difference	0.781	0.445	-0.103	1.701

These findings are partly in line with our initial hypotheses. Pupil size decreased over time, throughout the first block of the gap-overlap task, while SRTs generally increased over time throughout both blocks of the task. The time-related decrease in pupil size, accompanied by an increase in SRTs, is likely to indicate a reduction in tonic activity of the LC (Rajkowski, 1993; Murphy et al., 2014), probably indicating a gradual switch from the 'exploration' (tonic) mode to the 'exploitation' (phasic) mode. Our evidence seems to indicate that this switch was costly, and it was accompanied by a worsening of attentional performance over time. However, more research is needed to elucidate the relation between pupil size and SRTs, and to investigate the bidirectional influence between fluctuations in pupil size and eye movement latencies.

We had predicted that children with symptoms of ADHD would be more likely to display generally reduced allocation of attentional resources to the task, and that this would be reflected in a more flattened (less negative) slope of change in pupil size and

an increased slope of change in SRTs, during each block of the task. Interestingly, our findings partly confirmed these hypotheses, suggesting that children with symptoms of ADHD might have been allocating a reduced amount of attentional resources to the gap-overlap task over time (reflected in reduced negative pupil size slope). However, this was accompanied by an only marginal difference between children with or without ADHD on the increase of SRTs over time. It could therefore be that exploratory behaviours were more frequent in children with ADHD, while exploitation of task-related information was increased in those without ADHD, but there was not a clear effect of ADHD symptoms on time-related changes in eye movement latencies. Moreover, as initially predicted, these effects were not associated with the presence of symptoms of ASD, and children with ADHD+ASD showed a similar profile than children with ADHD-only.

The fact that we did not find a significant decrease in pupil size in the second block, is a limitation. However, this may be associated with the fact that the second block of the task was not different from the first in terms of structure and progression, therefore children might have become confident with the experimental situation during the first block. If this was the case, any time-related effects on the investigated measures might be reduced. Despite this limitation, we found some evidence showing that using time-related changes in pupil size might be useful to investigate fluctuations of arousal and vigilance in people with ADHD.

3.1.5. Intra-individual variability in reaction times

We investigated our hypotheses about RTV by carrying out, firstly, a univariate ANOVA on the standard deviation of SRTs (SD-SRTs) for the gap-overlap task and, secondly, a univariate ANOVA on the standard deviation of RTs (SD-RTs) for the POP task, with *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

When investigating the intra-individual variability of SRTs, there was a marginally significant main effect of ADHD ($F_{1,89}$ = 2.941; p = 0.090; η_p^2 = 0.032). When investigating this through Bayesian statistics, we found anecdotal evidence against the presence of this main effect, and therefore it was not followed up (BF10 = 0.914). The main effect of ADHD was significant on SD-RTs during the POP task ($F_{1,68}$ = 9.221; p = 0.003; η_p^2 = 0.119) and, more specifically, children with ADHD (ADHD-only and ADHD+ASD) had increased variability in RTs, compared to children without ADHD (TD and ASD-only) (Table 14).

Table 14. Comparison of SD-RTs values during the POP task, in children with and without ADHD

	Mean (msec)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ADHD-no	276.471	10.506	255.507	297.435
ADHD-yes	321.114	9.623	301.912	340.315
Difference	44.643	14.702	15.306	73.979

Although we found no effect of ASD or ADHD on intra-individual variability of SRTs, the reaction-time-variability of motor responses during the POP task was affected by the presence of symptoms of ADHD, so that intra-individual variability in RTs was increased in children with ADHD compared to children without ADHD.

Children with co-occurring ADHD+ASD were not different from children with ADHD-only, therefore our findings are in line with previous evidence showing that increased RTV is specific to ADHD and reflects difficulties in maintaining an optimal level of vigilance and alertness to the environment which are not directly associated with the presence of symptoms of ASD (Adamo et al., 2019; Karalunas et al., 2014; Kofler et al., 2013; Lundervold et al., 2016; Tye et al., 2016).

3.1.6. Overall summary of Question 1

Based on the findings presented in the present paragraph, we found evidence in support of the hypothesis that hypo-arousal, reduced vigilance and alertness are associated with ADHD. These atypicalities seem to mainly derive from under-functioning of the ANS (which might be causing reduced activity of the SNS and reduced changes in baseline pupil size) and increased variability in response reaction times. Conversely, signs of hyper-arousal have been found associated with ASD, ad these seem to derive from reduced functioning of the parasympathetic nervous system, which could cause increased arousal, reduced HRV and increased sensory reactivity in children with ASD. The co-occurring presence of ADHD and ASD seems to be associated with an additive profile of these atypicalities separately reported in ADHD and ASD. The implications of these findings will be further discussed in paragraph 5.2, in light of results of the other two primary investigations (see paragraphs 3.2 and 3.3) and the secondary analyses (paragraphs 4.1 and 4.2).

3.2. Question 2. Are atypicalities in visual attention orienting more associated with ASD-symptomatology, than ADHD, and what is the profile of children with co-occurring ADHD+ASD?

The second investigation was aimed at verifying the presence of deficits in visual attention orienting in people with ASD, which we expected not to be mainly associated with ADHD, even in the comorbid group. We therefore analysed eye movement latencies, i.e., SRTs, in the gap-overlap task. Although our findings indicate that this task elicited the predicted effects in our sample of children and adolescents, most of the hypotheses related to clinical symptoms of ADHD and ASD were not supported and are further discussed in this paragraph.

We analysed SRTs through a repeated measures ANOVA, with *Condition* (2-levels; baseline and overlap trials), type of peripheral *Stimulus* (2-levels; social and non-social stimulus) and *Modality* of presentation (2-levels; static/unimodal and dynamic/multimodal) which were added to the model as within-subjects factors, while *ADHD* and *ASD* (2-levels: yes/ no) were included as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

As predicted, SRTs were reduced, indicating faster orienting of attention, during baseline trials, compared to overlap (effect of Condition: $F_{1,86} = 217.180$; $p < 0.001$; $\eta_p^2 = 0.716$; Table 15) and towards social stimuli, compared to non-social (effect of Stimulus: $F_{1,86} = 77.372$; $p < 0.001$; $\eta_p^2 = 0.474$; Table 16). We then investigated if there was any effect of ADHD and/or ASD on SRTs. We found a significant interaction between Modality and ADHD ($F_{1,86} = 7.575$; $p = 0.007$; $\eta_p^2 = 0.081$): only children without ADHD (TD and ASD-only) displayed a significant difference between SRTs in static/unimodal and dynamic/multimodal trials (i.e., orienting of attention was slower

in static trials, vs dynamic; mean difference = 20.729 msec; p = 0.004), while in children with ADHD (ADHD-only and ADHD+ASD) this effect was not present (mean difference between static and dynamic trials = 6.591 msec; p = 0.329; Figure 25). No other main effects or interactions resulted statistically significant (p > 0.05).

Table 15. Comparison of SRTs during baseline and overlap trials

	Mean (msec)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
Baseline	193.027	4.476	184.128	201.925
Overlap	292.301	9.238	273.937	310.665
Difference	99.274	6.736	85.883	112.666

Table 16. Comparison of SRTs to social and non-social stimuli.

	Mean (msec)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
Social	220.815	6.306	208.279	233.351
Non-social	264.512	7.433	249.736	279.289
Difference	43.697	4.968	33.821	53.573
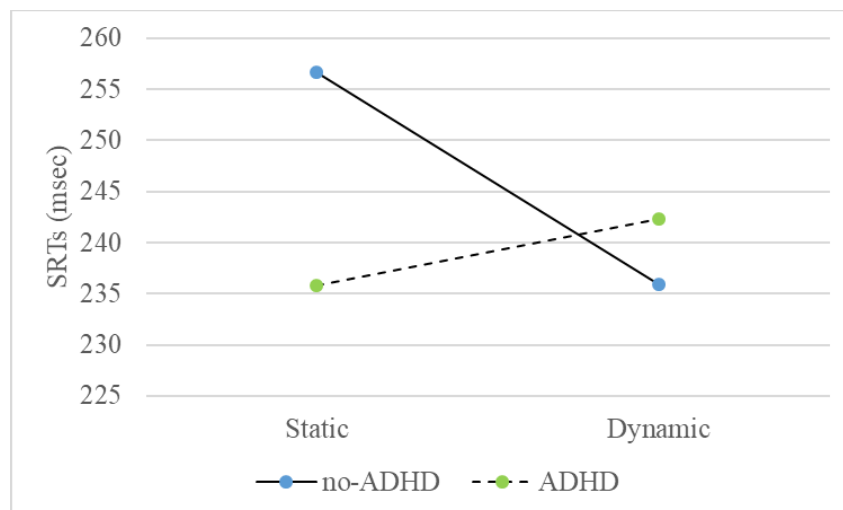
Figure 25. Comparison of SRTs in static and dynamic trials, in children with and without ADHD

Summarising, orienting of visual attention was faster during baseline trials, compared to overlap, and towards social stimuli, compared to non-social. This is in line with our expectations and suggest the involvement of different neural systems in the gap-overlap task, so that reflexive orienting of attention elicited faster eye movements, compared to voluntary orienting which was more time-consuming, as previously reported in literature (Kingstone & Klein, 1993; Reuter-Lorenz et al., 1991). Similarly, social stimuli were more salient than non-social, and children were faster to orient their visual attention towards the face stimuli, compared to the non-social three-dimensional objects, replicating previous findings (Morand et al., 2010).

Unexpectedly, we did not find any clear effect of neither ASD or ADHD on SRTs during the gap-overlap task, apart from the fact that only children without ADHD displayed a significant difference between SRTs in static and dynamic trials, so that orienting of visual attention was slower in static trials, compared to dynamic. It is difficult to draw any firm conclusions from this result, which might derive from the fact that children with ADHD could have been generally less engaged with the experimental situation. This conjecture is partly sustained by our findings about time-related changes in pupil size during the gap-overlap task (see paragraph 3.1.4), which suggested how children with ADHD seemed to generally allocate less attentional resources to the task. However, I am aware that this interpretation may be perceived as speculative and should therefore be further verified in future research. Overall, evidence of atypical oculomotor mechanisms in our sample of children with ADHD and/or ASD was not found, and this did not support our initial hypotheses.

3.3. Question 3. Are electrophysiological and behavioural measures of executive function and cognitive control more severely affected in children with co-occurring ADHD+ASD, compared to those with a single condition?

The third investigation was aimed at analysing behavioural and electrophysiological indices of executive functions and cognitive control, and their association with ADHD and ASD-symptomatology. We also investigated at what level executive function atypicalities were found in children with co-occurring ADHD+ASD, and which model (additive or interactive) was better supported by our data.

