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RAPID-ACTING ANTIDEPRESSANT DRUGS: AN EMERGING APPROACH FOR THE TREATMENT OF MAJOR DEPRESSIVE DISORDER

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SUMMARY

Rapid acting antidepressants

ABBREVIATIONS

1M-MRS = hydrogen spectroscopy study

5HT2R = serotonine receptor 2

ABD = agonist binding domain

ACC = anterior cingulate cortex

AD = Alzheimer's Disease

ADS = antidepressant disoder synthome

AKT = activity protein kinase B

ALCAR = acetyl L-carnitine

AMPA= α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AMPAR= AMPA receptor

AMYG = amygdala

AN = ventromedial affective network

ATD = Amino Terminal Domain

BBB= Blood Brain Barrier

BD = bipolar depression

BDNF= Brain-Derived Neurotrophic Factor

CA3 = cornus ammonis 3

CaMKII = calmodulin-dipedent kinasi II

cAMP = cyclic adenosine monophosphate

CAN = central frontovaginal automic network

CEN = excutive network

CHRM2 = cholinergic receptor type 2

CMS = chronic unpredictable mild stress

CNS = Central Nervous System

COPE = Centers of Psychiatric Excellence

CSDS = chronic social defeat stress

CTD = Carboxyl Terminal Domain

dACC = dorsal anterior cingulate cortex

DAG = diacylglyceral

DLPFC = dorsolateral prefrontal cortex

DMN = default mode network

DMT = N-dimethyltryptamine

DNRI = dopamine and serotonin reuptake inhibitor

DOI = 2,5 - dimethoxy-4-iodamphetamine

ECT = electroconvulsive therapy

eEF2K = eukaryotic elanagation factor kinase 2

EPSC = excitatory postsynaptic currents

ERK = enhancement signal-regulated kinase

ES = epiletic state

fMRI = functional magnetis resonance imaging

GABA = Gamma Amino Butyric Acid

GBCr = regressive global brain connectivity

GLX = glutamate – glutamine compound

GPCR = G-Protein-Coupled Receptor

GSK3β = glycogen synthase kinase 3 β

HAM-D = Hamiton Rating Scale for Depression

HO-1 = heme oxygenase 1

HPA = hypothalamic – pituitary- adrenal

HPC = hippocampus

HPT = hypothalamus

ICN = intrinsic connectivity network

iGluR = ionotropic Glutamate Receptor

IL-6 = interleukin 6

IP3 = inositol 1,4,5 – triphosphate

iPSC = Induced Pluripotent Stem Cell

L-Glu = L-glutamate

LBD = Ligand-Binding Domain

LPS = lipopolysaccharide

LSD = lysergic acid diethylamide-25

LTD= Long-Term Depression

LTP = Long-Term Potentiation

mAChR = muscarine acetylcholine receptors

MADRS = Montgomery Asberg Depression rating scale

MAO = monoamine oxidase

MAPK = mitogen-activated protein kinase

MDD = Major Depressive Disorder

MEG = magnetoencephalography

mGluR = metabotropic Glutamate Receptor

mOFC = medial orbitofrontal cortex

MORs= μ-Opioid Receptors

mPFC = medial prefrontal cortex

mTOR = rapamycin complex 1

NAc = nucleus accumbens

NF = nuclear factor

NLRP3 = NLR family pyrin domain containing 3

NMDA = N-methyl-D-aspartic acid

NMDAR = N-methyl-D-aspartate receptor

nNOS = neuronal Nitric Oxide Synthetase

NO= Nitric Oxide

NSRI = norepinephrine serotonin reuptake inhibitors

OPG = osteoprotegerin

PAG = periaqueductal grey

PCC = posterior cingulate cortex

PD = Parkinson's Disease

PFC = prefrontal cortex

pgACC = pregenual anterior cingulate cortex

PI3K = phosphatidylinositol 3 kinase

PKC = Protein Kinase C

PLC= phospholipase C

PP2A = protein phosphatase 2A

PPD = postpartum depression

PSD = postsynaptic density

PTSD = posttraumatic stress disorder

RANKL = nuclear factor recpetor activator KB ligand

REMS = Risk Evaluation and Mitigation Strategy

RIMA = reversible inhibitors of MAO

RN = ventral frontostriatal reward network

SAR= Structure-Activity Relationship

SARI = serotonin antagonist/reuptake inhibitor

SCFA = short-chain fatty acids

SERT = serotonin transporter

SI = suicidal ideation

SMN = frontocerebellar sensorimotor network

SN = anterior cingulo-insular

SNC = substantia nigra par compacta

SNOC = S-nitrocysteine

SNRI = selective norepinephrine reuptake

SP = serotonergic psychedelics

SSRI = selective serotinin reuptake

STN = subthalamic nucleus

TH = thalamus

TMD = transmembrane domain

TNF = tumor necrosis factor

TRD = Treatment-Resistant Depression

TRP = tryptofan

VMPFC = ventromedial prefrontal cortex

VNS = vagus nerve stimolation

1. Riassunto

In questa tesi, viene proposta ed elaborata l'evoluzione terapeutica contro la depressione resistente al trattamento convenzionale con una maggiore focalizzazione su una nuova e promettente classe di farmaci denominata Rapid-Acting-Antidepressant. Inizialmente, si propone una presentazione in cui vengono definite le varie tipologie di depressioni diagnosticabili insieme alle loro rispettive caratterizzazioni e sintomi e, successivamente, si illustrano le terapie attualmente prescritte contro questa patologia. Proseguendo, si introduce una nuova ipotesi denominata ipotesi glutammatergica in quanto si ritiene che la presenza di una maggiore concentrazione del neurotrasmettitore glutammato sia coinvolta nella manifestazione dei sintomi nei pazienti depressi. Questo neurotrasmettitore eccitatorio è il più abbondante nel sistema nervoso centrale dei mammiferi adulti e possiede un ruolo importante nella neuroplasticità. In base alla neurotrasmissione, i recettori glutammatergici possono suddividersi in: metabotropici (mGluR) i quali sono recettori accoppiati a proteine G implicati nella plasticità sinaptica, nell'eccitabilità e nella connettività neuronale; e ionotropici (iGluR) i quali sono canali cationici ligando dipendenti. I recettori ionotropici sono proteine di membrana composte da quattro subunità che costituiscono il canale ionico il quale consente l'afflusso di cationi calcio a seguito del legame con il glutammato. Le subunità sono essenziali per la sinaptogenesi, il rimodellamento sinaptico dipendente dai cambiamenti nella potenza sinaptica. Nella depressione, è presente un'eccessiva stimolazione di questi recettori causata da elevate concentrazioni di glutammato portando ad una condizione di eccitotossicità.

Le terapie famacologiche basate su questa teoria si pongono come obbiettivo l'inibizione del recettore *N*-metil-D-aspartato (NMDA). Il principale capostipite dal punto di vista farmacologico è la ketamina, diffuso come anestetico locale, la quale ha mostrato una rapida e sostenuta attività antidepressiva. Tuttavia, la ketamina non può essere considerata come un farmaco sicuro in quanto la sua somministrazione richiede l'ambulatorizzazione del paziente, a causa della comparsa di effetti collaterali dissociativi e del potenziale di abuso. Per questo motivo, la ricerca si è focalizzata sullo sviluppo di altri composti attivi. Oltre alla ketamina, altri antagonisti del recettore NMDA sono: la memantina, farmaco prescritto per il morbo di Alzheimer; norketamina, derivato della ketamina; MK-801, destrometorfano, destrometadone e lanicemina. Tuttavia, sono presenti anche altri farmaci aventi diverso meccanismo d'azione come il rapastinel, un agonista parziale del recettore NMDA. Oltre al sistema glutammatergico, negli ultimi anni è stato

dimostrato il coinvolgimento di altri sistemi tra cui il sistema serotoninergico, il sistema colinergico, e l'asse ipotalamo-ipofisi-surrene. Ognuno di questi sistemi presenta almeno un candidato farmaco in fase di clinica di sperimentazione per il trattamento del disordine depressivo maggiore. In conclusione, questo lavoro di tesi propone un confronto tra le terapia attualmente prescritte e nuovi approcci terapeutici per la depressione paragonandone le principali caratteristiche.

1.1 Abstract

In this thesis, therapeutic evolution against depression resistant to conventional treatment is proposed and elaborated with a greater focus on a new and promising class of drugs called Rapid-Acting-Antidepressant. Initially, a presentation is offered in which the various types of diagnosable depression are defined along with their respective characterizations and symptoms, and then the currently prescribed therapies against this condition are outlined. Continuing, a new hypothesis called the glutamatergic hypothesis is introduced as it is believed that the presence of increased concentration of the neurotransmitter glutamate is involved in the manifestation of symptoms in depressed patients. This excitatory neurotransmitter is the most abundant in the adult mammalian central nervous system and possesses an important role in neuroplasticity. Based on neurotransmission, glutamatergic receptors can be divided into: metabotropic (mGluR) which are G-protein-coupled receptors implicated in synaptic plasticity, excitability and neuronal connectivity; and ionotropic (iGluR) which are ligand-dependent cation channels. Ionotropic receptors are membrane proteins composed of four subunits that constitute the ion channel which allows the influx of calcium cations following glutamate binding. The subunits are essential for synaptogenesis, the synaptic remodeling dependent on changes in synaptic potency. In depression, there is overstimulation of these receptors caused by high concentrations of glutamate leading to an excitotoxic condition.

Phamacological therapies based on this theory target *N*-methyl-D-aspartate (NMDA) receptor inhibition. The main progenitor from the pharmacological point of view is ketamine, widespread as a local anesthetic, which has shown rapid and sustained antidepressant activity. However, ketamine cannot be regarded as a safe drug because its administration requires ambulatory treatment of the patient, due to the occurrence of dissociative side effects and the potential for abuse. For this reason, research has focused on the development of other active compounds. In addition to ketamine, other NMDA receptor antagonists include: memantine, a drug prescribed for Alzheimer's disease; norketamine, a ketamine derivative; MK-801, dextromethorphan,

dextromethadone, and lanicemine. However, there are also other drugs having different mechanism of action such as rapastinel, an NMDA receptor partial agonist. In addition to the glutamatergic system, the involvement of other systems including the serotonergic system, the cholinergic system, and the hypothalamic-pituitary-adrenal axis has been demonstrated in recent years. Each of these systems has at least one drug candidate in clinical trials for the treatment of major depressive disorder. In conclusion, this thesis work proposes a comparison of currently prescribed therapies and new therapeutic approaches for depression by comparing their main characteristics.

2. Introduction

2.1 Definitions of depression

Depression is an affective disorder, i.e. the person presents excessive sadness or euphoria that either persists beyond the foreseeable impact of a stressful event or arises in the absence of a stressor [1].

The diagnosis of this disorder is made by means of the Hamilton Test, which is based on symptomatology, family history, course and response to somatic treatments. Depression is more frequently diagnosed in women; it may also be associated with anxiety and/or stress, and with suicide risk. Suicide is the cause of death in 15-25% of patients with such disorders without therapeutic treatment or in cases of undiagnosed or inadequately treated depression. The risk of suicide has the highest incidence in the young and elderly who lack good social support and tends to occur within 4-5 years of the first diagnosed clinical episode [1,2].

Depression can be diagnosed based on the extent of the symptoms manifested:

- major depressive disorder or unipolar depression (MDD). Symptoms must be present for at least two weeks. This disorder may be associated with vegetative signs and delusions congruent with mood; hallucinations may also be present. The patient must present with five or more of them including:
    - depressed mood for most of the day,
    - anhedonia i.e. loss of pleasure, reward and motivation, as well as a reduction in the amount and duration of positive affect after exposure to positive stimuli,
    - loss/gain of body weight,
    - insomnia/hypersomnia,
    - psychomotor agitation/sluggishness,
    - loss of energy/tiredness,
    - recurrent thoughts of death and/or suicide,
    - decreased concentration,
    - feelings of worthlessness and/or excessive or inappropriate guilt [1].

Various studies hypothesize that the debilitating effects of MDD result from dysfunction of the interconnected network of brain regions that collectively regulate mood. Clinical and preclinical data show that depressive behavior is caused by dysfunction manifesting in several integrated brain regions, including the prefrontal cortex (PFC) and the hippocampus (HPC). Many factors make this type of depression difficult to treat adequately, including the etiopathogenesis and the dearth of available pharmacological treatments. In recent years, there have been advances in molecular biology, especially in the field of Induced Pluripotent Stem Cell (iPSC) technology, which expands the possibilities of possible studies of the molecular mechanisms underlying MDD. These studies are very important as pharmacological screening systems could be implemented which would be of great help in identifying the appropriate patient-specific therapy [3].

- Dysthymic disorder: this is a mild depression and is defined as depressive neurosis. It became chronic in women and can be brought on by substance abuse. To be diagnosed as such, the patient should present at least two of the following symptoms: poor appetite, hyperphagia, sleep disturbances, easy fatigability, low self-esteem, poor ability to concentrate or difficulty in making decisions and feelings of hopelessness [1].
- Reactive depression: linked to a triggering event and does not have chronic features. Symptoms are present for a period of less than two months [1].
- Bipolar disorder: this is a combination of depression and mania (cyclothymia). The symptoms associated with it increase with age [1].

The treatment of depression is drug therapy that must last for at least six months as there is a high risk of patients relapsing. For some patients who show resistance, the therapy is multi-drug. The downside of this treatment is the appearance of undesirable effects before the therapeutic effects, which appear after 2-4 weeks of treatment [1].

The neuronal systems involved in this disorder are serotonergic, dopaminergic, adrenergic and cholinergic (especially muscarinic receptors). Serotonin is the neurotransmitter most involved in depression and this is apparent from three factors:

1. Low urine concentrations of 5-hydroxyindoleacetic acid (main metabolite);
2. Low concentrations of serotonin transporter (SERT), a presynaptic transporter with the function of reabsorbing released serotonin;

3. Low concentrations of tryptophan (TRP) at synapses [1].

There are several hypotheses that have been formulated to understand the mechanism of action of drugs:

- Monoaminergic hypothesis. In this hypothesis, depression is due to the inefficiency of synapses induced by serotonin, noradrenaline, and dopamine. The major drugs used in therapy are monoamine oxidase (MAO) inhibitors and reuptake inhibitors. MAO enzymes are involved in the metabolism of serotonin, norepinephrine, and dopamine, so inhibition of them favors an increase in their concentrations [1].
- Receptor sensitivity hypothesis. Not only are important monoamine concentrations, but also the sensitivity of the post-synaptic neuron. The receptor may be absent after hyperstimulation due to the action-reaction principle [1].
- Permissive hypothesis. The concentrations of the monoamines noradrenaline and serotonin are not important but their balance [1].
- Hormonal hypothesis. Variations in the hypothalamic-pituitary-adrenal axis influence the release of noradrenaline, serotonin, and acetylcholine by CNS neurons. In depressed patients, higher cortisol levels are detected [1].
- neurotrophic theory (BDNF = brain derived neurotrophic factor). The forming neuron emits certain neurotrophic peptides so that only a few neighboring neurons form dendrites for synapses. Therefore, the antidepressant effect is seen after months. BDNF regulates neuroblast proliferation, axonal growth, and neuronal plasticity. Several studies have shown that depression is associated with a reduction in the size of brain regions that regulate mood and cognition such as the prefrontal cortex and hippocampus; with a decreased number of neurons in these areas.

2.2 The current therapy

There is considerable agreement on treatment for acute major depressive episodes, although recommendations based on depression severity vary. All guidelines recommend psychotherapy (cognitive-behavioral or interpersonal) as a treatment option for mild to moderate depression. However, pharmacotherapy is usually the first choice for treating major depressive episodes because of a lack of immediate access [4].

If symptom severity, defined as a decrease in the Hamilton Depression Rating Scale score, does not improve at least moderately, the first strategy is usually to optimize acute treatment by increasing the dose of first-line drugs. Switching to another medication of the same class or another class of drugs, usually with a complementary medication (e.g., lithium or an atypical antipsychotic) or an "add-on strategy" with a second antidepressant, is also recommended [4]. For depressed patients treated with antidepressants, several adjunctive therapies may be prescribed that may be useful in enhancing the effects of antidepressants. The most common adjunctive treatments are mirtazapine and bupropion, which are prescribed in addition to existing SSRI/SNRI therapy to alleviate residual symptoms of sleep disturbance and lack of energy, respectively. Some patients do not respond satisfactorily to antidepressant treatment. For those who do not or partially respond, triiodothyronine or lithium are prescribed. In addition, other medications are added to existing antidepressants, or dietary supplements are prescribed to supplement antidepressant therapy. However, patients who do not improve after at least two courses of antidepressant treatment are referred to as "treatment-resistant depression." Patients with treatment-resistant depression are treated with additional (increased) doses of one of the second-generation antipsychotics, such as aripiprazole, olanzapine, or quetiapine. [5]. In scientific studies, the criteria for remission of depression have been defined as follows: patients with major depressive disorder (a) have a Hamilton depression rating below 7, (b) return to pre-disease functional levels, and (c) maintain this improved state for at least two months. In a survey of outpatients with major depression, patients in remission after treatment showed high agreement in four major definitions from their own perspective: (A) positive mental health (optimism, energy, confidence), (B) habitual self-perception, (C) general well-being, and (D) absence of depressive symptoms. Through both objective and subjective determinations, experienced psychiatrists should be able to easily determine when clinic patients are in remission from depression during clinic observation. [5]. Once in remission, treatment should be maintained at the same dose for at least one year. For people with three or more risk factors for relapse, long-term (one to two years) maintenance treatment with pharmacotherapy and/or evidence-based psychotherapy is recommended. During follow-up, clinicians should ask about residual symptoms, medication-related side effects, treatment adherence, and functional and psychosocial outcomes. [4].