3.3.1. RTs and percentage of correct responses – POP task

Indices of performance during the POP task, including RTs in correctly performed trials and percentage (%) of overall correct responses, have been investigated in order to clarify the third research question. More specifically, separate repeated measures ANOVA on RTs and % of correct responses, were carried out, with *Cognitive Demand* (2-levels; low- and high-demand trials) as within-subjects factor, while *ADHD* and *ASD* (2-levels: yes/ no) were included as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

RTs in the POP task were affected by the trial type, as predicted, so that high-demand trials (which included an incongruency between the direction of the arrow and the required manual response) elicited increased RTs, compared to low-demand trials (where the response was done congruently with the arrow direction) (effect of Cognitive Demand: $F_{1,68} = 10.966$; $p = 0.001$; $\eta_p^2 = 0.139$; Table 17). RTs were significantly longer in children with ADHD (ADHD-only and ADHD+ASD; mean = 901.094 msec; S.E. =

21.334) compared to children without ADHD (TD and ASD-only; mean = 823.774 msec; S.E. = 23.292) (effect of ADHD: $F_{1,68}$ = 5.627; p = 0.021; η_p^2 = 0.076; mean difference = 77.321 msec; S.E. = 32.595; 95 % C.I. = [12.279 – 142.362]). This effect was better interpreted when investigating a significant interaction ADHD * ASD ($F_{1,68}$ = 4.236; p = 0.043; η_p^2 = 0.059), which showed that TD children could be distinguished from children of the three clinical groups, who generally showed slower RTs than TD, as following:

- Typically developing children had reduced RTs compared to children with ASD-only (mean difference = 97.050; p = 0.042; 95 % C.I. = [3.798 – 190.301]) and compared to children with ADHD-only (mean difference = 141.501; p = 0.002; 95 % C.I. = [51.807 – 231.196]) (Table 18);

- There were no differences between children with ASD-only and comorbid ADHD+ASD (mean difference = 13.140; p = 0.772; 95 % C.I. = [-77.194 – 103.474]), or between children with ADHD-only and comorbid ADHD+ASD (mean difference = 31.312; p = 0.464; 95 % C.I. = [-53.615 – 116.238]) (Figure 26).

Table 17. Comparison of RTs to low- and high-demand trials.

	Mean (msec)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
Low	849.975	14.864	820.315	879.635
High	874.892	16.549	841.870	907.914
Difference	24.917	7.524	9.903	39.931

Table 18. Summary of RTs in the four experimental groups

| | Mean | | 95% Confidence Interval | |
	(msec)	S.E.	Lower Bound	Upper Bound
TD	775.249	30.611	714.166	836.332
ASD-only	872.298	35.213	802.031	942.565
ADHD-only	916.750	31.677	853.540	979.960
ADHD+ASD	885.438	28.505	828.557	942.319
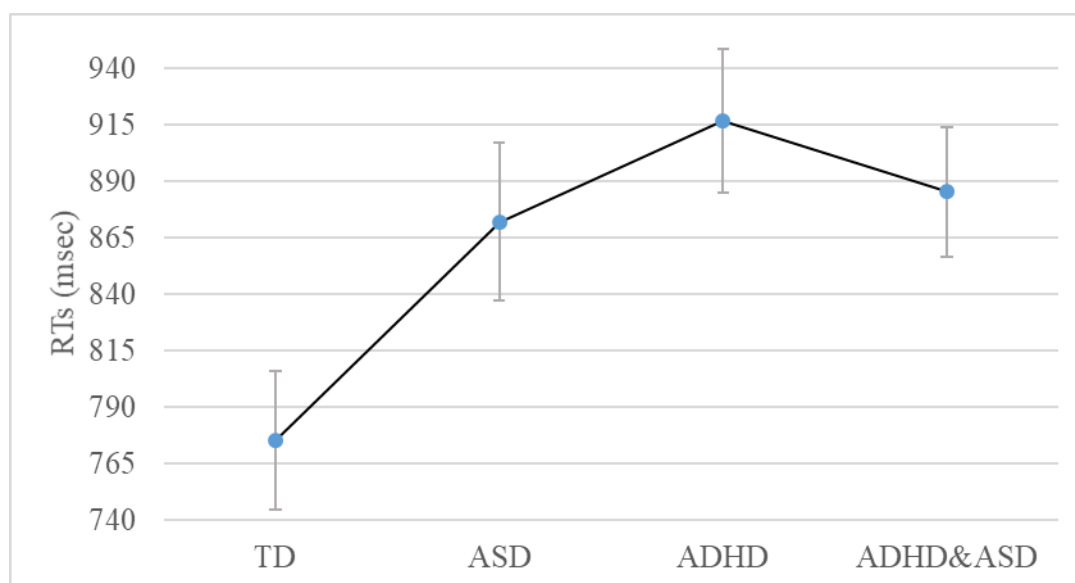
Figure 26. Comparison of RTs during the POP task in children with ADHD, ASD, ADHD+ASD and typically developing controls. Error bars indicate the S.E. of the mean

While the percentage of correct responses was not dependent on the trial type (effect of Demand: $F_{1,68} = 0.006$; $p = 0.936$; $\eta_p^2 < 0.001$), there was a significant main effect of ASD ($F_{1,68} = 6.009$; $p = 0.017$; $\eta_p^2 = 0.081$) on this measure. Children with ASD (ASD-only and ADHD+ASD) had a reduced percentage of correct responses during the POP task, compared to children without ASD (TD and ADHD-only). There was also a marginally significant effect of ADHD ($F_{1,68} = 3.123$; $p = 0.082$; $\eta_p^2 = 0.044$), which was further investigated with Bayesian statistics. This analysis showed that there

was strong evidence in support of the presence of this effect (BF10 = 39.234), indicating that children with ADHD (ADHD-only and ADHD+ASD) had a reduced percentage of correct responses, compared to children without ADHD (TD and ASD-only) (mean difference = 5.9 %; S.E. = 3.4; 95 % C.I. = [-0.8 ; 12.6]). We therefore tried to disentangle these two simultaneously present effects (main effect of ADHD and of ASD) and found that:

- There was no significant difference between typically developing children and children with ADHD-only (mean difference = 4.7 %; p = 0.309; 95 % C.I. = [-4.5 – 14.0]), and between TD children and children with ASD-only (mean difference = 6.9 %; p = 0.157; 95 % C.I. = [-2.7 – 16.5]) (Table 19);

- There was a significant difference between children with ADHD-only and children with comorbid ADHD+ASD (mean difference = 9.3 %; p = 0.038; 95 % C.I. = [0.5 – 18.0]), so that children with comorbid ADHD+ASD showed reduced percentage of correct responses than those with ADHD-only (Figure 27).

Table 19. Summary of % of correct responses in the four experimental groups

| | Mean | | 95% Confidence Interval | |
	(%)	S.E.	Lower Bound	Upper Bound
TD	83.00	3.20	76.70	89.20
ASD-only	76.10	3.60	68.8	83.30
ADHD-only	78.20	3.30	71.70	84.70
ADHD+ASD	68.90	2.90	63.10	74.80
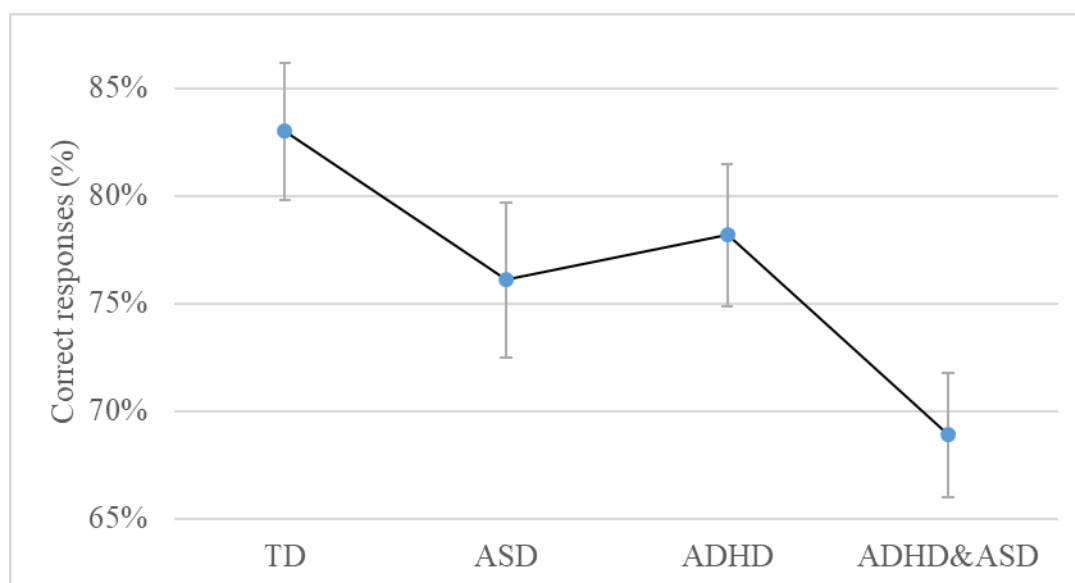
Figure 27. Comparison of the percentage of correct responses to the POP task trials, in children with ADHD, ASD, ADHD+ASD and typically developing controls. Error bars indicate the S.E. of the mean

3.3.2. P3 and N2 – POP task

The electrophysiological measures investigated in the POP task included the latency and amplitude of the parietal P3 in response to cue stimuli, the fronto-central N2 and the parietal P3 in response to target stimuli. These measures were investigated through separate repeated measures ANOVA, where *Cognitive Demand* (2-levels; low-

and high-demand) was the within-subjects factor, while *ADHD* and *ASD* (2-levels: yes/ no) were the between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

While no significant effect of Cognitive Demand (low vs high) was found for the amplitude of the cue-P3, the target-N2 and the target-P3, there was a significant effect of Cognitive Demand on the latency of the parietal cue-locked P3 ($F_{1,68} = 11.920$; $p = 0.001$; $\eta_p^2 = 0.149$), the latency of the fronto-central target-locked N2 ($F_{1,67} = 12.070$; $p = 0.001$; $\eta_p^2 = 0.153$) and the latency of the parietal target-locked P3 ($F_{1,68} = 3.979$; $p = 0.050$; $\eta_p^2 = 0.055$). More specifically:

- Cue-locked P3 peaked earlier on high-demand (mean = 340.007 msec; S.E = 3.721) compared to low-demand trials (mean = 346.954 msec; S.E = 3.858) (Figure 28)

- Target-locked N2 peaked earlier during low-demand (mean = 167.335 msec; S.E = 1.234) than high-demand trials (mean = 170.514 msec; S.E = 1.250) (Figure 29)

- Target-locked P3 peaked earlier during low-demand (mean = 350.503 msec; S.E = 3.691) than high-demand trials (mean = 354.118 msec; S.E = 3.813) (Figure 30)
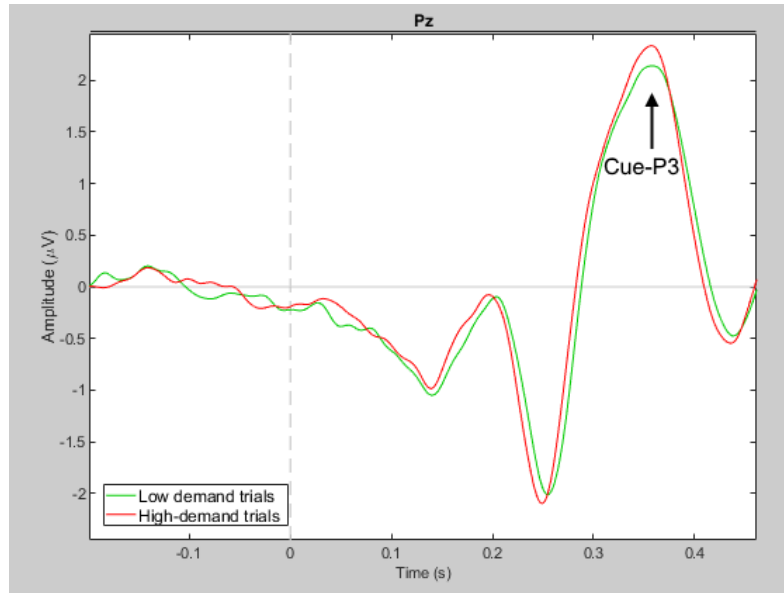
Figure 28. Visual representation of parietal P3 in response to cue-stimuli during low-

and high-demand trials of the POP task
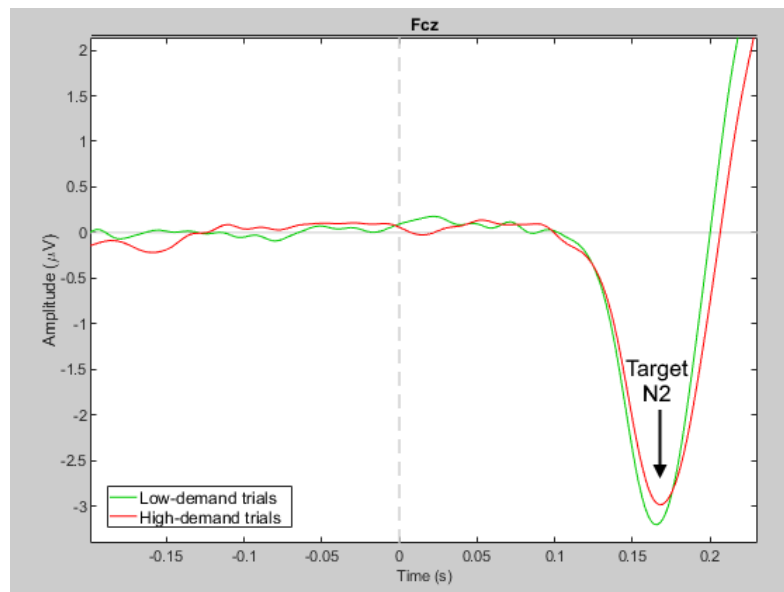
Figure 29. Visual representation of fronto-central N2 in response to target-stimuli

during low- and high-demand trials of the POP task
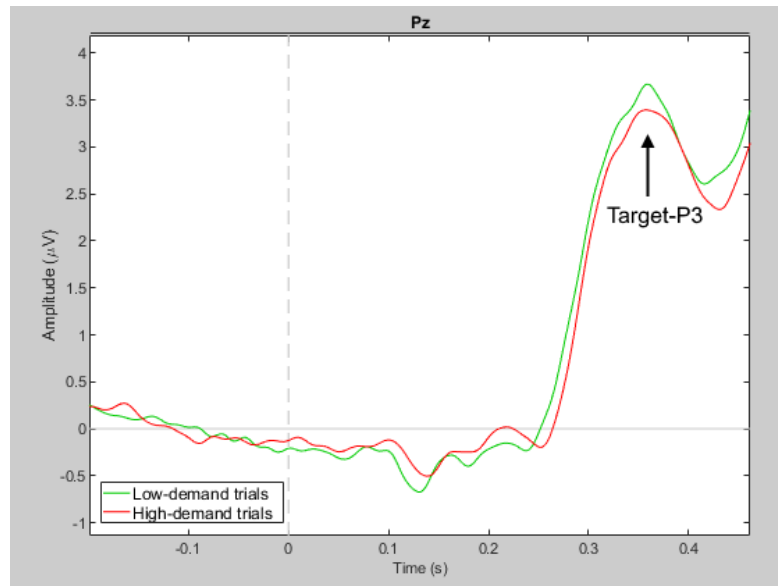
Figure 30. Visual representation of parietal P3 in response to target-stimuli during
low- and high-demand trials of the POP task

Although no effect of ASD and/or ADHD was found on target-P3 latency or amplitude, we found a significant main effect of ASD on cue-P3 latency ($F_{1,68} = 5.789$; $p = 0.019$; $\eta_p^2 = 0.078$), cue-P3 amplitude ($F_{1,67} = 11.914$; $p = 0.001$; $\eta_p^2 = 0.151$) and target-N2 latency ($F_{1,67} = 5.219$; $p = 0.026$; $\eta_p^2 = 0.072$). More specifically, the parietal P3 in response to cues peaked earlier in children with ASD (ASD-only and ADHD+ASD), compared to children without ASD (TD and ADHD-only) (Table 20 and Figure 31), and it had reduced amplitude in children with ASD, compared to children without ASD (Figure 31). Furthermore, the fronto-central N2 in response to targets had longer latencies in children with ASD than children without ASD (Table 21 and Figure 32). Following up a marginally significant interaction Cognitive Demand * ADHD on target-N2 amplitude ($F_{1,68} = 3.323$; $p = 0.073$; $\eta_p^2 = 0.047$), showed that children with ADHD (ADHD-only and ADHD+ASD) had reduced target-locked N2 (less negative amplitude) during high-demand trials, compared to children without

ADHD (TD and ASD-only) (mean difference = 1.108 µV; S.E. = 0.549; p = 0.048; 95

% C.I. for difference = [0.012 – 2.204]; Figure 33 and Figure 34). Interestingly, during

low-demand trials the difference between children with ADHD and children without

ADHD on target-N2 amplitude was not significant (mean difference = 0.458 µV; S.E.

= 0.555; p = 0.412; 95 % C.I. for difference = [-0.650 – 1.566].

Table 20. Comparison of latency of parietal P3 in response to cue-stimuli (POP task),

in children with and without ASD

	Mean (msec)	S.E.	95% Confidence Interval	
			Lower Bound	Upper Bound
ASD-no	352.697	5.160	342.399	362.994
ASD-yes	334.264	5.424	323.440	345.089
Difference	18.432	7.661	3.145	33.720
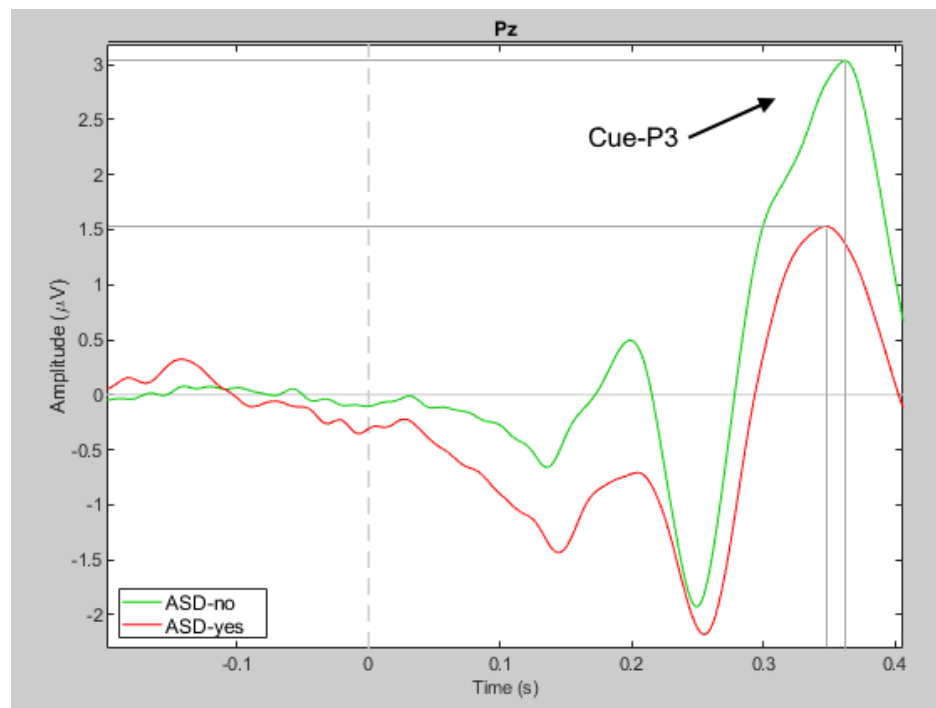
Figure 31. Visual representation of the P3 in response to cue-stimuli during the POP

task, in children with and without ASD

Table 21. Comparison of latency of fronto-central N2 in response to target-stimuli (POP

task), in children with and without ASD

| | Mean (msec) | S.E. | 95% Confidence Interval | |
			Lower Bound	Upper Bound
ASD-no	166.164	1.620	162.931	169.398
ASD-yes	171.684	1.721	168.249	175.119
Difference	5.520	2.416	0.697	10.343
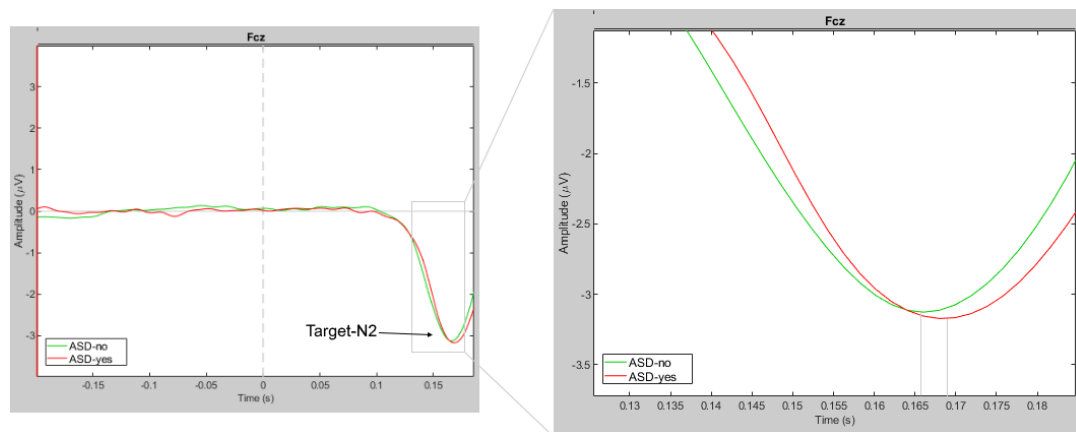
Figure 32. Visual representation of the N2 in response to targets during the POP task,

in children with and without ASD
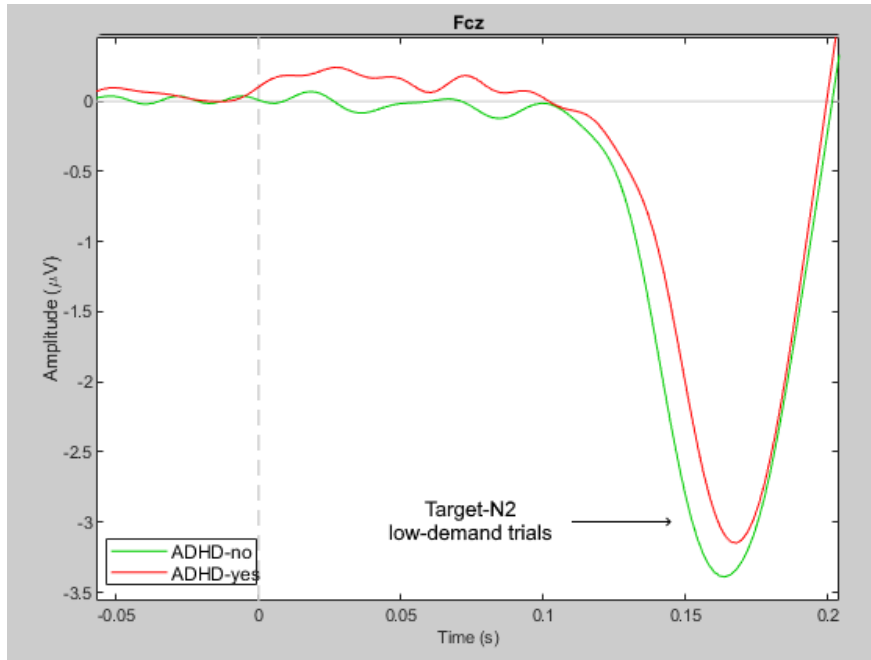
Figure 33. Comparison of amplitude of the N2 during low-demand trials, in children

with ADHD, ASD, ADHD+ASD and typically developing controls
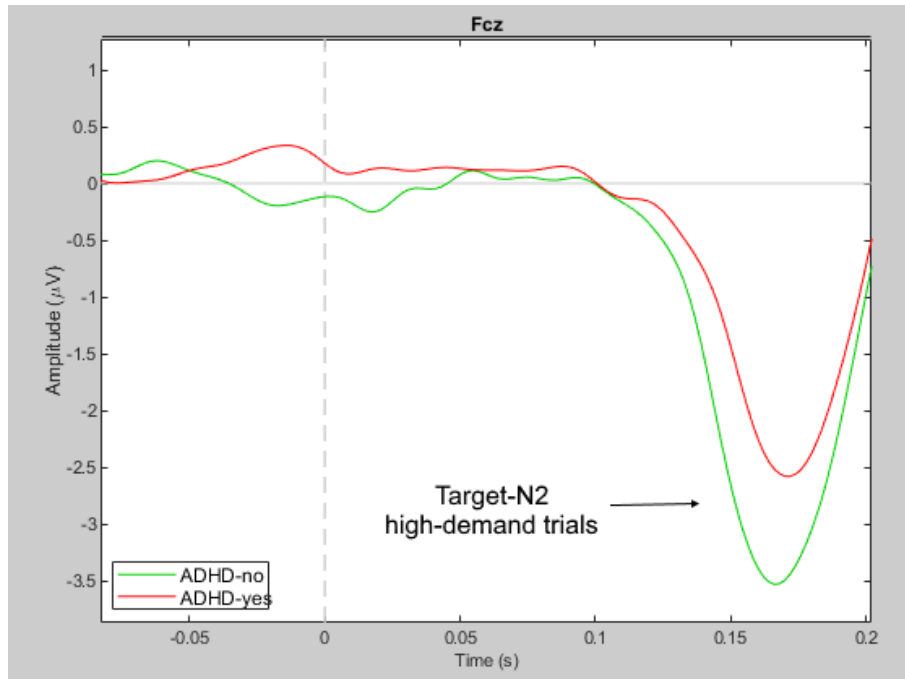
Figure 34. Comparison of amplitude of the N2 during high-demand trials, in children

with and without ADHD

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3.3.3. Overall summary of Question 3