In particular, drug therapy comprises several groups of drugs divided according to the target neurotransmitter, i.e. the substance released to mediate a message by neurons in its synaptic loop. The classes of drugs in therapy are:

- NSRIs (Norepinephrine Serotonin Reuptake Inhibitors): also called thymoleptics. These are first-generation drugs that are non-selective for the reuptake of norepinephrine and serotonin. Their mechanism of action involves blocking post-synaptic receptors and blocking sodium channels. This leads to many side effects including sedation, arrhythmias, weight gain, etc. They have a sedative action so can be administered in anxious or agitated patients. The drugs that represent this class are imipramine, amitriptyline, doxepin [1].

- SSRIs (Selective Serotonin Reuptake Inhibitors): very selective drugs that inhibit the reuptake of serotonin. These drugs are the most widely used in therapy. The drugs that represent this category are citalopram, escitalopram, sertraline [1,6];

- SNRI (Selective Norepinephrine Reuptake Inhibitors): selective drugs for norepinephrine which is involved in the regulation of temperature and sleep. Nortriptyline and desipramine are two of the drugs that belong to this class [1,6];

- RIMA (Reversible Inhibitors of MAO-A): reversible inhibitors of MAO-A. MAOs (monoamine oxidases) catalyze the oxidative deamination of serotonin, dopamine and noradrenaline. Inhibition ends when the drug is discontinued [1];

- TIMERETINS: These non-selective drugs inhibit metabolism in the pre-synapses so there is an increase in serotonin and noradrenaline concentrations. These molecules form a covalent bond with MAO. Due to the inhibition of MAO-B, there is a failure to metabolize tyramine, a derivative of tyrosine decarboxylation capable of stimulating catecholamine secretion; and this leads to possible hypertensive crises also with the intake of foods containing tryptophan. In this case, patients must avoid foods containing hydrolyzed proteins, cheese, wine and sympathomimetic compounds (amphetamines, ephedrine). The effect sets in slowly and lasts a few weeks after the end of therapy. This class of drugs has many side effects, so they are not the first choice. They are also used in the pharmacological treatment of Parkison's disease [1];

- SARIs (Serotonin Antagonist/Reuptake Inhibitors): these are 5HT2 receptor antagonists and serotonin reuptake inhibitors. These drugs modulate the amount of serotonin in the brain. They are also commonly used in the treatment of schizophrenia. The parent drug of this class is mirtazapine [1,6];

- DNRIs: are dopamine and serotonin reuptake inhibitors; bupropion is a drug that represent this class [1,6];
- ATIPICs: are drugs used in the treatment of depression that has a different mechanism of action to the other classes but affect the synaptic activity of monoamine neurotransmitters i.e., dopamine, noradrenaline and serotonin. The drugs that represent this class is aripiprazole [1];
- Humor stabilizers: these are drugs used in the treatment of bipolar depression or bipolar affective disorder. Their mechanism of action involves interaction with inositol triphosphate (IP3), in particular, inhibition of the enzyme involved called inositol monophosphatase by reducing the availability of free inositol. The best-known drug representing this class is lithium [1].

In addition to drug therapy, there are therapies that do not involve a pharmacological mechanism such as electroconvulsive therapy (ECT), psychotherapy and magnetic stimulation [1].

In addition, the correlations between each adverse reaction tended to be weak. As a result, an overall score on the adverse reaction measurement scale is not a sufficient statistic, and specific complaints should be evaluated individually. Individual side effects change over time during antidepressant treatment and have predictive value for discontinuation of antidepressants, which is not accounted for by the sum of weakly correlated items. Patients with severe depression are more likely to experience physical side effects from antidepressants. This may be due to an increased sensitivity and attention to the physical discomfort that accompanies a depressed mood [7]. Serotonin-transmitted antidepressants have three main side effects-gastrointestinal symptoms (nausea, upset stomach, abdominal discomfort), agitation (difficulty falling asleep, vivid dreams), and sexual dysfunction (reduced desire, orgasm suppression, delayed ejaculation, difficulty getting an erection or vasodilation/lubrication). Antidepressants with norepinephrine transmission have side effects in the form of constipation, dry mouth, paresthesia, nausea, or headache. However, all these side effects are easily tolerated by patients [5]. Dry mouth was the most reported side effect. This side effect occurred more frequently during treatment with nortriptyline or escitalopram than in the no-drug condition, and was positively related to the dose of both antidepressants. Anticholinergic side effects such as dry mouth, constipation, orthostatic dizziness, and blurred vision were seen more frequently in people taking nortriptyline than in those taking escitalopram. Increased appetite and weight gain

with nortriptyline and decreased appetite and insomnia with escitalopram can be interpreted either as side effects or as differential effects of both drugs on the neurotrophic symptoms of depression. Reduced appetite and insomnia have been reported more frequently in untreated depression than during treatment with escitalopram, suggesting that at least part of this difference may be explained by differences in efficacy [7]. The most reported side effects were not associated with the discontinuation of antidepressants. However, some side effects were strong predictors of discontinuation. For example, dysuria, although relatively rare, was associated with a doubling of the frequency of discontinuation within 12 weeks of nortriptyline treatment. Because urinary retention is treatable, special attention may be needed to ensure shared decision-making and maximize the chances of receiving effective medication treatment while minimizing the burden of side effects [7].

Rebound phenomena refer to the increased susceptibility of the body after discontinuation of the drug. In this phenomenon, depressive symptoms return to a greater extent than when the drug was first administered, or there is a greater risk of relapse than in patients who did not receive the drug. Signs of rebound phenomena is often confused with symptoms related to the depressive disorder, as they too can change in severity over the course of its natural course [9].
In particular, selective serotonin reuptake inhibitors (SSRIs) are studied because of this phenomenon. Fluoxetine does not present particular problems, even with abrupt discontinuation, whereas, sertraline, citalopram, and escitalopram present a reduced risk. The most common symptoms of SSRI withdrawal syndrome are described as flu-like or a sudden return of anxiety or depression. They may include diarrhea, dizziness, fatigue, headache, insomnia, nausea, vomiting, psychosis, and suicidal thoughts. However, these symptoms are rarely severe. In fact, most people experience only mild or moderate forms of SSRI discontinuation syndrome [9]. Discontinuation of paroxetine, escitalopram, citalopram, and fluvoxamine, paroxetine is marked with anxiety and panic disorders, sleep disturbances, and cyclothymic/bipolar disorders [8].
The minimum duration required for the onset of treatment withdrawal symptoms has not been sufficiently demonstrated; it is hypothesized at least 4 weeks seem necessary. For SSRIs and SNRIs, there is strong evidence for the risk of antidepressant discontinuation syndrome (ADS) starting at 8 weeks and that this risk does not change significantly with longer treatment. ADS appears to develop independently of the primary disorder [8].
From a clinical point of view, most of the symptoms of ADS correspond to the picture of serotonin syndrome, particularly with SSRIs, which can be explained at least in part by the effects of

antidepressants on serotonin transporters. This is due to the fact that some antidepressants not only block serotonin and norepinephrine transporters, but also cause a reduction (rather than a counterregulatory increase) in these transporters when used long-term, which can lead to persistent serotonin hyperfunction after discontinuation (transporters reduce the level of serotonin in the synaptic cleft) [8].

Within drug classes, there is a correlation between the plasma elimination time of drugs and the severity and time of onset of ADS. Therefore, antidepressants with a short half-life have a higher risk of developing (more severe) withdrawal symptoms. Consequently, those with rapid metabolization are also likely to have a higher risk of ADS. The onset of withdrawal symptoms appears to occur in about three to five half-lives after withdrawal. The increased risk of ADS at higher doses appears to affect only the high-dose range (duloxetine 120 mg/day; escitalopram 20 mg/day) [8].

Misinterpretation of symptoms can result in unnecessary and potentially harmful medication (e.g., if ADS is misinterpreted as a manic episode and later diagnosed as bipolar affective disorder). Similarly, when switching medications, ADS due to the discontinued medication may be misidentified as an adverse reaction to the new medication. A guiding criterion for this differentiation may be the time course, which is characterized by early onset and fluctuations and tends to be transient. The most likely time course is onset in the first week after drug withdrawal and resolution in the second week [8].

The most important therapeutic approach is probably prevention. Since in most cases the symptoms are mild and self-limiting, detailed patient education is often sufficient; if necessary, patients can receive symptomatic treatment in the form of hypnotic agents or antimuscarinic substances for TCA and cholinergic rebound. If symptoms are severe, antidepressants can be resumed, which usually leads to complete remission of symptoms within 24 hours. This also applies to extrapyramidal symptoms and paradoxical activation/mania. Gradual tapering can then be carried out. Although tapering antidepressants cannot completely rule out the risk of ADS, it appears to reduce its severity [8].

Fluoxetine has been shown to be a "rescue" substance for withdrawal symptoms from other SSRIs and venlafaxine. This drug can be given instead of the discontinued drug in case ADS emerges after weeks [8].

When an antidepressant is prescribed, there is a concern that once started it cannot be stopped due to the risk of relapse and, in some cases, resistance to treatment [8].

In conclusion, if you want to stop taking an antidepressant or reduce the dose, you should always consult your doctor. Gradual reduction of the SSRI dose over a period of time is often regarded as the best way to minimize the effects of SSRI withdrawal syndrome.

Should the patient notice the return of any symptoms of anxiety, and depression in the weeks and months following the discontinuation of the antidepressant drug, he is obliged to call his doctor [9].

3. Glutamatergic hypothesis

In recent decades, in addition to the assumptions described above, a new hypothesis has been theorized on the basis of the mechanism of action of certain drugs targeting the *N*-methyl-D-aspartate receptor called the glutamatergic hypothesis.

"The increased production of NMDA receptor agonists could induce a state of activation of the glutamatergic system that is currently associated with the development of depression." - Müller N. 2008

Considering this hypothesis, a possible therapy against treatment-resistant depression is suggested. Treatment-resistant depression is defined as such if two or more previously listed drug therapies fail on the patient. The first drug that supports this theory is ketamine, a local anesthetic drug that interacts with glutamate receptors [58].

Neurotransmission by the glutamate receptor occurs with the release of glutamate that involves the activation of G-proteins coupled to the receptor or the opening of the ion channel. Glutamate is an amino acid neurotransmitter that exhibits excitatory activity on the nervous system. This neurotransmitter is most abundant in the CNS of adult mammals and plays an important role in neuroplasticity [10,11].

3.1 Glutamate receptor

Depending on neurotransmission, glutamate receptors can be subdivided into:

- Metabotropic (mGluR): these are G-protein-coupled receptors implicated in synaptic plasticity, excitability, and neuronal connectivity. The group I receptors (mGluR1 and mGluR5) are coupled to G_{aq} proteins, i.e. proteins characterized by the presence of αq, a subunit, that activates phospholipase C resulting in the synthesis of the second messengers inositol triphosphate (IP3) and diacylglycerol (DAG) causing an increase in intracellular Ca^{2+}. In addition, these receptors are coupled to phospholipase C. They are involved in central sensitization and chronic pain. Group II receptors (mGluR2/mGluR3) and group III receptors (mGluR4 and mGluR6-mGluR8) are coupled to G_{ai} proteins and inhibit adenylate cyclase [10];
- Ionotropic (iGluR): these are ligand-dependent cation channels structurally divided into AMPA receptors, kainate receptors, and NMDA receptors. Several properties distinguish

between them such as the voltage-dependent blockade by extracellular Mg^{2+}, the high permeability of the Ca^{2+} cation, and the presence of two co-agonists useful for the channel activation request [10].

3.1.1. NMDA receptor

The NMDA receptor is the focal point of the glutamatergic hypothesis and it's the most studied of the ionotropic receptors. The NMDA receptor is a ligand-dependent ion channel that mediates a Ca^{2+}-permeable component of excitatory neurotransmission in the CNS. There can be various subtypes based on the variation in the assembly of the seven subunits present in tetrameric receptor complexes. The subunits are: GluN1, GluN2A-D, GluN3A-B [11]. They are highly permeable to Ca^{2+} and Ca^{2+} influx through NMDAR is essential for synaptogenesis, experience-dependent synaptic remodeling and changes in synaptic efficacy. These receptors mediate a current that is activated more slowly with a time course lasting tens to hundreds of milliseconds [11]. At rest, the pore is strongly blocked in a voltage-dependent manner by extracellular magnesium. The latter can be removed by activation of AMPA receptors. The mediated current depends on both the membrane potential and the frequency of synaptic release. Two co-agonists are always present: extracellular glycine or d-serine. The distinction depends on the brain region in addition to the subcellular location of the receptor [10,11].

Subunit structure-function

In this section, the structure of the receptor and their corresponding function will be described in detail to illustrate the possible binding sites by the rapid-acting antidepressants.

The GluN1 subunit, which binds glycine and d-serine, is a mandatory part of all functional NMDA receptors and is, therefore, widely expressed in central neurons. Three exons, or coding sequences, in the GluN1 subunit can be alternatively ligated to synthesize eight different isoforms. Exon 5 encodes 21 amino acids in the GluN1 amino-terminal domain (ATD), exon 21 encodes 37 amino acids in the carboxyl-terminal domain (CTD) and exon 22 encodes 38 amino acids in the CTD [12]. An important property of GluN1-containing NMDA receptors (e.g. GluN1-1b) with residues encoded by exon 5 is the reduced agonist potency. Consistent with the effect on agonist potency, the GluN1-1b-bound variant accelerated the inactivation of the NMDA receptor response after glutamate removal, resulting in reduced duration of excitatory postsynaptic currents (EPSCs). These effects probably reflect the interaction between amino terminal domain ATD and agonist binding domain (ABD) GluN1 and GluN2 generated by residues

encoded by exon 5. In addition, GluN1-1b attenuated the inhibition of NMDA receptor function by the antagonist-selective GluN2B, such as ifendil, reduced the extracellular inhibition of Zn^{2+} and protons, and almost abolished the potentiation of extracellular polyamines [13]. Unfortunately, the relationships between functional roles and structural features of the residues encoded by exons 21 and 22 of GluN1 are still not understood [14].

The four glutamate-binding GluN2A-D subunits provide the CNS with a means to control NMDA receptor properties depending on developmental period and brain region. GluN2 subunits influence the potency of glutamate [14]. Interestingly, intracellular allosteric interactions make the potency of glycine and d-serine in the GluN1 subunit sensitive to the identity of the GluN2 subunit. GluN1/2A and GluN1/2B demonstrate different properties compared to GluN1/2C and GluN1/2D such as higher single-channel conductance, and higher Ca^{2+} permeability and are more sensitive to Mg^{2+} blockade. These biophysical differences are important, as the sensitivity to voltage-dependent Mg^{2+} blockade can influence the period for spike-time-dependent plasticity [10,15]. Furthermore, the probability that the channel is open when all agonist binding sites are occupied by agonists is strongly dependent on the identity of GluN2. GluN2 subunits are involved in the inhibition of NMDA receptors by endogenous modulators, such as protons and extracellular Zn^{2+}. The amino acid sequence of intracellular c-terminal domain CTD is highly variable between GluN2 subunits. The latter variation influences cell surface expression, subcellular localization and recycling/degradation of NMDA receptor subtypes [10,11,16].

All glutamate receptor subunits share a similar structure formed by four domains: a large extracellular amino-terminal domain ATD, a bilobed agonist binding domain ABD (or ligand binding domain), a transmembrane pore-forming domain (TMD) and an intracellular CTD, as shown in Fig. 1.

The TMD consists of three transmembrane helices (M1, M3 and M4) and a reentrant loop (M2). In iGluRs, the reentrant loop lines the intracellular portion of the ion channel pore, while the elements of the third transmembrane segment (M3) form the extracellular region of the pore. Among the NMDA receptor subtypes, residues in the pore region are highly conserved as they influence ion permeation. Permeability to bivalent ions and Mg^{2+} blockade is controlled by a region residing at the apex of the M2 reentrant circuit, which is referred to as the Q/R/N site based on the amino acids at this position in AMPA, kainate and NMDA receptors [10,17].