I designed a cognitively challenging paradigm, i.e., the POP task, to investigate electrophysiological and behavioural measures of executive functions and cognitive control in our sample of children and adolescents with ADHD and/or ASD. More specifically, we analysed task- and symptoms-related effects on specific neural indices such as the latency and amplitude of the parietal P3 (in response to cues and targets) and the fronto-central N2 (in response to targets), besides focusing on response reaction times (RTs) and percentage of correct responses to analyse task performance.

Our results indicated that the parietal P3 peaked earlier in response to the red fixation cross (high-demand trials) than the green (low-demand trials), and this was followed by delayed fronto-central N2 and parietal P3 in response to the targets during high-demand trials, and longer response reaction times. These findings suggest that there was a difference in how children processed the cues and responded to the targets in high- and low-demand trials, both at behavioural and neural level. The analysis of electrophysiological measures showed that information processing was quicker when children were asked to prepare to inhibit a prepotent response (i.e., when they saw the red fixation cross). However, as soon as the arrow-target was presented on the screen, and children had to inhibit the prepotent response in favour of an alternative, indices of conflict monitoring and orienting of attention to the stimuli appeared slightly delayed, as an effect of the preparation to inhibit the prepotent response. This had a cascading effect on the actuation of the motor response, which was slower in high-demand trials, compared to low-demand. These results are in line with previous literature showing how response preparation is likely to recruit different neural systems according to the congruence or the incongruence between an expected stimulus and the associated required response (Barber & Carter, 2005; Kieffaber & Cho, 2010).

Children with comorbid ADHD+ASD displayed a profile of additive deficits found in children with ADHD- and ASD-only. While children with ADHD-only, ASD-only and comorbid ADHD+ASD similarly showed a more sluggish performance to the POP task, than typically developing children, those with comorbid ADHD+ASD showed a worse performance, in terms of correct responses, than children with ADHD-only. This suggests that while the separate presence of ADHD and ASD was associated with slower performance to a task challenging response inhibition and executive functions, only the co-occurring presence of ADHD+ASD was related to a specific impairment in performance accuracy (this findings supports the interactive model of ADHD/ASD comorbidity). Like children with ASD-only, children with comorbid ADHD+ASD showed an earlier parietal orienting response to cues (cue-P3), resembling the effect found for P3a latency during the oddball task (see paragraph 3.1.1.4) and probably indicating quicker reactivity and increased responsivity to the cues. However, the amplitude of the P3 in response to cues was reduced in children with ASD, suggesting that although they might have oriented earlier to the cues, they did not allocate sufficient attentional resources for processing them. These atypicalities in electrophysiological indices of cue processing were accompanied by atypicalities in indices reflecting conflict monitoring. In particular, we found that children with ASD had a delayed fronto-central N2 in response to targets, suggesting that the automatic monitoring of potential conflicts between the target stimulus and the associated response was slightly delayed in children with ASD. Therefore, delayed conflict monitoring, together with quicker but less effective processing of cues, may have had negative consequences on performance speed and accuracy in children with ASD. In addition to this, we found that during high-demand trials (but not low-demand), children with ADHD showed a reduced N2 (less negative amplitude) in response to targets,

compared to children without ADHD. This is likely to indicate the presence of atypical performance and conflict monitoring in children with ADHD, but only during more cognitively demanding trials. Interactive effects of deficits found in ASD and ADHD, including atypicalities in both cue-processing and conflict monitoring, might therefore underlie atypicalities in performance accuracy in children with comorbid ADHD+ASD.

Chapter 4. Results and discussion - Secondary investigations

4.1. Are there associations between autonomic arousal, attentional and executive function measures, and clinical symptomatology?

I investigated the presence of any relations between measures of autonomic arousal, vigilance and alertness; the presence of associations between baseline pupil size and SRTs; and the relations between indices of autonomic arousal, vigilance and alertness, and electrophysiological/behavioural measures of executive function and task performance. I also investigated if these measures were specifically associated with specific clinical symptomatology in the comorbid group of children with ADHD+ASD. The analyses presented in the first section of this Chapter were carried out on the sub-sample of participants who completed all experimental tasks, i.e., gap-overlap, oddball and POP tasks. Sixty-seven children were included in the analysis, including 18 typically developing children, 15 children with ADHD, 14 children with ASD and 20 children with ADHD+ASD.

4.1.1. Relations between measures of autonomic arousal, vigilance and alertness

I analysed bivariate correlations between the outcome measures collected during the three experimental tasks, from which both intra- and across-task associations between measures emerged from the analysis. CSI was highly negatively correlated with CVI (r_{65} = -0.702, p < 0.001), indicating that children who had higher values on one of the HRV indices (CSI or CVI) had lower values on the other index. We also found some associations between CSI and alpha during the POP task. In fact, children who had higher CSI during the active POP task displayed lower alpha during the breaks (r_{65} = -0.375, p = 0.002) and before the targets' appearance (r_{65} = -0.334, p = 0.006). Similar correlations were found between CSI and alpha during the breaks of the POP

task (r_{65} = -0.279, p = 0.022), and alpha in anticipation of targets (r_{65} = -0.253, p = 0.039). Moreover, CSI during the oddball task was negatively correlated with alpha measured during the breaks of the POP task (r_{65} = -0.242, p = 0.049), indicating that children who had increased CSI during the oddball task had reduced alpha during the breaks of the POP task. There was a positive correlation between the slope of changes in pupil size during the gap-overlap task and alpha in anticipations of the targets during the POP task (r_{65} = 0.266, p = 0.029), suggesting that children who displayed increased alpha after the presentation of the cues (reduced alpha desynchronization) had a less steeper negative slope of change in pupil size during the gap-overlap task.

4.1.2. Association between pre-saccadic baseline pupil size and SRTs

Previous research has shown that fluctuations in tonic pupil size (PS) are likely to represent an indirect index of the activation of the LC-NE system (Rajkowski et al., 1993; Murphy et al., 2014), and that baseline pupil size recorded before a motor response is likely to predict speed and accuracy of the response (Gilzenrat et al., 2010; Murphy et al., 2011). This effect seemed to follow an inverted U-shaped curve, so that when baseline PS was either smaller or larger than the subject's mean PS, RTs were slower.

We therefore tried to replicate these results by analysing, in the gap-overlap task, baseline pupil size recorded during fixation of the central stimulus, and SRTs, an index of visual attention orienting. For each participant, we segregated trials into tertiles based on baseline pupil size (PS) and extracted mean SRTs for each of these, defining the first tertile as 'small baseline PS', the second tertile as 'medium baseline PS' and the third tertile as 'large baseline PS'. We carried out a repeated measures ANOVA on mean

SRTs, with pre-trial *PS Tertile* (three levels: small, medium, large) and *Condition* (two levels: baseline and overlap) as within-subjects factors. We carried out this analysis for each separate group of children (typically developing controls, ADHD-only, ASD-only and ADHD+ASD).

A significant linear effect of PS Tertile on SRTs was found for the ADHD-only ($F_{1,19}$ = 9.727; p = 0.006; η_p^2 = 0.339) and ASD-only groups ($F_{1,17}$ = 4.416; p = 0.051; η_p^2 = 0.206). In children with ADHD-only, SRTs were reduced in trials with large vs small baseline pupil size (mean difference = 51.354 msec; p = 0.006), and in trials with large vs medium baseline pupil size (mean difference = 32.103; p = 0.047). Similarly, in children with ASD there was a just significant difference between SRTs in trial with large vs small baseline pupil size (mean difference = 33.386; p = 0.051). These findings seem to indicate that trials with larger baseline pupil size elicited faster SRTs in children with ADHD- and ASD-only. There was no significant effect of PS Tertiles in typically developing children and children with comorbid ADHD+ASD (Figure 35).
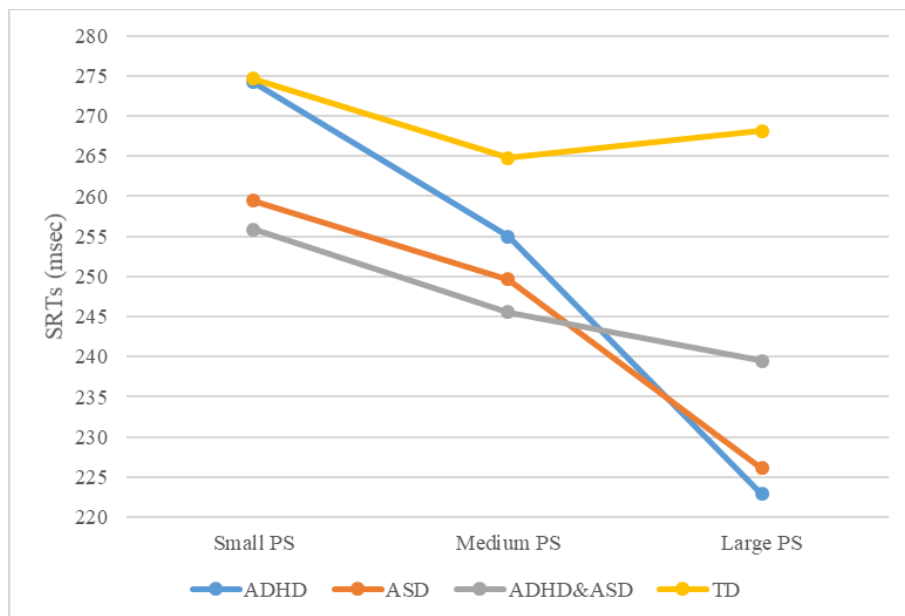
Figure 35. SRTs for trials of the gap-overlap task with low, medium and high baseline pupil size, for each group of children