ATDs play important roles in assembly and strongly modulate NMDA receptor function. These domains create binding sites for allosteric modulators, including extracellular Zn^{2+} and a diverse array of selective GluN2B antagonists [10,18,19].

The ABD is formed by segments S1 and S2 of the polypeptide chain, which are separated by segments M1, M2 and M3. These domains form bilobed structures containing an upper lobe (D1) and a lower lobe (D2) with the agonist binding site residing in the gap between these two lobes [10,20].

The structures of the ABD heterodimer GluN1-GluN2A in complex with various agonists, partial agonists and antagonists suggested a structural basis for their mode of action [10,21,22]. The binding of glycine and glutamate to the ABDs GluN1 and GluN2, respectively, produces a rapid ABD rearrangement that results in the reduction of the angle between the D1 and D2 lobes, producing a shell closure of the bilobed domain. This agonist-mediated ABD closure triggers the formation of hydrogen bonds between the upper and lower lobe residues, which are hypothesized to establish the agonist-bound ABD structure. The energy provided by agonist binding and ABD closure causes a series of conformational changes in the receptor to open the pores of the ion channel. Therefore, the closure of the ABD is the initial conformational change required for the ion channel gating process to be triggered [10,23,24].

The NMDA receptor has several unique structural features compared to AMPA and kainate receptors. Some examples are:

- There are extensive contacts between the two GluN1/2 ABD heterodimers that are only present in NMDARs which provide the structural basis for the GluN2 subunit dependence of glycine potency [10];
- The NMDA receptor ATDs show a different arrangement leading to distinct subunit interfaces compared to AMPA and kainate receptors [10].
- ATDs form strong contacts with the upper lobe of the ABD, whereas ATD-ABD interactions are weak in AMPA and kainate receptors. These interactions give the NMDA receptor a more compact 'balloon-like' appearance, a distinguishing feature from AMPA and kainate receptors. In addition to their shape, these interactions form a protein-protein interface where modulators can bind [10].

The structure of the NMDA receptor demonstrates unique intra- and interdomain contacts that illustrate a situation suitable for understanding allosteric interactions between subunits, as well as allosteric modulation by small molecule ligands [10].

The ATD adopts a bilobed structure, unrelated to the ABD, with R1 and R2 (indicating the upper and lower lobes, respectively). In addition to this structure, there is an unique dimer arrangement of the NMDA receptor ATDs compared to the ATDs in the AMPA and kainate receptors. This arrangement is characterized by a protein-protein interface formed by the upper R2 lobes of the GluN1 and GluN2 subunits, while the lower R1 lobes, which connect to the ABDs, are almost completely separated [10,20,25].

All three transmembrane helices (M1, M3, and M4) and the membrane-retracting pore-forming loop (M2) are involved in the pore-opening process. The M3 helix forms a helical bundle that physically crosses the pore, changing its position before the ions can cross the channel pore [10,20,25]. The M3 transmembrane helix contains nine amino acids (SYT ANL AAF) that are almost completely conserved in iGluRs throughout the animal kingdom [10,26].

Residues in the region connecting the S1 segment of the ABD with the M1 helix, i.e. the pre-M1 linker, are invariant in the healthy population and a locus for disease-associated mutations in various neurological diseases. In addition, the region linking the S2 segment of the ABD with the M4 helix, i.e. the pre-M4 linker, also appears to be implicated in patients with NMDA receptor missense mutations, and both the pre-M1 and pre-M4 linkers are close enough to link to the SYT ANL AAF sequence conserved in the M3 helical bundle junction. These three elements appear to be positioned to create a gating control mechanism and it is possible that kinetically distinct conformational states could be the result of rearrangements of this triad of interacting regions [10,26].

Agonist binding results in three conformational states:

- "inactive 1" and "inactive 2": the GluN2B ABD-M3 linker is too "relaxed" to disrupt the channel gate, this is reflected by the fact that the two GluN1-GluN2B ABD heterodimers are "coiled" and the R2 lobes of the ATDs of these heterodimers are distant [10,23,24,26];
- 'active': the ABD-M3 linker of the two GluN2B subunits assumes a 'striated' structure, creating a voltage that disrupts the gate of the closed channel. The gate is formed by a group of hydrophobic residues at the apex of GluN1-M3 and GluN2B-M3. In addition,

the GluN1-GluN2B ATDs orient themselves to bridge the gap between their R2 lobes [10,23,24,26].

Dysfunction of this receptor can lead to the appearance of neuropathologies such as Alzheimer's disease, depression, stroke, epilepsy, schizophrenia and in some cases even the appearance of tumors in the body [27].

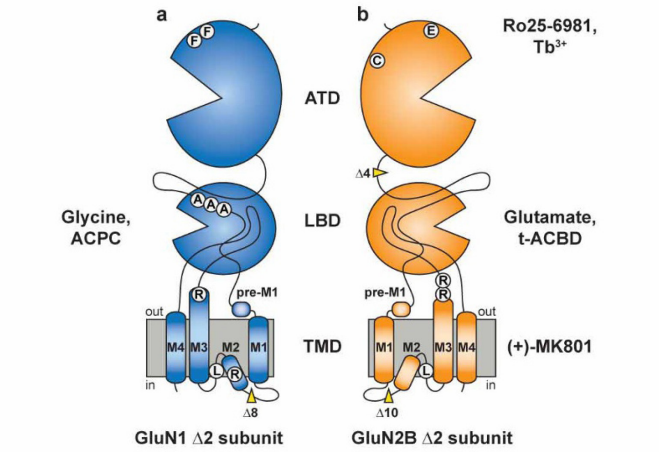

Fig 1: Representation of the NMDA receptor with more detail in the domains present in it. Map representation of the amino-terminal domain (ATD), ligand-binding domain (LBD) and transmembrane domain (TMD) for the GluN1 D2 (a) and GluN2B D2 (b) subunit constructs. The position of point mutations is highlighted by white circles. The position of deletions is highlighted with a yellow wedge. Mutated glycosylation sites are not shown.

3.1.2. AMPA receptor

These are alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors. They are channels that mediate a synaptic current with rapid rise and decay times. consists of homotetrameric subunits that may include different combinations of four different subunits. They are permeable to sodium, potassium and calcium cations depending on their composition as in Fig.2. They may play a critical role in pathologies from ischemia to neurodegenerative diseases [27,28].

3.1.3. Kainate receptor

These receptors are permeable to sodium and potassium cations as shown in Fig. 2. They have a tetrameric structure and their final composition is determined by the association and variation of five subunits. They are equally distributed in the CNS. Depending on their location, these receptors possess a certain function. If the receptor is post-synaptic, its function will be synaptic transmission; whereas if the receptor is pre-synaptic, its function is the release of neurotransmitters that influence synaptic plasticity through the activation of G-proteins that, in

turn, inhibit calcium currents. These receptors contribute to neurodegeneration by increasing excitotoxicity which, in turn, increases microglia activation and neuroinflammation [27].

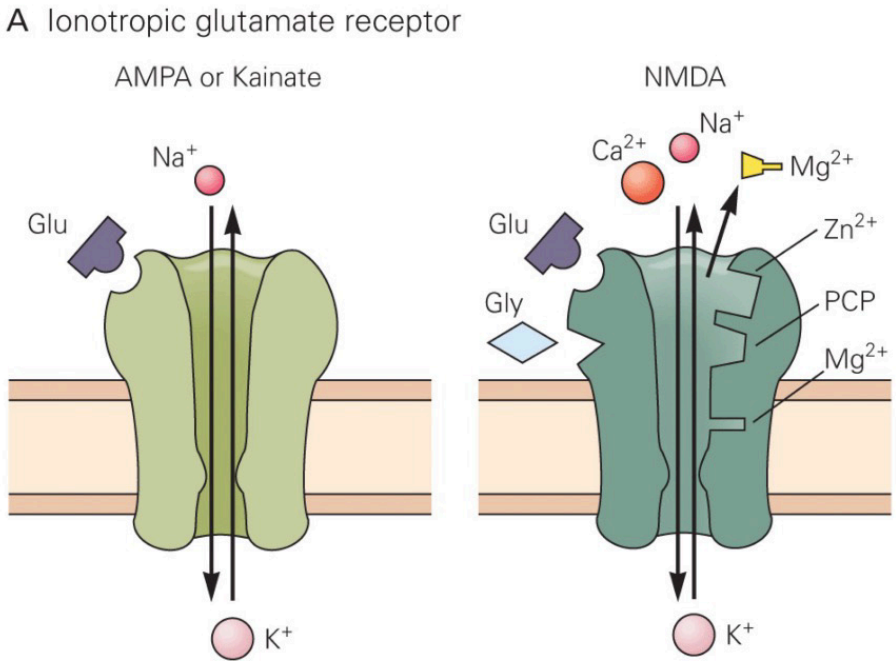

Fig 2: NMDA receptors physiological modulation.

3.2 Function of the glutamate receptor

In addition to the structure, it is also very important to understand the function of this receptor within the synapse. In the synaptic cleft, glutamate binds to AMPA, kainate and NMDA receptors, inducing the conformational changes necessary to trigger the opening of the ion channel pore [10,15]. This phenomenon is referred to as a gating process. At resting potential (negative internal cell potential -60/-70 mV) even if glutamate binds to the receptor, opening the channel, the current cannot flow because extracellular Mg^{2+} ions attracted by the internal negativity enter the channel and block it. Depolarization of the membrane removes this voltage-dependent blockade by magnesium, which is repelled by the positive internal potential. The current can only propagate through the channel if the two previous conditions: 1) glutamate binding and 2) membrane depolarization occur simultaneously. Only in this situation the channel is open and the calcium ions can pass through it freely and enter the cell. The NMDA receptor-mediated component continues to transmit a current for a period of 10-100ms even after the neurotransmitter has been removed. The functional consequences are highly dependent on the identity of the GluN2 subunit [10,15,21].

3.3. Neuroimaging

In this section, I would like to give an insight into how the irregularity of the glutamate receptor affects various parts of the brain. High concentrations of glutamate manifest an etiological picture by showing that alterations in its release, clearance and metabolism cause a prolonged accumulation in cortical and limbic brain areas. These brain areas are involved in the regulation of emotions, cognition and behavior, so their alteration promotes a depressive state. Considering this condition, one finds a situation where reduced synaptic connectivity persists in these regions, manifested by reduced synaptogenesis, imbalance of excitation-inhibition, neuronal loss and atrophy, and deficits in fine inhibitory regulation. However, clinical research consists of hypotheses and translational preclinical models are lacking. The effects of glutamate-mediated compounds on human brain connectomes remain inconclusive. This makes it difficult to identify reliable and reproducible biomarkers of therapeutic efficacy and rapid response to antidepressants, limiting the application of these findings in real psychiatric practice. To date, research has mainly focused on methods to quantify biological structure and function, such as multiomics (transcriptomics, proteomics and metabolomics), neuroimaging and network and pathway analysis [29,47]

In particular, neuroimaging is a technique that can provide robust and reproducible models of the functional neuroanatomy of the brain's network architecture. One of the most important advances of this technique is the demonstration that the activity of brain regions is organized in coherent networks that are functionally distinct from each other. These networks are termed intrinsic connectivity networks (ICNs) and represent paired brain regions related in time to spontaneous or activity-evoked fluctuations. ICNs are associated with human cognition and behavior undergoing either neuropsychological paradigms or at rest [29,30]. It is considered that the study of functional alterations in MDD- and TRD-specific ICNs could shed light on the heterogeneity of symptom manifestation and response to treatment, serving as predictive biomarkers of treatment resistance and influencing clinical and functional outcomes [29].

In the human brain, several areas associated with cognitive, behavioral and emotional functions are deeply interconnected through glutamatergic neurons. These key areas include the prefrontal cortex (PFC), anterior cingulate cortex (ACC), nucleus accumbens (NAc), hippocampus (HPC), amygdala (AMYG), thalamus (TH), hypothalamus (HPT), and brainstem neurotransmission centers (which regulate noradrenaline release) [29,31]. The abundance of glutamatergic neurons combined with the fact that glutamatergic transmission through cortical and subcortical limbic

pathways constitutes a large part of the brain's functional connectivity profile and this is a key mediator in the extensive control and expression of complex cognitive and emotional processes. Reduced glutamatergic neurotransmission is believed to disrupt synaptic connectivity, leading to reduced synaptogenesis and cell signaling. However, these abnormalities aren't generalized and are probably related to structural and functional abnormalities in specific nodes of the central network, such as PFC, HPC and AMYG [31]. The study of the entire brain connectome can be considered as a structure consisting of a set of key ICNs, each of which is functionally and behaviorally important. This concept would make it possible to better characterize local changes in absolute glutamate levels and how they might be reflected in specific symptoms of depression. Multimodal neuroimaging studies, including functional magnetic resonance imaging (fMRI), electroencephalography, magnetoencephalography and positron emission tomography, have identified several candidate ICNs functionally related to MDD symptoms and pathology. These particular ICNs number is seven and will be listed and described, emphasizing their altered function in MDD patients [29, 30, 32] and their modification under the influence of ketamine:

- the default mode network (DMN) shown in Fig.3: it is an interconnected and anatomically defined ICN that can be decomposed into at least three main sub-networks with twenty specific functions. These main sub-networks are: a midline 'core' sub-network (i.e., mPFC and PCC) that is involved in self-referential processing; the dorsal medial sub-network involved in mentalization and conceptual processing; and the medial temporal sub-network involved in constructive mental simulation and episodic/contextual retrieval [33,34]. Cortico-cortical glutamatergic connections would form the majority of synapses within the DMN, thus providing a substrate for its functional connectivity patterns within and between networks. High DMN connectivity and high nodal centrality are manifested by excessive self-referential processes and maladaptive rumination (Rumination consists of repetitive thinking about one's own state, which has adaptive or maladaptive consequences, depending on the processing mode involved. This is maladaptive when the mode is abstract-analytic and adaptive when it is concrete-experiential (Watkins, 2008)) associated with depression. The antidepressant response to drug and electroconvulsive therapy is associated with robust connectivity between the posterior (PCC) and anterior (mPFC) nodes of the DMN [30]. Furthermore, a recent coordinate-based meta-analysis concluded that DMN connectivity within the resting baseline network predicts antidepressant response regardless of treatment modality. Several studies point to DMN connectivity as a

candidate target of glutamatergic events for depression and support its role in predicting a rapid antidepressant response. A hydrogen spectroscopy study [(1H-MRS), is a specialized neuroimaging technique that allows the in vivo quantification of metabolites] confirmed that ketamine transiently increased levels of the compound glutamate+glutamine (GLX) in the mPFC in patients with MDD [35]; emphasizing that the mPFC is the central node of the midline subnetwork. However, despite the possibility of increased glutamate levels, neuroimaging studies generally show that glutamatergic compounds reduce DMN activity and connectivity, which is consistent with the antidepressant effects of other treatments on pathological DMN hyperfunction. There is reason to believe that treatment with glutamatergic drugs may normalize DMN hyperactivity in depression, not only at rest but also during periods of marked MDD psychopathology, such as emotion dysregulation [29,35,36].

- The ventromedial affective network (AN) shown in Fig.3: consists of the medial orbitofrontal cortex (mOFC)/VMPFC, the rostroventral portion of the ACC, including the sgACC, and limbic regions, including the AMYG, HPC and insula [29,32]. This ventral network is involved in emotional processing and regulation and therefore, if overstimulated, can be the basis for negative feelings or dysphoria. Individual nodes of the VMPFC are involved in the generation (sgACC) and regulation (mOFC) of negative affect [29,32]. The central AN node, called sgACC, was one of the first neural markers of MDD and response to antidepressant treatment. Increased AN connectivity is common in depressed patients especially AMYG and sgACC hyperactivity is present. From a functional point of view, increased sgACC activity is linked to the creation of negative mood states, at the same time, increased VMPFC activity is associated with its inhibition [29,32]. The increased response to ketamine was associated with increased VMPFC-AMYG and AMYG-ACC binding, supporting the idea that cortical nodes reduce glutamatergic AMYG activity in MDD. Functional binding of sgACC-AMYG and sgACC-right in the inferior lateral PFC has been shown to predict response to ketamine treatment. Furthermore, ketamine was shown to increase sgACC binding in the right lateral PFC. In brain models of depression, sgACC is believed to be abnormally hyperactive at baseline, and several studies that have examined the effects of ketamine on sgACC have confirmed this hypothesis and demonstrated a decrease in sgACC hyperactivity consistent with the effects of other forms of antidepressant treatment. Therefore, given the conflicting results on the effects of ketamine on sgACC responses,

it remains unclear whether sgACC function in MDD can be modulated directly through glutamatergic input. Ketamine suppresses AMYG hyperactivity, which also correlates with treatment response. AMYG activity may not be necessary for an antidepressant response to glutamatergic treatment [37,38].