We found significant interactions between Trial Condition and PS Tertiles, in the ADHD-only ($F_{1,19}$ = 5.934; p = 0.025; η_p^2 = 0.238) and ASD-only groups ($F_{1,17}$ = 4.311; p = 0.053; η_p^2 = 0.202), but not in the TD and ADHD+ASD groups. More specifically, in both children with ADHD-only and ASD-only, the reduction of SRTs in trials with large vs small baseline pupil size was specifically found in overlap trials (ADHD-only; mean difference = 82.204 msec; p = 0.006; ASD-only; mean difference = 59.517 msec; p = 0.025). However, in children with ADHD-only, during baseline trials (without overlap between central and peripheral visual stimuli) SRTs were similarly faster in trials with large vs medium baseline pupil size (mean difference = 29.957; p = 0.035). These findings suggest that attentional disengagement and re-orienting was affected by baseline pupil size in children with ADHD- and ASD-only, so that larger pupil size before the onset of the peripheral stimuli elicited faster orienting response. This effect was partly present in children with ADHD-only for baseline trials as well, probably indicating some effects of tonic pupil size on reflexive visual attention mechanisms, in this specific population (Figure 36).
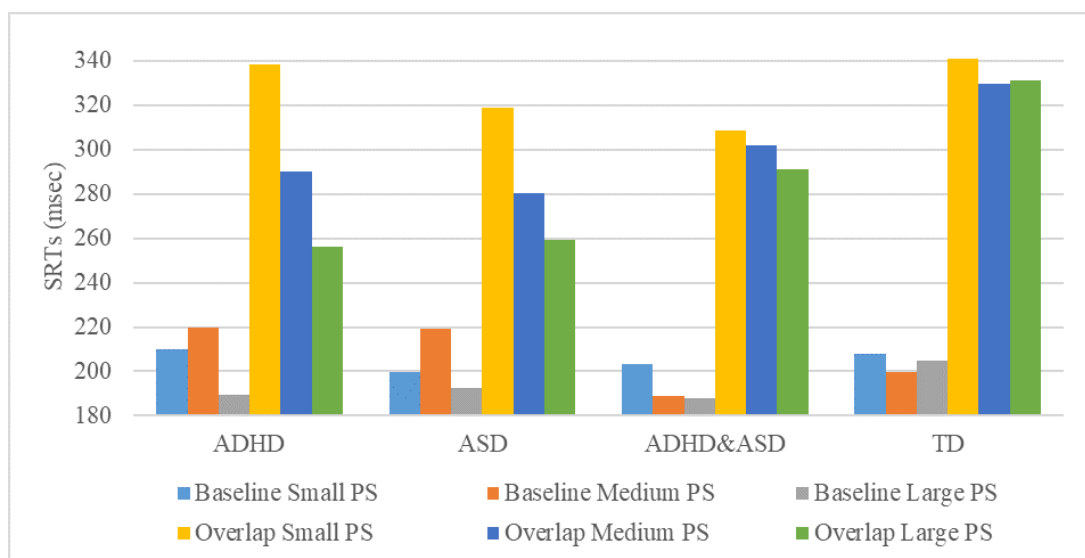
Figure 36. SRTs for baseline and overlap trials of the gap-overlap task, with low, medium and high baseline pupil size, for each group of children

4.1.3. Relations between indices of autonomic arousal, vigilance and alertness, and electrophysiological/behavioural measures of executive function and task performance

Children showing increased amplitude of the P3 in response to cues, during the POP task, had greater negative amplitude of the N2 in response to target stimuli (r_{65} = -0.347, p = 0.004), increased amplitude of the P3 in response to targets (r_{65} = 0.578, p < 0.001) and an overall better performance, in terms of percentage of correct responses (r_{65} = 0.311, p = 0.011). There was also a correlation between N2 and P3 amplitude in response to targets (r_{65} = -0.282, p = 0.021), so that children who displayed increased (more negative) N2 had increased P3, and those with a greater P3 in response to the targets displayed increased percentage of correct responses (r_{65} = 0.333, p = 0.006). The latency of the cue-P3 and the target-P3 were positively correlated, so that more delayed P3 in response to the cues was associated with more delayed P3 in response to targets (r_{65} = 0.563, p < 0.001).

The percentage of correct responses was correlated with the amplitude of the P3 both in response to the cues (r_{65} = 0.311, p = 0.011) and to the targets (r_{65} = 0.333, p = 0.006), suggesting that children who had increased P3 in response to the cues and to the targets performed better to the task. Similarly, children who displayed higher amplitude of the P3 in response to targets had reduced variability in RTs (r_{65} = -0.395, p < 0.001). We also found that both RTs (r_{65} = -0.469, p < 0.001) and the intra-individual variability of RTs (r_{65} = -0.667, p < 0.001) correlated with the percentage of correct responses during the POP task, so that children who had slower and generally more variable responses did perform worse to the active POP task.

A study by Kuiper et al. (2017) demonstrated an association between heart rate variability and performance to an experimental task tackling higher level cognitive functions and showed that reduced baseline HRV (in our study, this would be reflected by reduced CVI and, potentially, increased CSI) was associated with response inhibition difficulties. Our findings supported this study, as demonstrated by the presence of significant correlations between HRV and electrophysiological measures, and between alpha and EEG measures. More specifically, lower CSI during the breaks (r_{65} = -0.332, p = 0.006) and the blocks of the POP task (r_{65} = -0.362, p = 0.003) was associated with increased amplitude of the P3 in response to cues. This effect was found across tasks, so that children who had increased CSI during the oddball task had reduced Cue-P3 amplitude during the POP task (r_{65} = -0.383, p = 0.001). Since CSI and CVI were negatively correlated (see paragraph 4.1.1), higher amplitude of the cue-locked P3 was similarly predicted by increased CVI during the POP task (r_{65} = 0.242, p = 0.049), but also by higher alpha during the breaks (r_{65} = 0.270, p = 0.027).

Children with lower CSI during the POP task and higher alpha during the breaks displayed increased amplitude of the N2 and of the P3 in response to targets (CSI POP task - Target-N2 amplitude: r_{65} = 0.304, p = 0.012; CSI POP task - Target-P3 amplitude: r_{65} = -0.299, p = 0.014; Alpha during the breaks - Target-N2 amplitude: r_{65} = -0.344, p = 0.004; Alpha during the breaks - Target-P3 amplitude: r_{65} = 0.247, p = 0.044). We also found some associations between the P3a measured during the oddball task and the P3 measured during the POP task, so that the latency of the P3a (oddball task) was positively correlated with the latency of the P3 in response to cues (r_{65} = 0.249, p = 0.042) and in response to targets (r_{65} = 0.280, p = 0.022) during the POP task. Furthermore, children who displayed more delayed P3 in response to cues during the

POP task showed reduced amplitude of the P3a during the oddball task (r_{65} = -0.246, p = 0.045).

Overall, these findings are likely to indicate that children who displayed increased HRV, higher activity of the PNS during the POP task and reduced sympathetic arousal, during both breaks and task blocks, had increased electrophysiological indices of attention orienting and conflict monitoring, which predicted better performance to the task.

4.2. Associations between clinical symptoms and outcome measures

I was interested to investigate, in the subsample of children presenting clinical symptoms of ADHD and ASD (49 children; 15 ADHD-only, 14 ASD-only, 20 ADHD+ASD), if specific outcome measures were associated with specific measures of clinical symptomatology. Table 22 includes significant bivariate correlations between ADHD- and ASD-symptoms (inattention, hyperactivity/impulsivity, socio-communicative deficits, RRBs); comorbid symptoms of anxiety (generalised anxiety, social anxiety and specific phobia), depression, oppositional defiant behaviours (ODD/CD); and the outcome measures of the present study.

Associations between specific symptoms of ASD and ADHD, and HRV measures, emerged from this analysis. More specifically, while increased symptoms of hyperactivity (but not inattention) correlated with increased CVI and reduced CSI, socio-emotional difficulties (but not RRBs) correlated with increased CSI and reduced CVI. Similarly, children with increased symptoms of anxiety (social anxiety and specific phobias) had increased CSI and reduced CVI. Both reduced social abilities and communication skills, and anxiety symptoms were associated with delayed N2 in response to the target stimuli during the POP task, while inattention was associated with reduced target-N2 amplitude. Higher symptoms of depression were associated with reduced SD-RTs, indicating that children with less severe depressive symptoms had increased intra-individual variability in RTs. Lastly, children with higher oppositional-defiant behaviours had less negative slope of changes in pupil size during the gap-overlap task, and higher P3a amplitude during the oddball task.

Table 22. Significant correlations between clinical symptoms and outcome measures

		Pearson r	p
ADHD-Hyperactivity/Impulsivity	CSI	-0.282	0.05
ASD-Communication deficits	CSI	0.344	0.017
ASD-Social difficulties	CSI	0.312	0.031
Social anxiety	CSI	0.294	0.042
Specific phobia (anxiety)	CSI	0.407	0.004
ADHD-Hyperactivity/Impulsivity	CVI	0.297	0.038
ASD-Communication deficits	CVI	-0.35	0.015
ASD-Social difficulties	CVI	-0.469	<0.001
Social anxiety	CVI	-0.38	0.008
Specific phobia (anxiety)	CVI	-0.397	0.005
Depression	Intra-individual RTs variability	-0.375	0.009
ODD/CD	PS slope	0.295	0.042
ADHD-Inattention	Target-N2 amplitude	0.394	0.005
ASD-Total score	Target-N2 latency	0.324	0.023
Social anxiety	Target-N2 latency	0.291	0.045
Social anxiety	Target-P3 amplitude	0.339	0.019
ODD/CD	P3a amplitude	0.317	0.028

Based on findings that ADHD- and ASD-like symptoms seemed associated with different profiles of autonomic arousal, we used a Two-step cluster analysis to verify the presence of sub-groups of children with different arousal profiles among those with clinical symptoms of ADHD and ASD. Instead of specifying a-priori the number of clusters to be extracted, two-step cluster analysis investigates any possible combinations in the data (pre-clustering) before extracting the final number of clusters. Raw CSI and CVI measures were added to the two-step clustering algorithm, which identified two distinct clusters.

Fourteen participants were assigned to Cluster 1, which was characterised by increased CSI and reduced CVI, while 35 participants were assigned to Cluster 2, which was characterised by increased CVI and reduced CSI. Most of children with ADHD-only (14 out of 15) were categorised as showing an autonomic arousal profile characterised by increased CVI and reduced CSI. Children with ASD-only and,

interestingly, children with ADHD+ASD, did not show a predominant profile, so that some children in these groups showed a profile characterised by increased CVI (8 out of 14 children with ASD-only and 13 out of 20 children with ADHD+ASD), while others displayed a profile of increased CSI and reduced CVI (Figure 37).

I was therefore interested to investigate if specific clinical measures differentiated these newly created groups and, for a purely descriptive purpose, I carried out independent-samples t-tests to compare measures of clinical symptomatology between the newly created groups ('CVI-predominant profile' and 'CSI-predominant profile'). We found that children displaying signs of hyper-arousal (increased CSI and reduced CVI), compared to those showing hypo-arousal (increased CVI and reduced CSI), had reduced social abilities ($t(46) = 3.594$; $p = 0.001$) and more severe communicative deficits ($t(46) = 2.833$; $p = 0.007$); increased avoidance of sensory stimulation (SPQ-avoidance scale; $t(46) = 2.012$; $p = 0.05$); more severe generalised anxiety ($t(46) = 2.405$; $p = 0.020$), social anxiety ($t(46) = 2.500$; $p = 0.016$) and specific phobia ($t(46) = 3.014$; $p = 0.004$); and increased obsessive-compulsive symptomatology ($t(46) = 2.213$; $p = 0.032$).
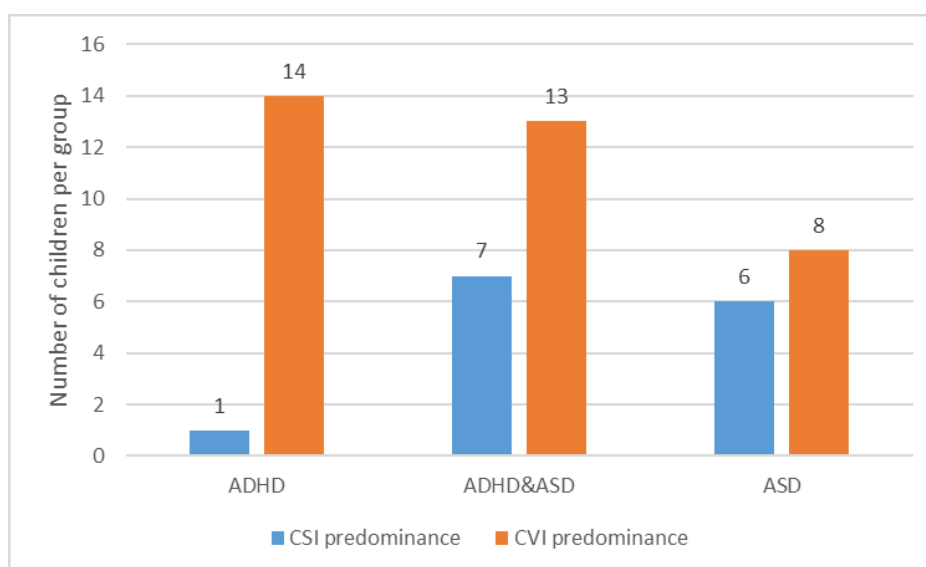
Figure 37. Number of children allocated to the newly created clusters, for each group

4.3. Summary of secondary investigations

Besides finding direct associations between specific symptoms of ASD and ADHD, and measures of HRV, we found that children who had higher values on one of the HRV indices (CSI or CVI) had lower values on the other index. This is likely to suggest that children who showed more balance between activity in the SNS and the PNS, were more likely to show less 'extreme' CSI and CVI measures, and less imbalance between activity in the two branches of the ANS, possibly indicating more efficient arousal regulation. It would be interested to investigate if and how CSI and CVI could be used to evaluate the balance of activity in the ANS, and if innovative composite scores of 'arousal regulation/dysregulation' could be extracted from these indices.

We found some associations between hyper-activity of the SNS during the POP task and reduced alpha during the breaks, suggesting that children who had increased sympathetic arousal during the active POP task displayed reduced alpha during the breaks. This is in line with a recent study (Barry et al., 2020) who confirmed an inverse relationship between some components of alpha and SCL, indicating that increased autonomic arousal (especially sympathetic) is likely to be linked with reduced alpha in resting-state-like situations. Moreover, children who displayed reduced post-cue alpha during the POP task, had a steeper negative slope of change in pupil size during the gap-overlap task. Better alpha desynchronization, to facilitate attention orienting to task-relevant information, might therefore be linked with stronger indices of exploitation of information.

Reduced activity of the SNS and increased activity of the PNS, together with increased alpha during the breaks of the POP task, were generally associated with

increased amplitude of the P3 in response to cues, indicative of better information processing, and increased amplitude of the N2 and of the P3 in response to targets, indicative of better conflict monitoring and increased orienting of attention to task-related information. This finding was found across tasks, so that higher SNS activity during the oddball task was associated with reduced processing of information from the cues during the POP task. Moreover, indices of HRV, alpha and electrophysiological measures were all found associated with task-related activity and performance. For example, increased electrophysiological indices of cue-processing were associated with increased indices of conflict monitoring and stronger orienting of attention to the target stimuli, with consequent more accurate and less variable motor responses. Slower brain responsivity to the cues was also associated with delayed orienting of attention to the targets, and this sluggish brain responsivity was also demonstrated by delayed and weaker automatic orienting of attention to auditory information in the oddball task. Similarly, children who had slower and generally more variable motor responses showed less accurate performance to the task.

Interestingly, direct linear associations between symptoms of ADHD and ASD, and performance accuracy were not found. However, there might have been interactive effects between ADHD/ASD symptoms, autonomic arousal and vigilance mechanisms, and electrophysiological/behavioural indices of performance. In fact, children with higher CSI generally displayed a reduced P3 response to stimuli during both POP and oddball tasks, and the amplitude of these electrophysiological indices of attention orienting and information processing, together with reduced intra-individual variability in RTs, was associated with increased performance accuracy (i.e., higher percentage of correct responses during the POP task).

Although we did not find any association between baseline pupil size an SRTs in typically developing controls and children with ADHD+ASD, we found that children with ADHD-only and ASD-only similarly presented a specific effect where larger tonic pupil size predicted faster orienting of visual attention. This is likely to indicate that during trials where baseline pupil size was smaller, orienting of attention happened more slowly in children with ADHD-only and ASD-only. In line with the rationale presented in paragraph 1.3.2, this seems to suggest that during exploration of the environment (when baseline pupil size was larger) eye movements were quicker to facilitate orienting of visual attention towards the peripheral stimuli. Conversely, in those trials when baseline pupil size was smaller, indicating exploitation of information and more focused attention on the central stimulus, orienting of visual attention was slower. It is not clear, though, why this effect was present in children with ADHD-only and ASD-only, and not in typically developing controls or children with ADHD&ASD. The relation between pupil size and response RTs should be further investigated, using precise measurements of pupil size (i.e., controlling for confounding variables such as light, distance from the screen, head movements) which would allow group comparisons on this measure collected at rest and during passive or active tasks.

While children with increased hyperactivity/impulsivity had an autonomic profile characterised by hypo-arousal (increased CVI and reduced CSI), those with more severe socio-emotional difficulties and symptoms of anxiety showed an opposite profile characterised by hyper-arousal (more increased CSI and reduced CVI). More severe symptoms of inattention predicted the presence of electrophysiological indices of reduced conflict monitoring (target-N2 amplitude), while in children with more severely impaired social abilities and communication skills, and increased anxiety symptoms, this specific ERP component (target-N2) was delayed. Children with higher

oppositional-defiant behaviours displayed a more flattened slope of changes in pupil size slope during the gap-overlap task, probably indicating the predominance of LC functioning in the tonic mode (Rajkowski, 1993; Murphy et al., 2014). Moreover, children with higher ODD symptoms displayed increased amplitude of the P3a in response to auditory stimulation, indicating higher automatic orienting of attention during the oddball task. Children with more severe depressive symptomatology showed reduced intra-individual RT-variability, such that increased variability in RTs was present in children with less severe depressive symptomatology.

Using a purely descriptive approach, I investigated if sub-groups of children with different profiles of autonomic arousal. Almost none of the children with ADHD-only showed an autonomic arousal profile resembling hyper-arousal, but instead showed a profile characterised by increased activity of the PNS and reduced activity of the SNS. On the opposite, among children with ASD-only and ADHD+ASD, those who displayed signs of hyper-arousal, such as increased CSI and reduced CVI, had more severe socio-communicative deficits, increased avoidance of sensory stimulation, higher anxiety (generalised, social and specific), and more severe obsessive-compulsive symptomatology, compared to those showing signs of hypo-arousal.

Chapter 5.   Final discussion and conclusions

5.1. Summary and discussion of results

The present doctoral project was aimed at investigating measures of autonomic arousal, vigilance and alertness, visual attention orienting and executive functioning, in children with ADHD and/or ASD. A secondary aim of this study was to clarify if atypicalities on these measures were condition-specific or shared between ADHD and ASD, and which model of ADHD/ASD comorbidity (additive or interactive) better explained the findings. As summarised in Table 23 (page 180), we generally found both ADHD-/ASD-specific and shared atypicalities, but our findings were dependent on the experimental situation and the measure investigated.

Children with ADHD showed general difficulties in regulating vigilance and allocating attentional resources to sensory information from the environment, which were associated with reduced arousal and vigilance, as mirrored by reduced activity of the sympathetic nervous system and increased variability in reaction times during the active POP task. These findings are in line with theoretical models that speculated how reduced vigilance and alertness might be core symptomatologic phenotypes of ADHD (Geissler et al., 2014; Kuntsi & Klein, 2012; Sergeant, 2000). When interpreting the results of the present study within the frameworks proposed by Aston-Jones and colleagues (2000; 2007) and Howells et al (2012), they might indicate that the LC is under-functioning in people with ADHD (i.e., generally firing at lower frequencies than expected), causing reduced release of norepinephrine. Therefore, situations where sensory stimulation is reduced or with slow pace might specifically elicit critical reductions of activity in the ANS (especially in the sympathetic nervous system), causing drowsiness and inattention in people with ADHD, who might adopt maladaptive regulatory strategies, such as hyperactivity, restlessness and fidgeting. For

example, it has been shown that inattentiveness and spontaneous mind wandering predicted fidgeting in a healthy sample (Carriere et al., 2013): it would be interesting to test if this association is similarly found in individuals with ADHD. Conversely, situations which are engaging or less boring might be beneficial and help them to regulate arousal and vigilance, while mentally challenging situations might trigger specific executive function deficits, due to difficulties in focusing and sustaining attention when the load of information to process is too high.

It would be therefore interesting to verify if the onset of hyperactive behaviours, but also deliberate control and suppression of motor behaviours, have some short- and long-term effects on ANS measures. Moreover, verifying if ANS functioning is atypical from early infancy in children later developing ADHD, might help to test the models which proposed the existence of a relationship between atypical pre-natal development of brainstem structures involved in autonomic arousal and consequent development of higher-level abilities and behaviours (e.g., Geva & Feldman, 2008; 2017). Inattention during early infancy might be in fact a non-specific precursor of ADHD and ASD (Johnson et al., 2015; Visser et al., 2014), mainly deriving from atypical development of basic strategies of arousal regulation.

Indices of hyper-arousal in ASD seem to suggest that children with ASD are more likely to experience excessive responsivity to sensory stimulation and might find it difficult to down-regulate autonomic arousal according to contextual demands. In mentally challenging or more stimulating situations, they may therefore find it difficult to effectively process information, with negative consequences on performance. Specific behaviours, including stereotyped/repetitive behaviours or movement patterns, and general avoidance of sensory stimulation, might therefore be consequences of temporary or chronic autonomic hyper-arousal in ASD. These strategies, which are

maladaptive because they limit social interactions and communication, have in fact been found somehow beneficial in reducing levels of dopamine in prefrontal system and, consequently, autonomic arousal and stress (Kinsbourne, 2011). These findings, interpreted in the light of theoretical models presented in Chapter 1 (including Aston-Jones et al., 2000; 2007; Howells et al., 2012), might indicate that reduced top-down control of the PNS-related systems (which, among all, includes the DMN) over the LC-NE system, might produce hyper-activation of the LC, causing a chronic exaggerated release of dopamine and norepinephrine, and higher levels of stress, in those with Autism.

For most of the measures considered in the present study, children with comorbid ADHD+ASD displayed an additive profile of condition-specific atypicalities reported in children with ADHD- and ASD-only. These findings are in line with previous studies who found that the phenotypical expression of ADHD- and ASD-symptomatology is likely to diverge early during development (Visser et al., 2014), despite a shared underlying susceptibility (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2019; Ghirardi et al., 2017; Rommelse et al., 2010). Children with co-morbid ADHD+ASD showed difficulties in maintaining optimal levels of vigilance and sustaining attention, especially during more passive tasks, but also hyper-arousal during sensory stimulation and mentally demanding cognitive tasks, distractibility and difficulties in focusing on task-relevant information. The co-occurring presence of ADHD and ASD might therefore affect autonomic arousal, however our secondary analyses seemed to show that only children with ASD-related symptomatology, including higher anxiety and more severe socio-communicative difficulties, displayed increased sympathetic arousal and reduced PNS functioning.

Conversely, children with predominant activation of the PNS (and reduced SNS acidity) were those with increased ADHD-related hyperactivity.