- the ventral frontostriatal reward network (RN) shown in Fig.3: is a ventral network involved in reward processing, reward prediction and reward-based reversal learning. glutamatergic stimuli are functionally related to reward learning and adaptive decision-making by VMPFC/mOFC on the striatum. Connectivity within the RN network, having its central node in the NAc, appears to be reduced in MDD, thus explaining anhedonia and avolition present in affected patients [29,39,40]. There remains a translational gap in the anhedonia literature, as findings predicting antidepressant response through neural markers of the reward system are inconsistent and clinical neuroimaging research in this area is still at a relatively early stage [29,41]. Ketamine administration has brought many benefits including increased activity of reward-related brain regions by increasing dopamine concentrations thus dopaminergic transmission; increased GNCr by supporting improved emotional processing. These effects are indicative of an improvement in connectivity within the RN network in TRD patients, where the favorable change in anhedonia scores was associated with increased connectivity between the dorsal caudate and ventrolateral PFC as well as between the dorsal caudate and pgACC [29,42,43].

- executive network (CEN) shown in Fig.3: is a network involved in a wide range of behaviors representing higher-order cognitive and executive functioning, including action planning, working memory, sustained attention, decision-making and problem-solving in the context of goal-directed behavior, behavioral inhibition and cognitive flexibility. One of the hallmarks of depression is the inability to effectively control cognitive processing of emotions, which is probably related to a decrease in CEN binding in the network [29,44,45]. DLPFC activity and binding during cognitive and working memory tasks are reliable biomarkers of a rapid response to antidepressants and repetitive transcranial magnetic stimulation [29,32]. However, it should be noted that CEN binding during glutamatergic interventions differs between functional activity and resting-state activity, so the results of activity and binding studies may depend strongly

on the study conditions. Ketamine administration suggests an inhibitory effect on anhedonia [29,37, 46].

- anterior cingulo-insular salience network (SN) shown in Fig.3: this is a network structured by dACC and AI, the activity of which is related to anxiety ratings and is responsible for attentional switching and goal-directed behavior. Four mechanisms are associated with it and defined: 1) identification of relevant external information or detection of salient stimuli; 2) shifting of attention to salient stimuli; 3) facilitation of autonomic responses to salient stimuli (via projections to the nodes of the CAN); and 4) initiation of goal-directed behavior via projections from the dACC to the premotor and motor areasn [29,37]. In depressed patients, a reduction in anhedonia-related activity and low incentive relevance was found, and the pre-treatment activity of dACC and AI during emotional processing was identified as predictors of the antidepressant response [29,32]. The therapeutic effect of ketamine appears to increase the activity and glucose metabolism of dACC while improving the symptoms of anhedonia. The drug's action reduces dACC connectivity with frontal and parietal brain areas, supporting the hypothesis that decreased high connectivity of the dorsal nexus is important in reducing depressive symptoms [29,48,49].

- frontocerebellar sensorimotor network (SMN) shown in Fig.3: is a network comprising central nodes within the primary motor and somatosensory cortices, extending to the supplementary motor area and subcortical structures, including the TH, basal ganglia, brainstem sensorimotor nuclei and cerebellum [29,50]. Its function involves the transduction of externally directed reactions and behavior in response to incoming input. The SMN is closely connected to other ICNs and has been implicated in a number of functions related to error detection, planning and initiation of motor actions, motor inhibition, subjective desire to move, body awareness and pain experience, as well as fine regulation of cognitive and executive functions. Neuroimaging studies have shown that SMN node activity and connectivity within the network are reduced in patients with MDD, and this was confirmed by a recent mega-analysis showing that SMN connectivity can predict the antidepressant response to ketamine [29,51,52]. TRD transmission to ketamine showed significantly increased binding between the cerebellum and basal ganglia at baseline compared to GC, and decreased binding during a course of continuous ketamine infusion [29,53]. Vagus nerve stimulation (VNS) is a new treatment

for chronic depression that involves applying electrical impulses to the vagus nerve, which sends signals to areas of the brain responsible for emotion and cognition. This evidence indicates an overlap between the mind-brain axis and the standard VNS involved in depression [29].

- Frontovagal central autonomic network (CAN) shown in Fig.3: this is an intricate, recently studied ICN that is incorporated into the network model of depression, where its hyperactivity could be related to a high reactivity to stress as well as to the general reactivity of the autonomic nervous system to central nervous system perturbations in the domains of affect and cognition. This network involves various structures such as the HPT, possible central node, the AMYG, the insula and the brainstem nuclei responsible for controlling the autonomic responses of the body [29,53]. The central node is responsible for integrating autonomic, endocrine and sleep functions and the hyperconnectivity of the CAN would explain the strong association between depression and physiological mechanisms inappropriately regulated by the autonomic nervous system, including the distinct markers of elevated heart rate, low heart rate variability, increased arousal and stress reactivity. Ketamine possesses a sympathomimetic action in that it is able to increase blood pressure, heart rate and respiration through direct stimulation of neural structures within the CAN [29,54].

Although communication within the network in each of the seven main ICNs may represent the neural basis for specific emotional, cognitive, behavioral and physiological configurations of depression, the interaction between each node is more complex and, when considering ICNs as biomarkers and biologically-based therapeutic targets, ICNs should be viewed as integrated functional circuits of the whole brain rather than discrete units [29]. In patients with MDD, glutamate levels may change dynamically depending on the involvement of the ICN during a particular mental state or task, demonstrating 'functional pathology' as opposed to static neurochemistry [29]. The changes observed in specific ICNs may provide mechanistic insights into the etiology of brain network abnormalities in depression. This is a first step towards a promising design of glutamate-focused whole-brain 1H-MRS-fMRI connectivity studies, which will facilitate the development of future glutamate-mediated therapies that selectively target these dysfunctions in the brain connectome. The link between ICN and glutamate signaling based on the pharmacological action of injected compounds is based on an understanding of

neuroimaging rather than a direct link to neurochemical indices, and therefore these results must be interpreted with caution [29].

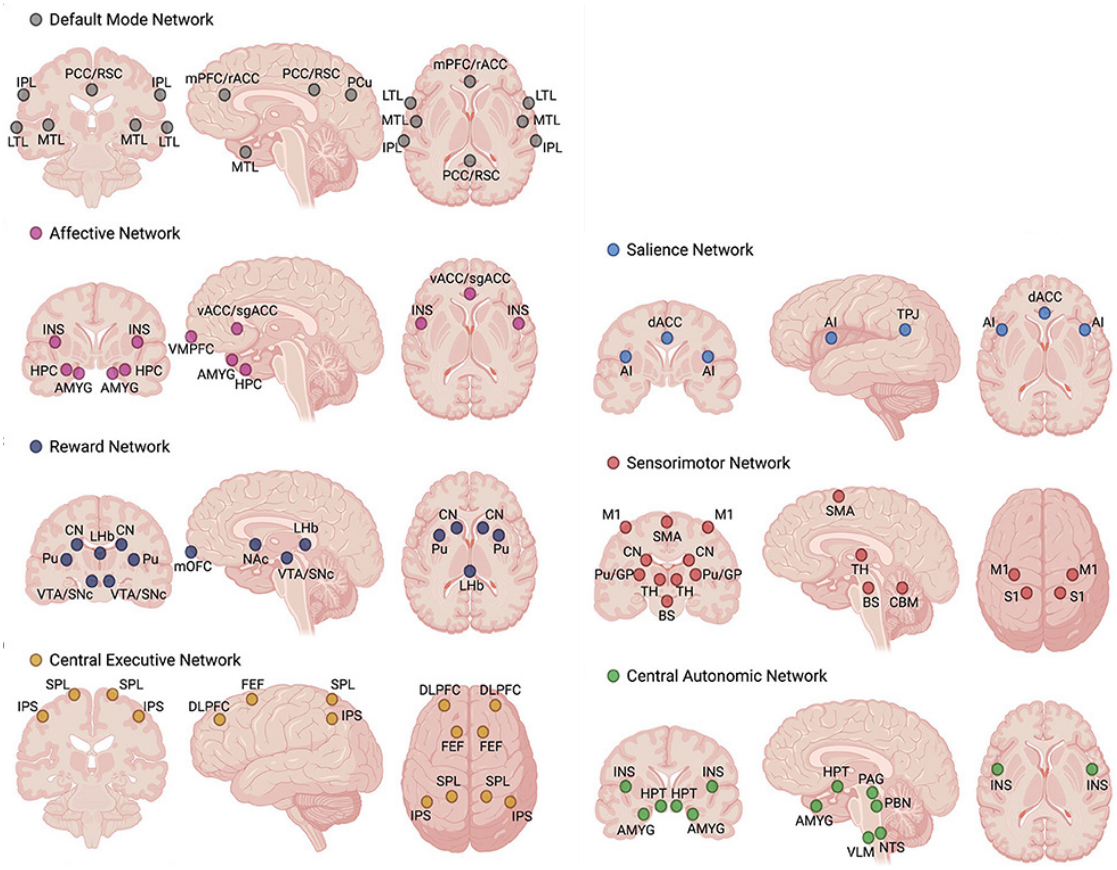

Fig. 3: Functional profile of seven intrinsic connectivity networks (ICNs), including the default mode network (DMN), ventromedial affective network (AN), ventral frontostriatal reward network (RN), frontoparietal central executive network (CEN), anterior cinguloinsular salience network (SN), frontocerebellar sensorimotor network (SMN), and frontovagal central autonomic network (CAN), has been associated with the pathophysiology and symptom manifestation in depression. Hyperconnectivity of the DMN, AN, and CAN and hypoconnectivity of the RN, CEN, SN, and SMN are the hallmark biomarker features that can differentiate depressed individuals from healthy controls. Disturbances in specific glutamatergic white matter pathways may form a neural substrate for aberrant functional connectivity within and between the core ICNs, serving as neuroanatomical targets for future mechanistic studies involving glutamate-based therapies.

The NMDA receptor can be modulated by molecules present in the human body or it can interact with other receptors, favoring or preventing their function. An example of each of the cases that have received most attention will be described next, as they may become possible therapies in various diseases.

3.4 Modulation of the NMDA receptor by molecules with a NOx group

The acronym NOx denotes a nitrous or nitrate group present in the molecular structure with the function of an endogenous mediator of important processes such as vasodilation and the transmission of nerve impulses. [1,55]

Activation of the NMDA receptor promotes an increase in the activity of neuronal NO synthase, which in turn promotes an increase in endogenous NO concentrations. This increase in concentration can be detected by electrochemical probes. With the increase in NO, there is activation of GMP cyclase causing Ca^{2+} ions to enter the synapse [57,58,59]. In the case of a pathology where there is excessive stimulation of the NMDA receptor, NO acts via negative feedback to compensate for the excessive activity. Modulation occurs through the presence of Cys residues only in the GluN2A subunit. These residues undergo endogenous *S*-nitrosylation, which is the basis for the regulation of NMDA receptor ion channel activity [55,58,59].

Based on this phenomenon, molecules capable of intervening in the modulation of NMDA receptors have been studied. Some of these molecules are:

1. S-nitrocysteine (SNOC): this is a molecule capable of reducing the currents evoked by the NMDA receptor. It is reversed by the reducing agent dithiothreitol via covalent binding of the Cys residues present in the GluN2A subunit [55];

2. Methantisulfonate and derivatives: these are molecules that react quickly and specifically with thiol groups to produce mixed disulfides. They are able to mask the NO's action on NMDA receptors [55];

3. 2-aminoethylmethanetiosulfate: causes current inhibition on NMDA receptors formed by the GluN1-GluN2A subunits. Inhibition can be avoided by administration of SNOC [55];

4. 2-trimethylammoniumethylmethanesulfate: can interact with Cys residues by blocking the interaction of NMDA receptors with SNOC-related species [55].

The extracellular modification of protein thiol by endogenous NO could cause further implications for autocrine and paracrine signaling pathways involved in cellular regulation [55].

3.5. Relationship between NMDA and μ-opioid receptors (MOR)

Chronic pain and depression share genetic and psychological risk factors and are frequent comorbidities in several clinical conditions. These conditions are bidirectional: the worsening or improvement of one variable predicts subsequent changes in the severity of the other. Neuropathic pain is caused by lesions or diseases of the somatosensory nervous system that cause and maintain spontaneous pain and stimulus-independent positive or negative sensory disturbances. This pain impairs cognitive functions, mood and quality of life [1,6,60]. Therapeutic treatment includes the prescription of antiepileptic drugs and antidepressants that act on abnormalities of the somatosensory nervous system [1,6]. The exact relationship between chronic pain and depression is still unclear and further analysis is needed. In chronic pain and depression, morphological and functional neuroplastic changes were more pronounced in the frontal and limbic areas. Furthermore, chronic exposure to stress is a common factor leading to long-term changes in highly sensitive brain areas, such as the prefrontal cortex and hippocampus, and their functional connectivity, which may underlie the cognitive and behavioral disorders associated with these conditions. Neuroplasticity refers to the brain's ability to change over time, specifically the ability to strengthen or weaken synaptic signals between neurons following various physiological stimuli, such as behavior, cognition and movement, or pathological events, such as pain, stress conditions or neurological diseases. The increasing prevalence of depression and pain in the world's ageing population makes the development of strategies targeting both disorders particularly important, in order to minimize polypharmacy and optimize therapeutic efficacy [60,61,62]. In this direction, antidepressants and fast-acting analgesics have taken an important step forward [60]. Opioid receptors are involved in pain and are associated with inhibitory G-proteins. Stimulation of G-proteins causes an inhibition of the enzyme adenylate cyclase thereby reducing cyclic adenosine monophosphate (cAMP). This decrease in cAMP leads to a reduction in the conductance of voltage-dependent Ca channels favoring the opening of K^+ channels and interrupting the transmission of the pain signal. There are several types of the opioid receptor including the μ-opioid receptor (MOR) [1,6,60].

The ability of opioids to relieve inflammatory pain is negatively regulated by the NMDA receptor. In various studies it has been shown that MORs and the NMDAR subunits GluN1 or NR1 associate with the postsynaptic structures of neurons in the murine mesenchymal periaqueductal grey (PAG) [60,62]. Upon morphine administration, this complex is disrupted by protein kinase-C (PKC)-mediated phosphorylation of the NR1 C1 segment and enhances NMDAR-calcium and

calmodulin-dependent kinase-II (CaMKII) activity. PKC inhibition restored the MOR-NR1 association and also rescued the analgesic effect of morphine. This combination can be interrupted by the administration of *N*-methyl-D-aspartic acid. In addition, the administered dose may also lead to an increase in the phosphorylation of a MOR's serine, a reduction in MOR binding to G-proteins and a decrease in the antinociceptive capacity of morphine [60,62,63].

In the case of neuropathic pain, opioids that activate MOR don't provide effective relief. Neuropathic pain is characterized by tactile allodynia and hyperalgesia, which decrease with drug therapy that antagonizes NMDAR function, e.g. administration of ketamine, methadone and memantine [60].

A better study of the relationship between MOR and NMDAR is necessary for the selectivity of an effective and safe therapy against neuropathic pain. This relationship can be defined as bidirectional in that morphine tolerance develops as a consequence of MOR-induced potentiation of NMDAR-CaMKII activity [60].

In the situation of neuropathic pain, it has been theorized that the nociceptive signal exceeds a stimulation threshold causing activation of NMDA receptors. This activation leads to a decrease in stimulation by the MORs, in turn decreasing the analgesic effect of morphine. Morphine stimulates the separation of the MOR-NMDAR complex probably by PKC acting on the C1 segment of the C-terminal NR1 subunit, enhancing the calcium permeation of NMDA receptors. In the CNS, PKC enhances receptor function and negatively regulates morphine analgesia. In addition, PKC promotes the sustained enhancement of Ca^{2+} currents of NMDA receptors. Subsequently, CaMKII regulated by NMDAR promotes phosphorylation of MOR and its uncoupling from regulated G-proteins. The MOR-NMDAR association indicates that these concatenated processes are confined within the tight environment of both receptors. PKC-mediated disruption of MOR by NMDAR is observed when the analgesic activity of morphine is suppressed by the triggering of PKC activity by it [60,65,66]. The administration of NMDA receptor antagonists leads to a benefit with regard to the analgesic effect from opioid drugs. This could be useful for the therapy of pain states that show resistance to opioid treatment. An example of a possible therapeutic route may be through the selectivity provided by bifunctional drugs that by binding to the MOR reach and antagonize the function of the associated NMDAR [60,64].

3.6 The role of NMDA receptors in Parkinson's disease

Parkinson's disease is caused by degeneration of dopaminergic neurons located in the substantia nigra pars compacta (SNC), a mesencephalic nucleus included in the basal ganglia circuit, which is responsible for the modulation of voluntary movement [27,67]. glutamatergic stimulation in the basal ganglia has two main sources: projections from the subthalamic nucleus (STN), the system's only excitatory nucleus, and the motor cortex. Secondary glutamatergic afferents to the SNC proceed from the amygdala, pontine pedunculus and laterodorsal tegmental nuclei. In MP, the altered neurotransmission observed within the basal ganglia influences the glutamatergic system, assuming a critical involvement of glutamate-mediated excitotoxicity in the pathogenesis and progression of the neurodegenerative disease process [27,68].