While atypicalities in performance speed were shared between ADHD and ASD, suggesting that these might be a non-specific phenotypical expression of ASD and ADHD, there seemed to be an interactive effect of co-occurring ADHD and ASD on performance accuracy, so that children with ADHD+ASD had reduced performance accuracy than children with ADHD-only. This is in line with previous studies, including Rommelse et al. (2017), who proposed that specific domains of impairment might be affected by the co-occurring presence of ADHD and ASD at a greater level than that found in the single conditions. These might probably derive from more severe atypicalities in the early development of brain structures involved in executive functions, conflict monitoring and cognitive control.

Children with ADHD+ASD could also be distinguished from children with ADHD-only on the level of SNS activity during the resting-blocks of the oddball task. While in children with ADHD-only signs of hypo-activation of the SNS were found during these periods without auditory stimulation, children with ADHD+ASD showed higher SNS activity than ADHD-only, probably due to an interactive effect of different profiles of arousal dysregulation in ADHD and ASD. Children with ADHD+ASD might therefore struggle in environments with both too low or high sensory stimulation, making it even more difficult for them to regulate arousal and attention in line with environmental demands, with more severe consequences on performance accuracy and adaptive functioning than what is found in children with single conditions.

Our results also indicated that the CSI and the CVI are likely to reflect the balance of activity in the sympathetic and parasympathetic nervous systems. More specifically, we found an inverse correlation between these indices, suggesting for example that those children who have extremely high CSI had extremely low CVI, and vice versa. Moreover, we found some links between indices of brain arousal and activity (alpha and P3) and measures of autonomic arousal, such as CSI, CVI and time-related changes in pupil size, which were in parallel associated with speed and accuracy of performance in a mentally challenging executive task. Our findings also suggested that mechanisms of attentional disengagement and visual attention orienting might be affected by tonic autonomic arousal in children with ADHD- and ASD-only, who displayed faster orienting response when pupil size before the onset of the peripheral stimuli was large.

While ADHD-symptoms, and more specifically more severe hyperactivity, seemed to be associated with predominance of PNS activity and autonomic hypo-arousal, more severe socio-communicative deficits were associated with increased activity of the SNS and hyper-arousal. Those children who displayed a predominant profile of hyper-arousal were those who were more clinically severely impaired and showing increased ASD-symptomatology, such as more severe socio-communicative deficits, increased avoidance of sensory stimulation, higher anxiety (generalised, social and specific), and more severe obsessive-compulsive symptomatology. The implications of findings from the present study will be now discussed.

Table 23. Summary of the results of the present study

Measure	Task-related findings	ADHD	ASD	ADHD+ASD	Phenotype
CSI - POP	Reduced during task vs breaks	--	Increased CSI during 3rd task block and 4th break	Similar to ASD-only	ASD-specific
CVI - POP	--	--	Reduced CVI	Similar to ASD-only	ASD-specific
RMSSD - POP	--	--	Reduced RMSSD	Similar to ASD-only	ASD-specific
CSI - oddball	Increased during task vs resting; Increased in block 2 vs block 1	Reduced CSI	--	Reduced CSI, compared to ADHD-only, during resting blocks	ADHD-specific AND interactive effect in ADHD+ASD
CVI - oddball	Increased during task vs resting	--	Reduced CVI during 1st resting and 2nd task block	Similar to ASD-only	ASD-specific
RMSSD - oddball	--	--	Reduced RMSSD	Similar to ASD-only	ASD-specific
P3a - oddball	Increased for deviant vs standard tones (especially in 1st block)	--	Earlier P3a latency	Similar to ASD-only	ASD-specific
MMN - oddball	Increased for social vs non-social stimuli	--	--	--	
Alpha power - POP	Increased during task vs break, especially pre-cue vs post-cue	--	--	--	
Slope of change in baseline pupil size - gap-overlap	Within-block negative slope (only in Block 1)	Reduced slope of change in pupil size	--	Similar to ADHD-only	ADHD-specific
Slope of change in SRTs - gap-overlap	Within-block positive slope	--	--	--	

Measure	Task-related findings	ADHD	ASD	ADHD+ASD	Phenotype
Intra-individual variability of SRTs - gap-overlap		--	--	--	
Intra-individual variability of RTs - POP		Increased intra-individual variability in RTs	--	Similar to ADHD-only	ADHD-specific
SRTs - gap-overlap	Reduced in baseline vs overlap trials; Reduced for social vs non-social stimuli; Reduced for dynamic vs static trials (only in children without ADHD)	No effect of modality	--	Similar to ADHD-only	ADHD-specific
RTs - POP	Increased for high- vs low-demands trials	Longer RTs	Longer RTs	Similar to ASD-only and ADHD-only	Shared phenotype, additive model
% of correct responses - POP		Reduced %	Reduced %	Reduced % of correct responses than children with ADHD-only	Shared phenotype, interactive model
Cue-P3 - POP	Reduced latency for high-demand trials vs low-demand	--	Reduced cue-P3 latency; Reduced cue-P3 amplitude	Similar to ASD-only	ASD-specific
Target-N2 - POP	Reduced latency for low-demand trials vs high-demand	Reduced target-N2 amplitude during high-demand trials	Increased target-N2 latency	Similar to ASD-only and ADHD-only	Shared phenotype, additive model
Target-P3 -POP	Reduced latency for low-demand trials vs high-demand	--	--	--	

'--' indicates the absence of any task- or group-effect

5.2. Implications

5.2.1. Scientific impact

To our knowledge, the present project has been one of the firsts to investigate the effects of ADHD and ASD on measures of autonomic arousal and attention regulation in different experimental situations. Results from the present study can therefore be used to guide the design of future research studies on arousal and attention regulation in ADHD and ASD.

Our findings suggest that measuring the activity of the ANS in various experimental situations might help to better explain the relationships between autonomic arousal, brain functioning and human behaviour. The LC-NE and other brainstem-systems seem to interact with and affect functioning of brain systems involved in attention and behaviour regulation. Atypical pre- and post-natal development of subcortical systems might therefore be associated with later atypical structural and functional development of brain systems responsible for more complex behaviours, as proposed by some theoretical models (e.g., Geva & Feldman, 2008; 2017).

I suggest that future studies should focus on carefully designing experimental situations where autonomic (including HR, EDA and pupillometry), neuroimaging (EEG or fMRI) and behavioural measures are collected during periods of resting-state, cognitive or attentional tasks, and in task-to-break transitions, but also during everyday-life activities. This, in fact, might be useful to investigate the interactions between different brain systems and their functioning in association with real -life situations, possibly focusing on those situations where patients have more difficulties. This would probably make possible to translate the results of these studies in the development of

more tangible and impactful interventions for people with ADHD and ASD. I would also like to invite researchers to work towards extrapolating innovative measures which could be used to evaluate the balance of activity in the sympathetic and parasympathetic nervous systems, and the hypo/hyper-arousal continuum. Moreover, I suggest measuring arousal over both short- and long-periods, to obtain reliable measures of tonic and baseline autonomic arousal, and HRV.

Our findings suggest that children displaying co-occurring ADHD+ASD displayed both similarities and differences with those presenting ADHD- or ASD-only. Recruiting large samples of individuals with different levels of symptoms of ADHD and ASD (both clinical and subclinical), and considering the potential impact of co-occurring symptoms instead of excluding participants with a complex symptomatology, would help to obtain a better picture about the heterogeneity of ASD and ADHD, and to provide further knowledge about the shared and overlapping mechanisms in people with these conditions. Overall, our study found that both the additive and the interactive models of ADHD/ASD comorbidity were supported by the empirical data, indicating that further research is needed on this topic. It would be interesting to integrate data from different domains, including genetic data, data from longitudinal studies, data on infants at risk of developing ADHD and ASD, young and older adults with ADHD and ASD, besides data collected through different techniques, including physiological and electrophysiological measures, behavioural and clinical data, to further disentangle the comorbidity between ADHD and ASD and identify condition-specific and shared atypicalities, and their additive or interactive effects in people with co-occurring ADHD and ASD.

5.2.2. Impact on the everyday life of people with ADHD and/or ASD

Although I am aware that the results of this doctoral project may not have an immediate and direct impact on life of children with ADHD and/or ASD, and their families, this study provided some new knowledge about these conditions.

It was demonstrated how ADHD and ASD might be differentially associated with specific profiles of arousal, vigilance and attention. While people with ADHD seem to be struggling more with maintaining their attention to the environment and extracting relevant information, people with ASD are more likely to experience hyper-reactivity and increased sensory sensitivity, resulting in difficulties to down-regulate physiological arousal according to the contextual demands. Therefore, while people with ADHD may be inattentive and have a more 'sluggish' cognitive style in less engaging situations or when attention should be maintained for long time, children with ASD are likely to benefit from those situations where sensory stimulation is reduced or with a slower pace. Conversely, in more demanding settings such as cognitive tasks under time pressure, they might experience excessive autonomic arousal and reactivity, which undermine information processing and performance. People with co-occurring ADHD and ASD might therefore find it difficult to maintain attention in different situations, and together experience excessive distractibility and hyper-reactivity to sensory stimulation, struggling to focus on the task or the ongoing activity and displaying general difficulties in carrying out everyday life activities.

Manipulating specific characteristics of the setting and the environment, might prove useful to help individuals with clinical symptoms of ADHD and/or ASD in regulating their level of autonomic arousal and vigilance. For example, allowing period of movement and physical activity, teaching self-regulation strategies to up- or down-regulate arousal (including breathing exercises, mindfulness, etc.), or using engaging

stimuli, positive rewards and reinforcers, might be helpful for people with ADHD to up-regulate their level of arousal and to better regulate and sustain attention, benefitting their performance at home, at school or in the workplace. On the opposite, changing specific environmental features to reduce the sensory load, might be useful and beneficial for people with ASD to effectively process sensory information. Further research is therefore needed to specifically understand how individuals with ADHD and/or ASD might benefit from manipulations of the environment. It would be also interesting to understand if 'external' strategies of arousal regulation (e.g., driven by parents, teachers or employers) or more 'internal' strategies (e.g., physical activity, breathing exercises, mindfulness) have different effects in optimising arousal and vigilance based on contextual characteristics and demands, in people with these conditions. Future research should engage and involve people with ADHD and ASD, together with their carers and the clinicians involved, since this cooperative work might result fruitful in identifying the most impactful consequences of a clinical diagnosis on patients' everyday life, and in developing new strategies and interventions to reduce its negative effects.

5.2.3. Impact on the clinical setting

The findings from the present study might have some implications for the clinical setting, although it is unlikely that they will directly affect clinical practice in the short-term.

Having demonstrated that ADHD and ASD might be associated with different profiles of autonomic arousal, might suggest the possibility of integrating innovative diagnostic tools which could guide and support clinicians in the diagnostic process, especially when asked to carry out a differential diagnosis. It has already been demonstrated how the QbTest, a 20-minutes-long computerised test, might improve the diagnostic process by providing the clinicians with an objective measure of attention and impulsivity (Hollis et al., 2018). It would be interesting to implement wearable devices, such as smartwatches or Fitbit™ that people with ADHD can use at school or in their workplace, or integrate the QbTest with recordings of heart rate through smaller devices that rely on two electrodes to extract heart rate (as done for the present study). This could provide clinicians with objective measures of physiological arousal in both life- and laboratory-settings, which could be used to drive the choice of medication and potentially predict the outcome of the medical treatment. For example, the prescription of stimulants for people with ADHD+ASD and a profile of autonomic hyper-arousal, might result less beneficial, or even deleterious, since this type of medication seems to increase autonomic arousal, at least in people with ADHD (Bellato et al., 2020). It is therefore necessary that studies aimed at assessing the impact of different medical interventions (for example, comparing stimulant and non-stimulant medication for ADHD) on autonomic arousal and ANS functioning, will be encouraged and supported by public and private funding bodies anytime soon.