Excitotoxicity is defined as the pathological process by which neurons are damaged and killed after excessive stimulation of NMDA receptors by glutamate. This condition is due to intracellular processes, which increase the oxidative load by activating apoptosis. Studies have shown that selective activation of extrasynaptic NMDARs alone isn't sufficient and suggest that the origin of excitotoxic processes rather depends on the extent and coactivation's duration of synaptic and extrasynaptic NMDARs. It has recently been shown that kainate receptors also favor neurodegeneration by promoting excitotoxicity, but also microglia activation and neuroinflammation. Excitotoxicity is mediated by overstimulation of the NMDA receptor and can be triggered in two ways: directly, i.e. influenced by glutamate concentration; or indirectly where the stimulus is generated in the absence of high glutamate concentrations and is therefore associated with any process that may interfere with the neuron's membrane potential [27,69,70].

Ca^{2+}-mediated neurotoxicity is closely linked to ion input as it causes excessive stimulation of the NMDA receptor. After glutamatergic stimulation, Ca^{2+} can enter directly through NMDA receptor activation [27,71]. The introduction of the Ca^{2+} ion is followed by an overload due to its release from the endoplasmic reticulum and mitochondrial stores. This event is also regulated by transmembrane mGluRs and is responsible for secondary cascades involving calpain and activation of pathways leading to necrotic or apoptotic cell death [27,72]. Overload causes stimulation of nitric oxide synthase activity as well as mitochondria functions. This leads to the synthesis of reactive oxygen and nitrogen species (ROS and RNS). Increased levels of ROS inhibit the activity of mitochondrial complex I, pyruvate dehydrogenase and critical enzymes involved in the tricarboxylic acid cycle, thus leading to reduced ATP synthesis and energy crises. In addition, glutamate-induced oxidative stress leads to mitochondrial fragmentation, promoting

the over-regulation of NMDA receptors and contributing to excitotoxicity and neuronal death. Overall, these phenomena lead to an increase in energy metabolism and ATP synthesis and, above all, to the depletion of antioxidant molecules in neurons [27,73]. The relationship between Ca^{2+}-induced dynamics and dysregulation of glucose metabolism contribute to the neurodegenerative process triggered by glutamate excitotoxicity. Glutamate-mediated neurotoxicity is a side effect of dopaminergic neuron susceptibility, molecular/bioenergetic defects and altered neurotransmission associated with cell death in this nucleus [27,74]. Furthermore, it is shown that aggregated α-synuclein, the main pathological marker in PD, increases the frequency of spontaneous NMDA receptor-mediated synaptic currents and increases both pre- and post-synaptic transmission, thus exacerbating the disruption of intracellular Ca^{2+} homeostasis in murine neuronal cultures [27,75].

Finally, neuroinflammation and activated microglia play an important role in disease progression as they also contribute to neurotransmitter release by enhancing glutamate receptor-mediated responses. This evidence suggests that neuroinflammation and excitotoxicity support each other by creating a vicious circle, which ultimately worsens nigrostriatal degeneration in MP [27,76]. Both in animal models and in the brains of patients, the loss of nigral dopaminergic neurons together with the depletion of striatal dopamine locally influences the balance between excitatory and inhibitory neurotransmitter-mediated and synaptic plasticity. It's likely that these changes affect the threshold for glutamate stimulation and Ca^{2+}-dependent synaptic plasticity in the striatum. Competitive NMDAR antagonists have shown neuroprotective properties, although they show some serious side effects associated with their administration [27,77].

4. NMDA receptor blockers

In recent decades, in addition to the formulation of the glutamatergic hypothesis, studies have also focused on the identification of possible drugs capable of supporting this hypothesis. Furthermore, it has been possible to classify these drugs according to their interaction with the NMDA receptor.

The NMDA receptor can be inhibited in a voltage-dependent manner by a wide range of organic cations with different chemical structures. There is a classification according to the interaction with the receptor [10]:

1. 'foot-in-the-door' or sequential blockers: they can only bind to the channel when it is open and prevent it from closing after binding [10];
2. partial entrapment blockers: they obstruct channel closure but are unable to prevent it completely (memantine)[10];
3. entrapment blockers: they are trapped within the canal pore as it closes, and the agonists may dissolve while the blocker remains bound (ketamine, MK-801)[10].

Some blockers may exhibit bifunctionality, i.e. they have two approaches towards the receptor. An example is nitromemantine and its derivatives that bind the pore facilitating the targeting of an NO group to the regulatory site mediated by redox reactions at the NMDA receptor [10,26].

A classification of drugs according to their affinity towards the NMDA receptor will now be illustrated their characteristics will be described in detail. Interactions with other pharmacological substances or molecules in the body, and their hypothetical mechanism of action causing the rapid antidepressant action will also be discussed.

4.1 Non-selective NMDA-receptor antagonists

These antagonist drugs non-selectively block glutamate access or binding to receptors, thereby preventing or reducing cellular responses to it. We will now list some drugs that have this type of interaction with the NMDA receptor. These drugs include the progenitor of the rapid-acting antidepressants, ketamine.

4.1.1 Ketamine

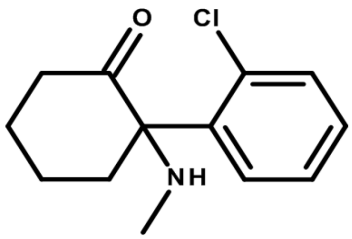

Fig. 4: Molecular structure of 2-(2-chlorophenyl)-2-(methylamino)-cyclohexanone (ketamine).

Ketamine, or (*RS*)-2-(2-chlorophenyl)-2-methylamino-cyclohexan-1-one, has a structure similar to phencyclidine (PCP or angel dust) and belongs to the category of arylcyclohexylamines [3]. This drug was synthesised in 1962 known as CI-581. Currently, it is used as a short-term anesthetic in humans but with certain limitations as it manifests side effects such as psychomimetic effects and behavioral dissociation [78]. Today, ketamine is widely used as a dissociative anesthetic for surgery and is sold as a racemic mixture in which the two enantiomers (*S*)-ketamine and (*R*)-ketamine are present in equal parts [79].

In recent decades, ketamine has shown antidepressant action with rapid effects as various studies have discovered antagonism towards the NMDA receptor. Antidepressant effects were manifested in patients suffering from major depressive disorder or bipolar depression, as well as in cancer patients. Ultimately, ketamine is a non-competitive NMDA receptor antagonist [78]. Results have described that the antidepressant effects are dependent on and activation of a TrkB receptor via binding to Brain-Derived Neurotrophic Factor (BDNF) [3]. BDNF is a 27 kDa polypeptide derived from the brain that plays a role in the survival, differentiation, and growth of neurons in the developing organism. BDNF can to cross the blood-brain barrier (BBB) and is also present in the blood. In treated depressed patients, BDNF levels are altered, i.e. there is an immunoreactivity of BDNF expression in the brain. This fact materializes the peptide's role in the physiology of mood disorders to such an extent that it’s a point of interest in the development of new mood-stabilizing drugs. Furthermore, BDNF is shown to be involved in both excitatory and inhibitory transmission and to play a role in the regulation of AMPA receptor surface expression [80]. Two possible hypotheses regarding the mechanism of action are suggested:

1. inhibition of NMDA receptors by ketamine would induce the inactivation of eukaryotic elongation factor kinase 2 (eEF2K) causing its dephosphorylation. Reducing phosphorylation of this factor in dendrites would increase the translation of mRNA

coding for BDNF by increasing its synthesis. The released BDNF would be able to enhance AMPA receptor activity and, consequently, glutamatergic synapses [3];

2. the antagonism of NMDA receptors on a subpopulation of GABAergic interneurons that control the activity of pyramidal and hippocampal neurons, which would increase glutamate release, resulting in stimulation of non-NMDA postsynaptic receptors, including AMPA receptors. The latter stimulation would promote a signaling cascade that would increase mRNA levels coding for BDNF, which would activate the TrkB receptor. This action would rapidly activate the mammalian target of rapamcycin (mTOR) complex and stimulate the activity of protein kinase B (AKT) and extracellular signal-regulated kinase (ERK), resulting in an increase in the number of synapses and enhancement of their activity, especially in the prefrontal cortex. After these steps, local protein synthesis would be activated, which is impaired in patients with MDD [3].

Other effects that are attributed to ketamine include modulation of specific astrocyte metabolic pathways underlying the increase in extracellular glutamate levels in the prefrontal cortex, all documented in animal and human models of MDD; and inhibition of glycogen synthase kinase 3β (GSK3β) through its phosphorylation [3].

GSK3β is a major player in many critical intracellular signaling mechanisms including the phosphatidylinositol-3 kinase (PI3K)/AKT cell survival pathway, which inhibits GSK3β activity. GSK3β itself inhibits the activation of several transcription factors that act as important cell survival factors [80] and would limit the negative regulatory action on the mTOR complex pathway [3]. The rapid activation of mTOR signaling, associated with the rapid and prolonged increase in synaptically implicated proteins and the number of spines in the PFC, represents a mechanism for the rapid action of ketamine. The mechanisms underlying the induction of mTOR signaling are unclear, but it is the basis for ketamine's mechanism of action as described in the hypothesis presented above [81].

In addition to NMDA antagonism, ketamine has other effects on neurotransmitters including:

- increased synaptic concentrations of acetylcholine in the spinal cortex, hippocampus, and neocortex;
- increased glutamate, serotonin, and noradrenaline in the prefrontal cortex;
- increased dopamine in the basal ganglia and neocortex [78].

Studies concluded that ketamine showed rapid antidepressant actions in patients with refractory bipolar depression (BD), as well as a significant reduction in suicide risk in patients with MDD [78]. Interestingly, anxious and non-anxious BD patients had a significant antidepressant response to ketamine, but the group of anxious and depressed patients responded less than the group of non-anxious patients. Anxiety is a predictor of poor response to other therapies in BD. Given this, this finding is not surprising and suggests that new approaches to treating anxious BD should be explored further [2].

Suicide is a growing public health crisis. Wilkinson et al. conducted a systematic review and meta-analysis of data examining the effect of a single dose of ketamine on suicidal ideation (SI) in studies of patients with TRD, with studies included on post-traumatic stress disorder (PTSD) treatment. Overall, the effect of ketamine was positive in reducing SI compared to saline and midazolam, suggesting a rapid antisuicidal effect lasting up to seven days [2].

The problem with the use of this drug is the occurrence of side effects, i.e. patients present with dissociative symptoms such as blurred vision, impaired hearing, dizziness, proprioceptive disturbances, and delusions [78]. The risk of dissociation and other psychological side effects with ketamine is known, although these are usually transient and subside 90 minutes after injection. There are also known concerns about hemodynamic reactions and respiratory depression [2]. In addition to the occurrence of adverse reactions, another problem is that ketamine is defined as a substance of abuse so its use is limited and only for emergencies [78].

To avoid the occurrence of these effects, many studies have the necessary aim of finding predictable biomarkers that distinguish responder and non-responder patients from ketamine. To date, candidate biomarkers are:

- the Val/Val vector (rs6265) in the neurotrophic factor gene BDNF in MDD patients shows a higher response to ketamine than Met vectors. This makes the Val66Met polymorphism in the BDNF gene a potential genetic biomarker for the ketamine responder [79]. Given the existence of a dose-response relationship for ketamine and the lack of clear evidence of reduced efficacy in this population, the impact of preclinical evidence that BDNF is necessary for antidepressant response is questionable [2];
- baseline interleukin-6 (IL-6) could be a predictive blood biomarker for the rapid antidepressant effects of ketamine [78];

- low baseline levels of fibroblast growth factor-2 could be related to the antidepressant response of ketamine [78];
- an increase in blood levels of osteoprotegerin (OPG)/nuclear factor receptor activator kB Ligand (RANKL). ratio and osteopontin after ketamine infusion, suggesting that the OPG/RANK/RANKL system may be involved in the antidepressant effects of ketamine. (*R*)-ketamine attenuated higher blood levels of RANKL in a mouse model of chronic social defeat stress (CSDS). With this, likely, bone turnover markers may also be involved in the antidepressant effects of (*R*)-ketamine. Furthermore, the latter may be considered as a potential therapeutic drug for bone metabolism abnormalities in depressed patients [78].

Comparison of (S)-ketamine and (R)-ketamine

(*S*)-ketamine has a three- to fourfold higher affinity for the NMDA receptor than its enantiomer (*R*)-ketamine. This is explained by the fact that the *S*-enantiomer forms hydrophobic interactions and hydrogen bonds with Leu642 of GluN2A and Asn616 of GluN1. A mutation or modification of these residues results in reduced activity of the drug, and in some cases, this reduction is worse than with (*R*)-ketamine, such as the Asn614 mutation of GluN2A. The introduction of longer side chains of glutamine and arginine leads to a reduction in steric interaction [79,82]. In recent years, researchers have given a second evaluation to (*R*)-ketamine, which induces more potent therapeutic effects on reduced PSD-95 dendritic spine density and the BDNF-tropomyosin kinase B (TrkB) receptor cascade in the PFC and hippocampus from CSDS-sensitive mice than (*S*)-ketamine. Once administered, (*R*)-ketamine appears to present no psychotomimetic or abuse side effects in humans. By compressing this data, the researchers were able to establish a 'classification' of therapeutic and side effects. In a population of mice suffering from a condition known as 'Olney's lesion', in which lesions occur in the retrosplenial cortex of the encephalon, it was noted that the order of potency of the neuropathological changes induced by NMDAR antagonists correlated with the potency of these compounds. The order of potency of the antidepressant effects was (*R*)-ketamine > (*R,S*)-ketamine > (*S*)-ketamine; whereas the order of the side effects is the reverse of that described above. According to this classification, (*R*)-ketamine appears to be a safer antidepressant than its enantiomer and the mixture of them. Further evidence for the safety of (*R*)-ketamine is described using positron emission tomography, in which it was shown that (*R*)-ketamine significantly suppressed the metabolic rate in several brain areas compared with (*S*)-ketamine, which significantly increased

the metabolic rate of glucose in the frontal cortex and thalamus and contributed to the adverse reactions [78]. However, (*S*)-ketamine remains the enantiomer having a higher affinity for NMDAR than the *R*-enantiomer.

On 5 March 2019, the US FDA approved (*S*)-ketamine nasal spray for treatment-resistant patients [79] and patients at risk of suicide in August 2020 who have confirmed acute SI and/or self-harm. The drug may only be used in accredited healthcare facilities in accordance with the Risk Evaluation and Mitigation Strategy (REMS) Program [2]. Due to the risk of serious adverse effects, it is only available through a limited distribution system as part of the risk assessment and mitigation strategy. At present, the clinical study of (*R*)-ketamine in depressed patients with MDD or BD remains to be reported [78].

Oral infusions, more specifically the low bioavailability of ketamine may contribute to its slow-acting antidepressant effects in patients with MDD; but this is less effective than intravenous and intramuscular administration [78]. Intranasal ketamine and esketamine are effective in patients with TRD and/or SI, but there are many unresolved issues. However, it remains unclear whether there are groups of patients for whom ketamine treatment is particularly effective or should be excluded. Predictors of biological and clinical response are unknown. The Centers for Psychiatric Excellence (COPE), an alliance of six outpatient psychiatric clinics in the United States, has also developed a database registry. While researchers are considering the use of fast-acting antidepressants, it is important to emphasize a cautious approach to interpreting clinical data and to be mindful of any foreseen able dangerous side effects. Ultimately, the goal is to reduce the burden of depression and suicide worldwide through the safe and appropriate widespread use of new treatments such as rapid-acting antidepressants [2].

4.1.1.2 ketamine and μ-opioid receptors

Ketamine can interact with MOR as well as other opioid receptors and can therefore be prescribed as an antinociceptive. Furthermore, it has been determined that opioid receptors are required for the acute antidepressant effect of ketamine in humans [83,84].

Depression may be associated with a dysregulation of the endogenous opioid system in particular the MOR and kappa-opioid receptor (KOR). Researchers demonstrated that the administration of buprenorphine, a partial MOR agonist but KOR antagonist drug, produced antidepressant effects; therefore, it was hypothesized that administration of MOR agonists may also induce antagonist actions on NMDARs suggesting therapeutic potential for mood or anxiety

disorders. KOR is also emerging as a regulator of mood and motivation, with increased kappa opioid receptor activity associated with depression [86].

In ketamine-responsive patients with treatment-resistant depression, pretreatment with naltrexone, an opioid antagonist, profoundly reduced the antidepressant ketamine's effect. Unfortunately, naltrexone doesn't possess substantial selectivity for MOR compared to KOR, so their respective roles in mediating the antidepressant effects of ketamine cannot be distinguished, suggesting also that they do not play an important role in mediating side effects either [83,85,86].

Meta-analyses have consistently shown that ketamine has a clinically significant opioid drug-sparing effect, whereby concomitant administration of ketamine allows lower doses of traditional opioids to be used to achieve similar antinociceptive effects [85,86].

In addition to its antidepressant activity, ketamine has several effects: it enhances the analgesic effect of opioids; produces opioid receptor-dependent analgesia; reduces opioid-induced tolerance and hyperalgesia, and produces MOR-dependent respiratory depression [86].

The researchers suggest that ketamine-mediated analgesia involves direct action on MOR or an interaction between NMDAR antagonists and MOR. Based on this finding, the study was discontinued as the patients had no therapeutic effect and were highly exposed to the harmful risks caused by ketamine [86].

4.1.1.3 ketamine and its interaction with other drugs

In addition to interacting with other receptors, ketamine can interact with other drugs involved in the glutamatergic synapse synergistically like lithium; or by diminishing the effects of ketamine like naltrexone. Next, some cases that have received more scholarly attention will be proposed.

4.1.1.3.1 ketamine and GSK3 inhibitors

Lithium

Lithium, a mood stabilizer, may act through its ability to inhibit GSK-3, a ubiquitous serine-threonine kinase, considered necessary to support its numerous neuroprotective and mood-stabilizing effects [87,89]. This inhibition can follow two pathways: 1) direct action where the Mg2+ cation is antagonized; 2) indirect action through activation of the serine/threonine kinase Akt upstream of mTOR and destabilization of the β-arrestin/Akt/PP2A/GSK-3 complex [87].

By inhibiting GSK-3β, lithium was shown to activate the BDNF promoter in primary cortical neurons by upregulating BDNF expression in the rat brain. Presumably through BDNF-TrkB receptor signaling, lithium treatment can subsequently enhance the activation of its downstream effectors Akt and ERK. Moreover, the latter has the function of mediating GSK-3β phosphorylation and, at the same time, ketamine exerts its rapid antidepressant effects. Ketamine-induced BDNF synthesis was found to be dependent on eEF2, suggesting that eEF2 kinase inhibitors created an effect similar to a fast-acting antidepressant in mice. Lithium may accomplish multiple effects including regulation of eEF2 dephosphorylation; activation of a signaling pathway involved in eEF2 kinase inhibition; enhancement of ROS levels by suppressing markers of elevated oxidative metabolism, including thiobarbituric acid reactive substances and catalase in unmedicated manic patients. That said, treatment with a low therapeutic dose of lithium is assumed to prevent ketamine-induced hyper locomotor activity although further experiments are needed [88]. Given their inhibitory actions on mTOR, the low-dose combination of ketamine and lithium has been shown to increase the inhibitory phosphorylation of GSK-3. GSK-3's inhibition may be critical in protecting new synapses from destabilization by causing their loss. The ketamine-lithium combination produced an increase in serotonin- and hypocretin-induced EPSPs. The responses of these two neurotransmitters are mediated by cortico-cortical and thalamocortical synapses respectively, emphasizing that the combination treatment increases both types of synaptic connections. Other effects of this drug combination are: the increased density of spines on mPFC pyramidal neurons, as well as the number of large diameter 'mushroom' spines, a sign of high spine maturation and synaptic strengthening; the production of a robust and relatively prolonged antidepressant effect in the rat FST, comparable to that of a single effective dose of ketamine. In comparison, the administration of single acute doses of lithium or the selective GSK-3 inhibitor SB 216763 didn't prove sufficient to reproduce prolonged antidepressant-like effects [87]. In conclusion, the ability of lithium, administered together with ketamine, to increase and prolong both clinical efficacy and remission in the treatment of depression is highlighted. Therefore, the combination of ketamine with lithium could enhance the antidepressant effects of ketamine, as well as protect against side effects related to ketamine use in mouse models [88].

sb216763

This study [89] hypothesizes that GSK-3 inhibitors may produce antidepressant effects in rodents. The aim of the study was, therefore, to investigate the effects of ketamine and the GSK-3 inhibitor in the mouse model of chronic unpredictable mild stress (CMS). SB216763, a potent

and selective GSK-3 inhibitor was reported to cross the blood-brain barrier after its intravenous administration suggesting that SB216763 might inhibit GSK-3 in the brain. However, no antidepressant effect was found for SB216763 in the CMS mouse model and control mice, although the dose used could cause GSK-3 inhibition in the brain. In a comparison, ketamine, but not SB21673, was found to exhibit antidepressant activity in control mice 24 hours after a single administration, suggesting that it induces long-lasting antidepressant effects in control mice. As described above, ketamine increases the phosphorylation of GSK-3 as confirmed by the lack of response in mice with a knock-in mutation in which phosphorylation does not occur making them immune to the drug's effects. In conclusion, this research demonstrates the lack of antidepressant response of the GSK-3 inhibitor, SB216763; whereas ketamine can produce a rapid and sustained response in the CMS mouse model. Furthermore, it is suggested that within the CMS model, direct inhibition of GSK-3 may not underlie the antidepressant mechanisms of ketamine. However, SB216763 can block the effects of ketamine in mice [89].

4.1.1.3.2 ketamine and etosuximide

In this presented study, the antidepressant-like effects of ketamine by T-VSCC inhibitors are evaluated. However, further detailed studies on the performance of other T-VSCC blockers are needed. In the case of ethosuximide, no ketamine-like antidepressant effect was discovered in a CSDS model. Therefore, T-VSCC blockers are unlikely to have strong antidepressant effects similar to ketamine, although further studies are needed [90].

4.1.2 Norketamine

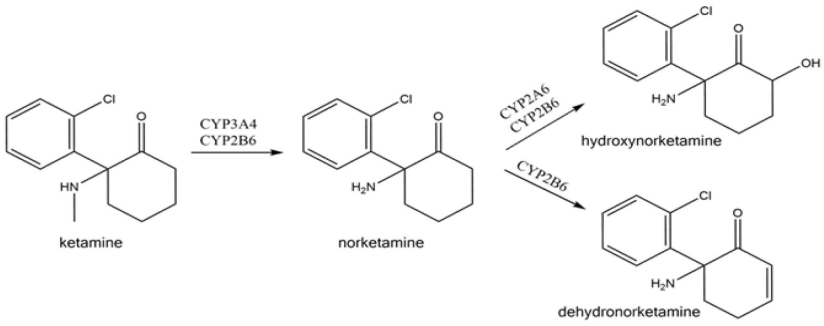

Fig. 5: metabolism of ketamine in norketamine by CYP enzymes.

(*S*)-ketamine is metabolized by CYP enzymes into (*S*)-norketamine. Hepatic first-pass metabolism is very present causing a reduction in bioavailability, but the analgesic effect is maintained to some extent. This metabolite shows a rapid and prolonged antidepressant effect in CSDS and LPS models of depression. The antidepressant potency of (*S*)-norketamine is similar to that of the parent compound (*S*)-ketamine, but lower than that of (*R*)-ketamine. (*S*)-norketamine induced

neither behavioral nor biochemical adverse reactions in mice. This event could indicate that the side effects of (*S*)-norketamine in humans might be significantly less than those of (*S*)-ketamine. Therefore, it can be concluded that (*S*)-norketamine acts as a weak non-competitive NMDA receptor antagonist in the rat cortex and spinal cord. After oral administration of ketamine, (*S*)-norketamine is present in rather high concentrations in human plasma, which explains some of the analgesic effects observed. Clinical studies are currently underway to determine whether oral administration of (*S*)-norketamine has fewer side effects than (*S*)-ketamine [78,91].

4.1.3 Dextromethorphan

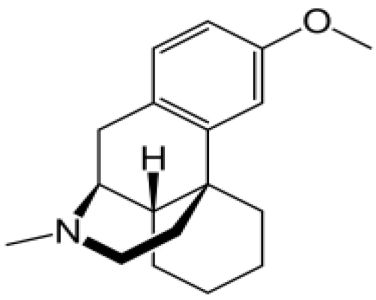

Fig 6: molecular structure of dextromethorphan.

Dextromethorphan, known as an antitoxic drug, is an NMDA receptor antagonist, in addition to being a sigma-1 receptor agonist and inhibitor of serotonin and norepinephrine transporters. There is little evidence of the dextromethorphan's use in the treatment of mood disorders, with one study on bipolar depression showing negative results. Several studies are looking at various combinations with this drug [92].

The combination of dextromethorphan and quinidine is approved by the FDA for the treatment of pseudobulbar affect and, at the same time, its role in the treatment of MDD is still under investigation. An open-label, proof-of-concept clinical trial analyzed the effects of the combination of dextromethorphan/quinidine on patients with treatment-resistant MDD. The intention-to-treat analysis revealed a response rate of 45%, with a remission rate of 35% [92]. The combination treatment significantly reduced Montgomery-Åsberg Depression Rating Scale (MADRS) scores and was well tolerated: the most common side effect was constipation [93]. However, various methodological restrictions, including the absence of group control, limit the generalization of the results. The rationale behind the combination of dextromethorphan and quinidine stems from the fact that the former is metabolized by the 2D6 fraction of the cytochrome P450 enzyme system and that the combination with quinidine (a potent 2D6 inhibitor) increases its bioavailability [92].

Following the same principle, the FDA granted a combination of dextromethorphan and bupropion (AXS-05) acceleration and breakthrough status for the treatment of treatment-resistant depression [92]. Bupropion has been prescribed for decades without fully understanding its mechanism of action as the inhibition of norepinephrine and dopamine transporters. The antidepressant action of the two compounds is additive while bupropion also acts as an enzyme inhibitor of CYP2D6 by increasing the bioavailability of dextromethorphan [93]. Preliminary results from phase 2 studies indicate positive effects on the treatment of MDD and a phase 3 clinical trial analyzing long-term efficacy and safety is currently underway [92].

Finally, the combination of dextromethorphan modified with deuterium and an ultra-low dose of quinidine (AVP 786) has recently been investigated in the treatment of MDD, as adjunctive therapy for patients with poor antidepressant response [90]. Modulation with deuterium, a hydrogen isotope, may lead to further attenuation of the first-pass effect and the dose of quinidine may also be reduced [93]. However, the results of this study are not yet available [92].

4.1.4 Methadone

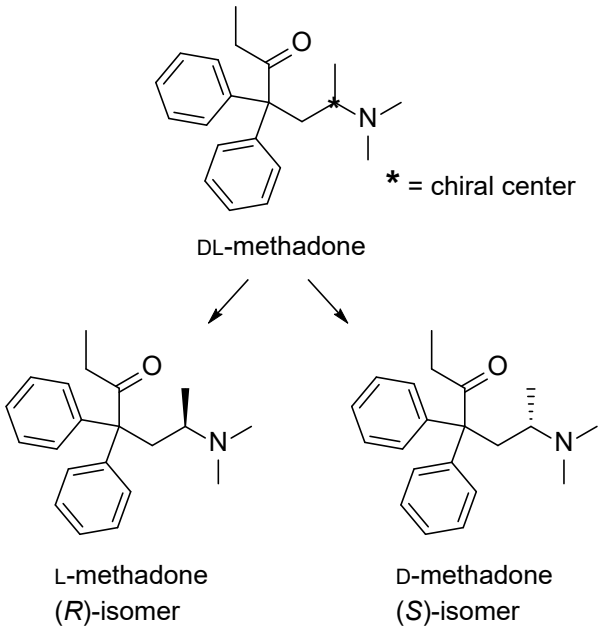

Fig. 7: Chemical structure of methadone and its isomers

Methadone is a synthetic opioid racemic mixture that is used as an alternative to morphine and hydromorphone to treat moderate to severe pain and in the rehab of patients with opioid dependence. Methadone has an asymmetric carbon atom resulting in 2 enantiomers, the *R* and *S* isomers (Fig. 7). In 1965 the pharmacology and addictiveness of the *dextro*- and *levo*-isomers

of methadone were firstly characterized [94]. It was found that D-methadone is a weak analgesic with low addiction while L-methadone was very potent in inducing morphine-like effects and in morphine withdrawal suppression. Although both isomers act as NMDA receptor antagonists, the focus will be on the D-isomer because of its safety. Dextromethadone has in fact a lower affinity for opioid receptors when compared to levomethadone and thus it does not produce typical opioid or psychotomimetic effects [95].

As mentioned in previous sections, currently available antidepressants have a delayed onset and limited efficacy, highlighting the need for new, rapid and more effective treatments. For this reason the use of dextromethadone, is currently under development [96]. Studies have in fact revealed the pharmacological activity both *in vitro* and *in vivo* of the D-methadone isomer as NMDA receptor antagonist [97], thus classifying it as a non-competitive NMDA receptor antagonist which binds to the MK-801 binding site with a similar affinity to ketamine and memantine, acting as a channel blocker apparently with a favorable safety and tolerability profile [96].

4.1.4.1 Dextromethadone

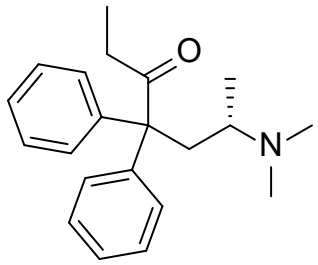

Fig. 8: molecular structure of Dextromethadone

Studies on dextromethadone activity showed interesting results: a preliminary preclinical study showed that a single dose (10–40 mg/kg) of D-methadone induces an antidepressant-like effect similar to ketamine decreasing the immobility of rats in the forced swim test. A single dose of D-methadone has also proved to produce rapid and sustained antidepressant actions in models of anhedonia, motivation, reward, and anxiety with long lasting effects. Two phase-1 studies also showed that D-methadone is well tolerated in humans and does not induce dissociative or psychotomimetic adverse events that are observed with ketamine [98]. These studies, the Single Ascending Dose (SAD) and Multiple Ascendind Dose (MAD), showed in fact favorable safety and tolerability profile. The safety and tolerability profiles that emerged demonstrated that single

doses up to 150 mg and repeated dosing up to 75 mg a day are well tolerated and without clinically significant opioid effects or psychotomimetic adverse events [99].

An interventional double-blind, placebo-controlled phase 2 clinical study (*REL-1017-202*) with 62 patients with TRD was conducted to examine the efficacy of dextromethadone. The results showed a significant improvement in depressive symptoms compared to placebo and this improvement was sustained within the following 14 days [95].

Relmada Therapeutics, that is currently developing dextromethadone (investigational name REL-1017), hypothesize that REL-1017 has the potential to be the first single agent oral NMDAR antagonist for the adjunctive treatment of depression and potentially for front line monotherapy treatment. Both Phase 1 and Phase 2 trial for "Adjunctive treatment of Major Depressive Disorder" were completed. In the Phase 2 trial, both doses of REL-1017 25 mg and 50 mg demonstrated statistically significant differences compared to placebo on all efficacy measures. "Dextromethadone Phase II Study in Adjunctive Treatment of MDD" primary objectives were to establish safety and tolerability of 25 mg and 50 mg of REL-1017 vs placebo as adjunctive treatment; the secondary objectives were the evaluation of the efficacy of 25 mg and 50 mg of REL-1017 as adjunctive treatment in patients with MDD, to characterize pharmacokinetic profile of REL-1017 25 mg and 50 mg for 7 days. 60 patients were enrolled in this placebo-controlled trial; the two doses were tested (25 and 50 mg) once a day versus placebo during a 7-days treatment in clinic followed by a 7-days observation as outpatient. A first follow up was made at day 14 for evaluating efficacy and safety while the second one at day 21 was to evaluate safety only. The obtained results confirmed the favorable tolerability and safety profile observed in the Phase 1 studies. Only mild and moderate adverse events were observed with no evidence that the treatment induced dissociative, psychotomimetic or opiate withdrawal symptoms in the treated groups vs placebo.

REL-1017 can thus advance into Phase 3 registration studies without additional clinical studies. FDA and Relmada are aligned on all key aspects of Phase 3 program to be initiated: studies will assess REL-1017 as adjunctive treatment in MDD patients who have failed at least one prior treatment in current depression episode.

Regarding the mechanism of action, it has been found that there is convergence of the signaling and synaptic mechanisms with ketamine. The ability of D-methadone to produce sustained antidepressant behavioral actions is presumably due to the sustained induction of synaptic strength, as it has been previously described for ketamine [96]. Results are consistent with the

hypothesis that the initial trigger for the antidepressant action of D-methadone is antagonism of NMDA receptors, which in turn rapidly stimulates BDNF-mTORC1 signaling resulting in rapid and sustained increases in PFC synaptic connectivity and antidepressant behavioral responses [96]. Supporting this hypothesis, dextromethadone showed to increase BDNF plasma levels compared to placebo in healthy volunteers [100].