This may further lead to the development of new medications that target specific systems, such as the LC-NE, involved in autonomic arousal and in arousal regulation. Similarly, if specific associations between medication type and dosage, and changes in measures of autonomic arousal, were found, clinicians would be more able to prescribe specific drugs, or more balanced doses, basing their judgment on the effects of the drugs on objective measures of attention and autonomic arousal, and behavioural outcomes.

Lastly, it would also be important to investigate if and how non-pharmacological interventions, including tactile stimulation, physical activity, breathing exercises and mindfulness, or the combined use of pharmacological and non-pharmacological interventions, have some effects on autonomic arousal, vigilance and attentional mechanisms, both at short- and long-term, in people with ADHD and-or ASD.

5.3.   Limitations

Although we were able to recruit and test 106 children and adolescents, not every child completed the entire battery of tests (see Table 6). We realised to have specifically struggled to recruit children with ASD-only, so that the final number of children recruited for this group was lower than aimed before starting the study (see paragraph 2.1.1). This difficulty may have originated from different reasons, including a quite low response rate from the chosen recruitment sources (of 284 people who got in touch with the research group, only 133 attended the testing session). Moreover, we had to further exclude some participants from the final analyses (see Table 6, page 107), possibly lowering the power of the statistical analysis and increasing the risk of type II errors (false negatives). Deciding to use Bayesians statistics, in addition to more traditional frequentist approaches, seemed to have helped in further elucidating some marginal results, and possibly guiding decisions about the acceptance or rejection of null hypotheses. Besides this, I decided to apply Benjamini-Hochberg correction for multiple comparisons, which is less conservative than Bonferroni and controls for the proportion of false positives (Benjamini & Hochberg, 1995).

We are also aware that there may have been some biases in the selection of the final sample. In fact, children were recruited from local support groups, while psychiatrists and paediatricians in NHS services also helped to identify potential participants. This may have caused an imbalance in the number of children presenting comorbid ADHD+ASD, compared to children with ADHD-only or ASD-only. The fact that we provided to parents a report which summarised the results of some of the measures used in the clinical assessment, may have been caused an increased interest in the study by those families who were still under assessment in the NHS services, or

for whose children a clear diagnosis was not still confirmed. We could therefore have missed a portion of children presenting less severe presentations ASD and/or ADHD. Similarly, we could have also missed those children with more severe profiles of ADHD and ASD, for whom their parents did not consider the study suitable. For these reasons, our sample may not be fully representative of the population of children with ADHD and ASD. In order to compensate for this bias, I would suggest that future studies shall be designed and carried out by recruiting a more generalisable sample of children and adolescents with ASD and/or ADHD, for example involving special schools, charities and specialist NHS services. Although children receiving stimulant medication were withdrawn medication 24 hours prior the testing session, we could not include children on non-stimulants, reducing the representability and generalisability of our sample.

Since our clinical assessment was comprised of both direct observational and parent-report measures, it is important to consider that parent-reported measures are likely to contain biases dependent on the respondent's perspective. However, the measures chosen for the clinical assessment are standardized, validated and widely used in clinical settings in the UK and worldwide. When investigating the effects of ADHD and/or ASD on the main outcome measures of the present study, we used binomial between-subjects factors reflecting the absence or presence of ADHD and ASD, which could be considered less effective in detecting group-specific effects. However, I should say that this approach was useful to both highlight condition-specific and shared effects, therefore I suggest its use in future studies investigating the comorbidity of ADHD&ASD.

5.4.   Future directions and next steps

I would like to conclude this dissertation by presenting specific areas of research which should be targeted in future studies, based on the implications of the results of this project, as just discussed:

- Review, through a systematic approach, previous studies which investigated different domains (including genetic studies, longitudinal studies, infants- and lifespan-studies) and findings obtained through different techniques (physiological and electrophysiological measures, behavioural and clinical data, etc.) to further disentangle the comorbidity between ADHD and ASD and identify condition-specific and shared atypicalities, and their additive or interactive effects in people with co-occurring ADHD and ASD

- Design studies with batteries of experimental tasks where autonomic (including HR, EDA and pupillometry), neuroimaging (EEG or fMRI) and behavioural measures are collected, also including periods of resting-state to investigate the task-to-break and the break-to-task transitions

- Design research studies where different measures of ANS functioning (HR, pupillometry, EDA, etc.) are collected in typically developing participants, to clarify the relationships between these measures and their associations with different conditions of sensory and cognitive loads, before designing more specific research studies to investigate the relations between these measures and clinical symptoms of ADHD and ASD

- Design studies aimed at extrapolating composite scores reflecting the balance of activity in the sympathetic and parasympathetic branches of the ANS, and more general indices of 'arousal regulation/dysregulation' or 'hypo-/hyper-arousal'

- Design studies specifically focused on investigating the short-term effects of hyperactive behaviours and RRBs on measures of ANS functioning, and test if these strategies are effectively useful for people with ADHD and/or ASD in regulating their level of arousal

- Design clinical studies where smart-devices or simple ECG systems are implemented, since these might provide clinicians with measures of functioning of the ANS, which can result helpful for the diagnostic classification of complex cases presenting different profiles of comorbidities, but also in the choice of medication for ADHD

- Design non-laboratory studies which investigate autonomic arousal mechanisms and arousal regulation in situations of real life, to verify if the same relationships found in the laboratory settings are present in more complex environments. Similarly, investigate if and how the modification of the external environment is useful for people with ADHD and/or ASD, and which effects it has on measures of ANS functioning.

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Appendix A

Two separate repeated measures ANOVA were carried out on CSI and CVI with Task (2-levels; oddball and POP) and Activity (2-levels; resting/break periods and task blocks) as within-subjects factors, and *ADHD* and *ASD* (2-levels: yes/ no) as between-subjects factors. We controlled for the effects of age, gender, verbal and performance IQ.

We specifically analysed planned pairwise comparisons and found that there was a significant differences between CSI during the breaks of the oddball task and CSI measured during the breaks of the POP task (mean difference = 0.877; $p < 0.001$); between CSI measured during the blocks of the auditory oddball task and the blocks of the POP task (mean difference = 0.133; $p = 0.032$); and between CVI measures during the breaks of the oddball task and CVI during the breaks of the POP task (mean difference = 0.175; $p < 0.001$). There was not a significant difference on CVI measured during the blocks of the oddball and the POP task (mean difference = 0.007; $p = 0.691$) (see Table A1 and Figures A1 and A2).

Table A1. CSI and CVI calculated during resting/breaks and blocks of the oddball and the POP tasks

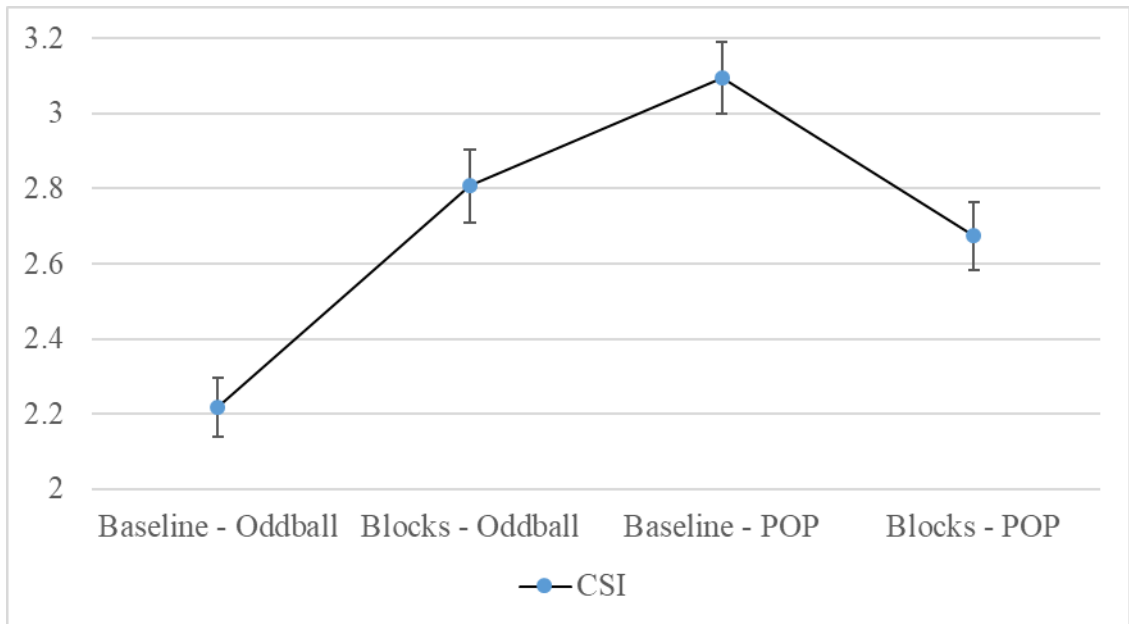| Measure	Task	Activity	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
CSI	Oddball	Resting	2.218	.079	2.060	2.377
		Task	2.807	.096	2.615	2.999
	POP	Break	3.096	.095	2.906	3.285
		Task	2.674	.090	2.494	2.854
CVI	Oddball	Resting	4.562	.052	4.459	4.665
		Task	4.688	.043	4.603	4.773
	POP	Break	4.737	.042	4.654	4.820
		Task	4.695	.043	4.609	4.781

Figure A1. Visual representation of CSI measured in resting/break periods and during

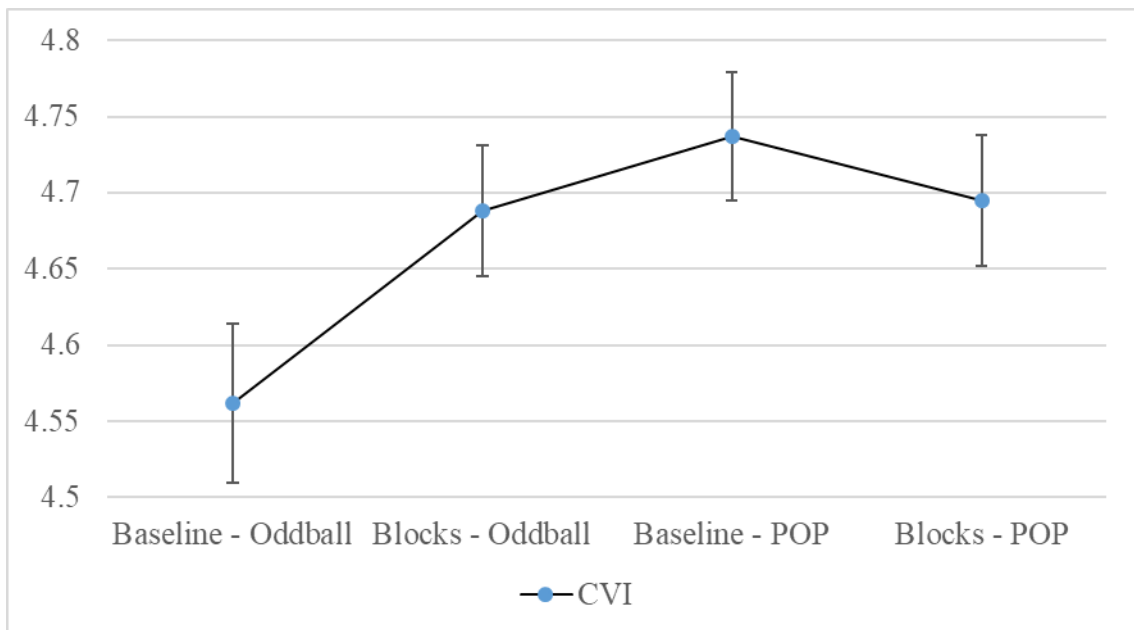the blocks of the oddball and the POP task.

Figure A2. Visual representation of CVI measured in resting/break periods and during

the blocks of the oddball and the POP task.