Moreover REL-1017 has been shown to preferentially block hyperactive NMDA receptor channels. Blocking hyperactive NMDAR channels represents a novel approach to treating depression with a mechanism that is different from the classes of currently approved drugs: REL-1017 shows preferential blocking of NMDAR subtypes (GluN1-GluN2C and GluN1-GluN2D) that may be of particular importance in MDD, while avoiding effects on those channels that are associated with normal physiological functions (GluN1-GluN2A and GluN1-GluN2B). With this mechanism, REL-1017 is being evaluated to determine whether it can provide the benefits associated with NMDAR channel block without psychotomimetic effects or other cognitive side effects associated with other drugs that work on the same receptors [101].

4.1.4 Memantine

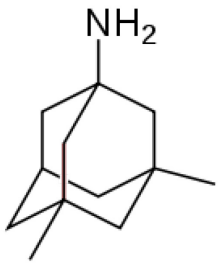

Fig 9: molecular structure of memantine.

Currently, memantine is an FDA (Food and Drug Administration) approved and prescribed drug for the treatment of moderate to severe Alzheimer's disease. In recent years, this molecule appeared to manifest antidepressant effects in depressed patients and to behave as a non-competitive NMDA receptor antagonist with low to moderate affinity [102,104].

Its possible mechanism involves blocking NMDARs containing GluN2C and GluN2D subunits located on extrasynaptic neurons by preferentially binding to NMDA receptor-activated calcium channels. This action leads to the blockade of the adverse effects caused by high glutamate concentrations which, without blockade, would lead to neuronal dysfunction. However, the results show that memantine has no treatment efficacy for depressive symptoms in patients with MDD and BD. Memantine is free of psychotomimetic effects at therapeutic doses, emphasizing

its greater safety in comparison with ketamine. Furthermore, it was well tolerated, with no significant differences in safety outcomes between the memantine and placebo groups; however, it was not superior to the placebo with regard to response rate, remission rate, improvement in depressive symptom scale scores and discontinuation due to ineffectiveness [103].

Recently, researchers evaluated the efficacy and tolerability of memantine in patients with severe mental illness. The results suggest that the drug significantly reduced depressive symptoms in patients with mood disorders with a small ES (epileptic state), whereas, in patients with schizophrenia, it tended to reduce. It is hypothesized that its efficacy is due to the administration of high doses. During this experiment, no serious side effects were observed. In depressed animal models, memantine administration could increase BDNF levels. However, further studies are needed to clarify the relationship between depression, cognition and duration of memantine treatment [104].

Memantine, unlike ketamine, has a lower affinity for NMDA receptors, a much higher open channel blocking/unblocking rate and exhibits a different type of channel closure (i.e. 'partial trapping' rather than 'trap blocking' properties). These differences may explain memantine's lack of antidepressant effect and thus discard the idea of it as a possible antidepressant. [102].

Comparison of Memantine and Ketamine

Ketamine and memantine are both NMDA receptor antagonists, but memantine, according to clinical data, doesn’t exhibit rapid antidepressant effects. In one study, researchers tested the antidepressant action of both ketamine and memantine in mice, confirming that the second-mentioned drug does not possess antidepressant-like effects [104].

Both drugs effectively block NMDAR-mediated excitatory postsynaptic miniature currents in the absence of Mg^{2+}. However, in the physiological levels of extracellular Mg^{2+}, substantial functional differences were found between ketamine and memantine in their ability to block NMDAR function at rest. This differential effect extends to intracellular NMDAR-coupled signaling at rest, as memantine does not inhibit phosphorylation of eEF2 or increase subsequent BDNF expression, critical elements of ketamine-mediated antidepressant efficacy. These results illustrate significant inequalities between the potency of ketamine and memantine on NMDAR-mediated neurotransmission, which affects downstream intracellular signaling, hypothesizing that it is the trigger for antidepressant responses. This study emphasizes that only ketamine can block NMDAR at rest when physiological Mg^{2+} concentrations are included in the external solution.

Recently, chronic memantine didn't induce an antidepressant response in depressed patients compared to a placebo [104,108,109]. After in vivo administration, ketamine exhibits faster pharmacokinetics and rapid attainment of its maximum concentration than memantine. In vitro studies hypothesize that ketamine exhibits slightly greater potency than memantine, necessarily emphasizing that the two compounds reveal no significant differences in their ability to block NMDAR-mediated synaptic or extrasynaptic currents in the absence of physiological Mg^{2+}. In the absence of Mg^{2+}, ketamine is dominant in comparison to memantine in the inhibition of the two GluN2 subunits most highly expressed in the hippocampus, namely GluN2A and GluN2B [104,105,106]. Under physiological conditions in the presence of Mg^{2+}, ketamine is even more influential than memantine underlining the observed difference in the ability of the two antagonists to block NMDAR-containing GluN2A and GluN2B; however, the inhibition does not extend to NMDAR-containing GluN2C and GluN2D. Furthermore, it's believed that there are no major detachments in the blockade of NMDAR-containing GluN2C and GluN2D [104,107]. Therefore, the current findings propose that only ketamine blocks the NMDAR-mediated component of EPSCs in the presence of Mg^{2+} providing clarification on the functional antidepressant mechanism of the two compounds [104].

4.1.4.1 Nitromemantine

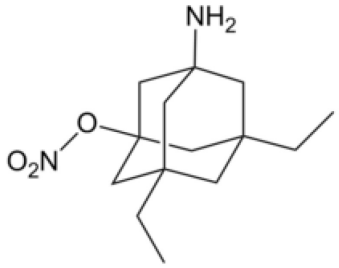

Fig 10: molecular structure of nitromemantine.

Nitromemantine is a drug that results from joining memantine with an NO group, leading to the manifestation of different effects than memantine alone. This derivative is regarded as a non-competitive, low-affinity voltage-dependent NMDAR antagonist. Furthermore, nitromemantine shows promising therapeutic effects for cerebrovascular and other neurodegenerative disorders [110,113,114]. Nitromemethanthine possesses an allosteric mechanism due to the presence of the nitrous group and the adamantane moiety sparing NMDA receptor activity, reducing side effects, and increasing neuroprotection [110,112]. During hypoxia, NO compounds react preferentially with the cysteine residues present in the receptor via an allosteric action, limiting hyperactivity. The memantine is intended to direct a NO-generating group to the redox

modulatory sites of these receptors where S-nitrosylation occurs causing channel inhibition [110,112]. Furthermore, the lipophilic properties of this drug should be clinically beneficial as its concentration increases due to its ability to permeate the blood-brain barrier. These events can be explained by the fact that aminoadamantane compounds possess a very high lipid-water partition coefficient. Based on the chemical properties, aminoadamantane nitrates could be accumulated in the lipid membrane as a reservoir, ready to enter the channel as soon as it opens, initiating the redox effect [110,112]. The beneficial features to ischaemic neurons are several including 1) voltage-dependent blockade of over-open, predominantly extrasynaptic NMDAR channels; 2) targeted delivery of the nitro group to NMDARs by the memantine scaffold; and 3) allosteric regulation of S-channel nitrosylation by hypoxia [110,111]. Nitromemantines exhibit good tolerability and excellent efficacy against cerebral infarction in rodent models due to a dual allosteric mechanism of open-channel blockade and NO/redox receptor modulation [110] .

4.1.5 MK-801

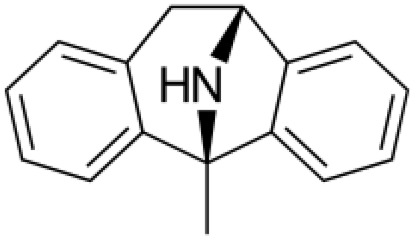

Fig 11: molecular structure of MK-801.

The compound MK-801 is a non-selective NMDA antagonist. However, this drug produced severe neurotoxic adverse effects in the brains of mouse models. Both of its enantiomers induced rapid antidepressant effects, but the effects were not long-lasting. This analysis illustrates that a single administration of (+)MK-801 or (-)MK-801 causes a rapid antidepressant effect in the depressive model of social defeat stress, although it is short-lived. MK-801 was compared with other NMDA antagonists confirming its antidepressant action, in particular: 1) ketamine and MK-801 can regulate the depression-like phenotype observed in the early childhood social isolation stress model, hypothesizing the involvement of the NMDA receptor in this model; 2) MK-801 induced an improvement in sucrose intake deficits under the chronic mild stress model, suggesting possible anti-anhedonic effects for NMDA receptor antagonists. Taking the above into account, it is likely that (+)MK-801 favors antidepressant effects in mouse models of depression. However, these data would appear to be inconsistent. Although the reasons for this discrepancy are still a mystery, the functional differences of ketamine and MK-801 on synaptogenesis may likely

contribute to the differences in the duration of antidepressant action between these compounds [115].

4.1.6 Lanicemine

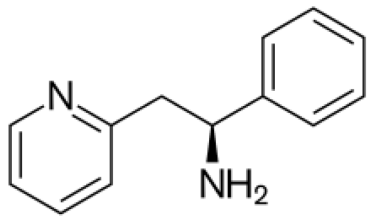

Fig 12: molecular structure of lanicemine.

Lanicemine is a low-trapping NMDA channel blocker that has been shown to perform antidepressant effects in patient studies. In the doses used in studies, this drug produces fewer dissociative and psychotomimetic symptoms than ketamine. In clinical trials, lanicemine expressed robust and significant efficacy without clinically appreciable dissociative and psychotomimetic adverse effects. These results are consistent with the pharmacological separation of efficacy and psychotomimetic side effects observed in preclinical and phase I studies. Importantly, in a 3-week placebo-controlled phase II-B study in patients with moderate to severe depression, repeated doses of lanicemine had sustained antidepressant effects without psychotomimetic effects [116]. Lanicemine produced a favorable outcome in an initial Phase II-B study with 152 participants, but failed to meet primary endpoints in a subsequent Phase II-B study. Notably, the active arm in both phases achieved similar improvements in depression, but the placebo response rate was considerably higher in the subsequent Phase II-B study than in the initial Phase II-B study. Recently, BioHaven Pharmaceuticals licensed an orally available compound, BHV-5000, from AstraZeneca. Lanicemine is the active metabolite of BHV-5000; the company plans to explore the development of BHV-5000 in TRD and other potential indications [93,116].

5. Partial NMDA receptor agonists

An NMDAR partial agonist is an agonist that has only moderate intrinsic activity compared to an agonist. So far, only one compound has shown this capability, Rapastinel.

5.1 Rapastinel

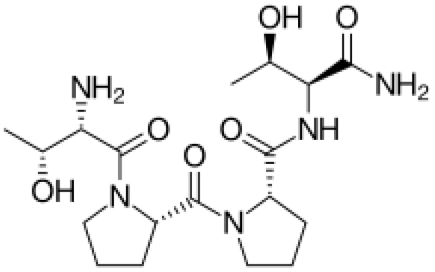

Fig 13: molecular structure of rapastinel.

Rapastinel, or GLYX-13, is a partial agonist compound at the glycine site of the NMDA receptor, but becomes an NMDAR antagonist in the presence of excessive glutamate release. This drug exhibits selectivity for receptors with the GluN2B subunit [92,93,119]. Unlike ketamine, this compound doesn't manifest any psychotomimetic, dissociative side effects or abuse. However, as is the case with ketamine, the antidepressant action expressed by GLYX-13 is inhibited by the AMPA/kainite receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo[f]quinoxaline-2,3-dione, emphasizing it as a requirement for glutamate-AMPA receptor activity. It is hypothesized that the GLYX-13's therapeutic action enhances neuroplasticity and, consequently, improves synaptic function. In animal models, the antidepressant effect given by a single administration of GLYX-13 can last up to 2 weeks suggesting the adaptive capacity of neuroplasticity. The mechanism of action of this drug involves the significant stimulation of mTORC1 signaling in the mPFC, including phosphorylated levels of mTOR, p70S6K, as well as the upstream kinases ERK and Akt. Together with the mechanism of action, GLYX-13 was found to significantly increase the frequency of hypocretin-induced EPSC in layer V mPFC neurons, unlike ketamine. The different synaptic and behavioral effects of GLYX-13 and ketamine are probably related to their respective primary sites of action and receptor selectivity since GLYX-13 is an NMDA modulator, whereas ketamine is a blocker of open NMDA channels. In addition, GLYX-13 also increases NMDA current conductance in a sustained manner. Selective GluN2B antagonists limit the effects of GLYX-13 on NMDA activity and LTP, suggesting that this receptor subtype is involved in the action of GLYX-13. These effects of GLYX-13 on plasticity-related processes can be combined with its cognitive reinforcing and antidepressant effects, as well as its ability to block the ketamine's effects in

declarative memory tasks [119]. However, unlike ketamine, GLYX-13 doesn't influence the response in inhibition or conditioned place preference or the impulsivity response of 5-HT2A-induced head contraction in a serial reaction time task [118]. Further studies are needed to clarify the mechanisms, including the relative importance of direct and indirect effects on glutamatergic transmission in PFC [119]. In a proof-of-concept study with 116 non-responsive MDD patients receiving antidepressant medication, a single intravenous dose of rapastinel was able to decrease depressive symptoms within 2 hours of infusion and the effect lasted for an average of 7 days. The drug received FDA designation as a rapid, breakthrough therapy for MDD treatment as an augmentation strategy [92,117]. In addition, an orally bioavailable analog of GLYX-13 has undergone Phase I studies, but data have yet to be published on the safety and tolerability of the drug candidate [118].

5.1.2 ketamine and rapastinel

In this study [120], the antidepressant action of rapastinel, or GLYX-13, and (*R*)-ketamine was compared in mouse models. Lower doses of (*R*)-ketamine and rapastinel were administered in the social defeat stress model. The results show that (*R*)-ketamine has a more lasting antidepressant effect than rapastinel at the same dose. The study shows a sustained antidepressant response 7 days after a single dose of (*R*)-ketamine or rapastinel. However, this differential antidepressant effect between (*R*)-ketamine and rapastinel is unlikely to be due to differences in pharmacokinetic profiles. Therefore, the rapastinel's pharmacokinetic profile in mice is still unknown. In conclusion, a single dose of either (*R*)-ketamine or rapastinel could reproduce rapid antidepressant effects in the depressive model of social defeat stress, although (*R*)-ketamine has a longer-lasting effect than rapastinel. Furthermore, the sustained antidepressant response to (*R*)-ketamine may be associated with increased synaptogenesis in the PFC, DG, and CA3 (cornus ammonis 3) of the hippocampus [120].

In recent years, drugs typically prescribed for other diseases have been identified that can interact directly or indirectly with NMDA receptors causing an antidepressant action in the animal model and humans. These drugs are involved in other synaptic systems such as cholinergic and GABAergic, or they are molecules partly already present in the body such as endogenous neuromodulators that have demonstrated protective activity against excitotoxicity. Some of these drugs will be mentioned below and their possible antidepressant interactions will be described.

6. Drugs acting on the opioid system

Opioid drugs have been taken into consideration as they manifest, in addition to an analgesic action, an antidepressant activity with rapid effects especially in cases with neuropathic pain [86]. The drugs that have manifested these actions are methadone and buprenorphine.

6.1. Buprenorphine

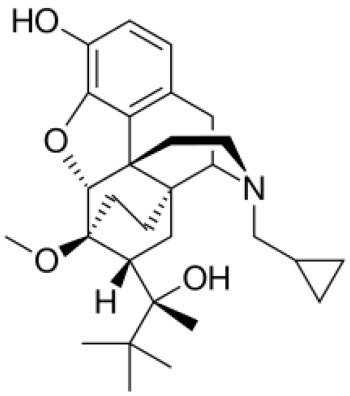

Fig 14: molecular structure of buprenorphine.

Buprenorphine is currently FDA-approved for the treatment of chronic pain and opioid dependence and has also been evaluated for its possible role in the treatment of MDD. Its mechanism of action involves partial agonism of MORs, as well as antagonism of KORs, which has been hypothesized to be responsible for buprenorphine's potential role in the treatment of depression. Early open-label studies with low doses of sublingual buprenorphine defined promising results, demonstrating a rapid onset of antidepressant effects in patients with treatment-resistant depression without a history of substance abuse. Recently, buprenorphine's efficacy as an augmentation strategy among elderly patients with treatment-resistant depression who don't respond to venlafaxine has been justified, not only with regard to depressive mood but also cognitive performance. In recent years, the combination of buprenorphine and

samidorphan (a MOR antagonist) has been studied for its efficacy in the treatment of MDD and is referred to as ALKS-5461. A series of phase III clinical trials produced conflicting results, as one trial failed to produce positive results at the established endpoints, while two other phase III trials demonstrated significant reductions in MADRS scores associated with ALKS-5461 compared to placebo. Despite initially being granted fast track status by the FDA, the drug saw its approval denied by the FDA in 2019, with requests for further evidence of its efficacy [92].

7. Drugs acting on the serotonergic system

Some natural substances with psychedelic properties have attracted interest as they have shown rapid antidepressant activity. These drugs can influence the glutamatergic synapse by stimulating the serotoninergic system. However, they are still under study.

7.1 Psychedelic substances

Recently, scholars have suggested that 'classic' serotonergic psychedelics (SPs) may possess antidepressant efficacy; in particular, they appear to manifest rapid and prolonged effects after a transient psychoactive period [121,122]. These particular compounds increase serotonin concentrations leading to the alteration of neural plasticity with positive effects such as improved resilience, and negative effects such as increased vulnerability to depression [121,123]. Furthermore, serotonergic psychedelics are drugs that induce psychoactive states similar to those exhibited by serotonergic 2A (5-HT_{2A}) receptor agonists and others [121,124]. Now, here are described the main compounds in this class, namely psilocybin, lysergic acid diethylamide-25 (LSD), 2,5-Dimethoxy-4-iodamphetamine (DOI), Ayahuasca and 5-methoxy-DMT currently being studied for the treatment of mood and anxiety disorders [121,125,126]. SPs are known for their 'mystical experiences' - psychological phenomena in which people report experiences of bliss, sacredness, transcendence of space and time, and encounters with greater truths. These experiences would seem to increase the likelihood and magnitude of reductions in depressive symptoms [121,127]. Therapeutic insights have not been reported in qualitative studies on ketamine treatment, which may help to explain the long duration of the antidepressant effect associated with SP. However, whether such insight is necessary for the therapeutic effect of SP and ketamine has not yet been systematically investigated [121,128,129]. It should also be noted that the effect of subjective experience on the overall drug effect was not found in all studies. The administration of any SPs is necessarily preceded by psychological preparation and dialogue focusing on their effects on the intended patients [121,130].

Some analyses speculate that the psychoactive experience may influence the outcome, but everything needs to be systematically investigated. Unfortunately, both the extent of the antidepressant and psychoactive effects and the minimum dose required to induce these effects are still unknown. Subsequently, DOI injections were found to influence the expression of BDNF in the murine neocortex; and both LSD and DOI administration increased the expression of BDNF, Arc, Nor1, egr-1, sgk, and Iκβ-α also in animal models [121,131,132]. All these genes were found

to be induced via the G-protein-coupled receptor pathway, which has some association with synaptic strength and neuronal growth and is mainly associated with 5-HT2A receptor stimulation. Induction of this gene is believed to promote neuroplasticity, leading to several key effects associated with RAADs; there is reason to believe that SPs also promote neuroplasticity. In addition, the administration of LSD and DOI induced synaptic enhancement in the form of increased amplitudes and frequencies of spikes resulting from recordings in animal brain samples [121,133]. Administration of DMTs similar to antidepressants caused spinogenesis in rat cortices, but this effect can be abolished by blocking mTOR or antagonism of the 5-HT_{2A} receptor or tropomyosin-related kinase B (TrkB), the main target of BDNF and an upstream activator of mTOR. All these experiments were carried out equally with ketamine and the results were subsequently compared with those obtained with SPs. From this comparison, SPs show greater potency and efficacy in promoting neurogenesis than ketamine. The main limitation of these studies is the lack of direct evaluation of BDNF activity in the brain. Similarly, studies show that SP causes an increase in glutamate in layer V pyramidal neurons, which mainly express 5-HT_{2A} receptors. Another study in mouse models proved that DOI administration increases the expression of the early activation gene cFos, a marker of neuronal activity, in a subset of neurons expressing 5-HT_{2A} receptors. This active population was mainly located in the mPFC, somatosensory cortex, orbitofrontal cortex, and clavicles, areas mainly composed of glutamate-releasing pyramidal cells [121,131]. There is evidence to suggest that SPs may have molecular and cellular effects similar to those of ketamine, but it should be noted that these data are only available for some SPs and mainly for DOIs, where no clinical studies have been performed. Studies on SPs haven't established a link between these molecular and cellular effects and antidepressant effects in rodent models of depression. The link between these effects and antidepressant efficacy in depressed mouse models hasn't yet been established and is probably a gap to be addressed in future preclinical studies. However, DMT, LSD, and psilocybin have similar antidepressant effects to rats in the forced swim test. Interestingly, in line with the clinical literature, LSD and psilocybin, but not ketamine, have been reported to cause antidepressant effects that persisted after 5 weeks [121,134,135]. It should also be noted that despite the similarities, important differences remain regarding the exact mechanisms of action of these drugs in the brain. The psychoactive effects of both ketamine and SPs are accompanied by acute and delayed electrophysiological and hemodynamic changes in brain activity. Thus, these effects of both drugs resulted in a higher signal complexity than normal waking consciousness, reflecting an elevated level of consciousness after their administration. MEG studies directly comparing

ketamine, LSD and psilocybin showed that all three drugs induced altered states of consciousness characterized by a decrease in spectral power and functional connectivity at low source levels [121,136,137]. Indeed, a decrease in low-frequency signal power measured with MEG/EEG in the resting state is the most commonly reported result after ketamine or SP administration, while a decrease in alpha-band power is more closely associated with psychoactive/hallucinogenic effects [121,138,139]. These interesting preliminary results suggest that the antidepressant effects of ketamine and SP are related to changes in brain network connectivity and function. However, due to the small sample size and lack of controls in SP studies, as well as the wide range of ketamine studies, only a few convergent results can be drawn with confidence at present. Further research is needed to further clarify these associations [121]. In conclusion, these illustrated data suggest that ketamine and SP may have a common mechanism that causes a rapid neuroplastic effect in a glutamatergic-dependent manner. Specifically, serotonergic RAADs exert their effects by mainly stimulating 5-HT_{2A} receptors, resulting in a glutamate-dependent increase in PFC pyramidal cell activity, thus modulating the activity of the prefrontal network [121,140]. The increase in extracellular glutamate also causes the activation of AMPARs, which are present in the same neurons throughout the cortex; increased AMPA production leads to the release of BDNF and mTOR signaling, causing the up-regulation of nerve growth-related plasticity genes, the strengthening of specific synapses and is thought to induce the formation of new synapses. It is therefore believed that the effects of SP and ketamine are linked to highly plastic brain states, allowing functional brain circuits to be 'reprogrammed' [121,141]. The RAADs currently being studied may share common pharmacological effects, suggesting that their antidepressant effects may be due to related mechanisms; studies examining the therapeutic mechanisms of SP, as well as those of ketamine, have shown that the 'glutamate augmentation' in SP increases the information processing capacity and plasticity of AMPARs. A more complete understanding of the cellular and molecular mechanisms of SP may lead to pharmacological targets that will merge with ketamine; in particular, understanding the mechanism by which SP provides lasting symptomatic relief may be critical in identifying treatments that offer the lowest risk of relapse. The amount and reliability of preclinical and clinical data on ketamine far exceed those currently available for SP, and further research is needed to determine clinical efficacy, optimal dosing, and mode of administration. Ultimately, identifying the common mechanisms of action of these fast-acting but pharmacologically different antidepressants may improve the treatment of depression and other stress-related brain disorders [121].

- Psilocybin: is an alkaloid naturally occurring in some species of mushrooms. In an open-label study, 12 patients with treatment-resistant depression were treated with a low initial safety dose of psilocybin and, one week later, a higher therapeutic dose combined with psychological support [92,93]. Treatment was associated with improvements in depression and anhedonia scores within 1-3 weeks of treatment, with more than half of the participants still showing sustained improvement at a 3-month follow-up. Furthermore, a subsequent 6-month follow-up analysis by the same group indicated the persistence of the observed improvements. Also, two randomized, placebo-controlled crossover studies evaluated the efficacy of psilocybin in the treatment of anxiety and depressive symptoms among cancer patients, reporting positive results and persistent antidepressant and anti-anxiety effects. Several phase 2 studies are currently underway to evaluate the effects of psilocybin in the treatment of MDD [92]. In a phase 2 trial involving participants with treatment-resistant depression, psilocybin at a single dose of 25 mg, but not 10 mg, reduced depression scores significantly more than a 1-mg dose over a period of 3 weeks but was associated with adverse effects. Larger and longer trials, including comparison with existing treatments, are required to determine the efficacy and safety of psilocybin for this disorder [142].

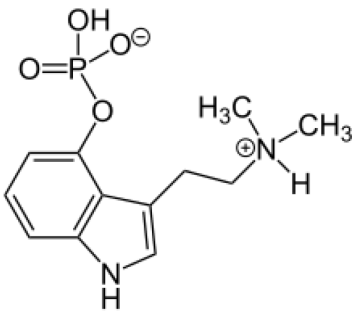

Fig 15: molecular structure of psilocybin.

- Ayahuasca: is a plant-based compound used by indigenous peoples in religious and medicinal procedures. Its components include a hallucinogen called N-dimethyltryptamine (DMT) which acts as a selective serotonin receptor agonist and β-carboline alkaloids. A small open-label study evaluated the efficacy of a single-dose administration of ayahuasca in the treatment of depression [92,93]. The reported results show reductions of up to 82% in depression scores following administration of the drug, as assessed at one-, two- and three-week follow-up. Furthermore, a randomized, placebo-controlled study investigated the efficacy of a single dose of ayahuasca in the management of treatment-resistant depression. The ayahuasca group showed

significant reductions in MADRS scores compared to the placebo, as well as significantly higher response rates at assessment. No other studies are currently investigating the role of ayahuasca in the treatment of MDD [92].

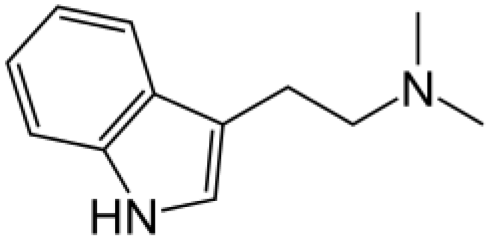

Fig. 16: *molecular structure of N-dimethyltryptamine*

- Lysergic acid diethylamide (LSD): is a semisynthetic hallucinogen, with evidence suggesting that it may play a role in promoting the improvement of mood and anxiety symptoms, especially in cancer patients. Safety concerns related to the risk of inducing a psychotic state are greater with LSD than with other psychedelics. This fact, coupled with regulatory restrictions, may partly explain the paucity of studies analyzing the potential use of LSD in the treatment of MDD [92].

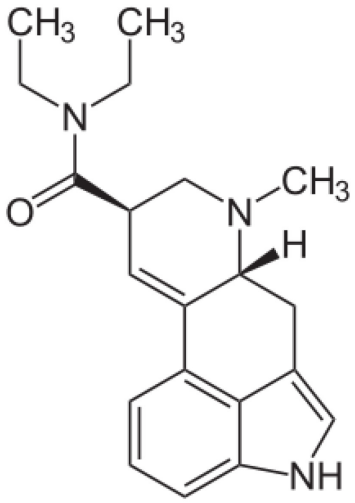

Fig 17: molecular structure of lysergic acid diethylamide.

8. Drugs acting in the cholinergic system

The involvement of the cholinergic system in the pathophysiology of depression has been repeatedly examined in recent decades. The cholinergic hypothesis of mood disorders suggests that an imbalance between noradrenergic and cholinergic neurotransmission in the central nervous system leads to mood dysregulation and that increased cholinergic transmission associated with decreased noradrenergic activity leads to the development of depression [1,6,92]. Increased cholinergic activity is believed to contribute to an increase in the hypothalamic-pituitary-adrenal (HPA) axis, which plays an important role in the development of depression. Furthermore, there is evidence to suggest that people with mood disorders have reduced availability and binding density of the cholinergic receptor type 2 (CHRM2), an autoreceptor that, upon stimulation, inhibits the release of acetylcholine into the synaptic cleft. Reduced CHRM2 activity or availability may therefore lead to increased acetylcholine release and thus to cholinergic hyperactivity [1,6,92]. Based on this hypothesis, several drugs acting on the cholinergic system have been tested for the treatment of depression [92]. One anticholinergic drug, which has shown antidepressant action, is scopolamine.

8.1 Scopolamine

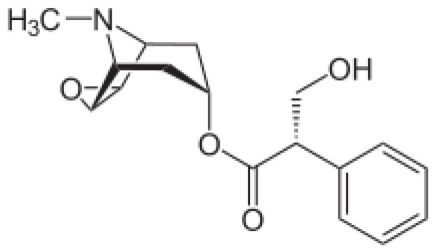

Fig 18: molecular structure of scopolamine.

Scopolamine, a non-selective anticholinergic drug, hasn't shown any significant antidepressant properties, although recent data showed that low doses of scopolamine administered intravenously showed positive effects in depressed patients with unipolar and bipolar depression. The non-selectivity of scopolamine may limit its therapeutic efficacy pharmacodynamically as a postsynaptic antagonist of mAchR M1 (which reduces cholinergic transmission) and mAchR M2 (which may increase acetylcholine release in the synaptic cleft). Allosteric mAchR M2 receptor modulators are of great interest because they reduce the central hypercholinergic state associated with depression and minimize the risk of peripheral anticholinergic side effects [92].

It has recently been shown that a single dose of scopolamine produces rapid antidepressant actions within a few days, but not as fast as ketamine. Scopolamine treatment causes a rapid and transient burst of glutamate in the mPFC and increases the number of synapses in the spine. Furthermore, it was shown that the antidepressant effects of scopolamine in rodent models are mediated by AChR M1, particularly on somatostatin interneurons in the mPFC, and are dependent on activity-dependent BDNF release. These data provide evidence that scopolamine increases glutamate by blocking AChR M1 in GABAergic interneurons via an inhibitory mechanism similar to that of ketamine that blocks GluN2B receptors in GABAergic interneurons [117].

8.1.2 ketamine and scopolamine

It's suggested that specific NMDA and mACh receptor subtypes on GABAergic interneurons are promising targets for new fast-acting antidepressant therapies. Furthermore, there is evidence that ketamine and scopolamine have inhibitory effects on these interneurons in the PFC leading to the disinhibition of pyramidal neurons and increased extracellular glutamate which promotes rapid antidepressant responses by these drugs. Subsequently, the synthesis of rapid and prolonged antidepressant responses by scopolamine in treatment-resistant patients is also discussed [143]. Compared to ketamine, scopolamine exhibits limited adverse reactions, particularly at the doses used for antidepressant therapy, however, it may cause cognitive deficits. Indeed, after ketamine and scopolamine therapy, the number and function of pyramidal neurons in the PFC improved that contrasts with the pathophysiology associated with stress and depression. The convergent mechanisms by which ketamine and scopolamine induce antidepressant effects suggest that common neurophysiological pathways underlie the action of these fast-acting antidepressants. The key element that initiates the molecular signaling required for a rapid response to antidepressants is the rapid rise or spike of glutamate in the PFC following ketamine and scopolamine administration [143]. The rapid and transient increase in glutamate after ketamine or scopolamine administration doesn't seem consistent with their role in neurotransmission, as they act by antagonizing receptors that normally increase neuronal activation. Based on these considerations, it has been hypothesized that interneurons secrete inhibitory neurotransmitters. GABA plays a key role in mediating the release of glutamate from pyramidal cells. In particular, a current hypothesis suggests that ketamine and scopolamine antagonize specific receptor subtypes on inhibitory interneurons that lead to the disinhibition of glutamatergic pyramidal neurons by stimulating excessive glutamate release in the PFC. The direct activation of pyramidal neurons seems paradoxical as NMDA receptor antagonists block

neuronal activation, however, clinical and preclinical studies show that the same antagonists increase the activity of pyramidal neurons in PFC. These results demonstrate that ketamine and scopolamine administered *in vivo* can act via GABA interneurons to stimulate the activation of pyramidal neurons. Recently, studies argue that mACh receptors in the PFC may not have a prominent role in the direct excitation of cortical pyramidal neurons; but this remains to be clarified [143]. Some evidence suggests that M1-ACh receptors modulate both cortical pyramidal and interneuronal activity. Against this, there is a possibility that scopolamine may not act directly through pyramidal neurons to increase glutamate concentration in the PFC. The increase in synaptic plasticity induced by ketamine and scopolamine is due to the antagonism of NMDA and mACh receptors, suggesting a transient inhibition of tonic neurotransmission of glutamate and acetylcholine in the PFC. Therefore, it's important to identify the neurons that regulate glutamate release in the PFC and to develop tools to control these processes. In addition, factors such as changes in dopamine and acetylcholine levels in the PFC during stress and depression, as well as in the presence of fast-acting antidepressants, need to be investigated. Despite efforts to better understand the molecular and cellular mechanisms underlying fast-acting antidepressants' action, significant elements remain to be examined [143].

9. Drugs acting in the hypothalamic-pituitary-adrenal (HPA) axis

The involvement of hypothalamic-pituitary-adrenal (HPA) axis dysfunction in the pathophysiology of depression is well known, and many data suggest a direct link between exposure to stressful events, increased cortisol levels, decreased neurogenesis in depressed patients, reduced plasticity and hippocampal atrophy. Evidence is also mixed, with some studies showing a direct link between HPA and depression. Furthermore, the association between HPA hyperactivity and early trauma is well known and appears to be related to a poor response to antidepressants. Not surprisingly, the HPA axis is considered a potential target for the pharmacological treatment of MDD [92].

9.1 Mifepristone

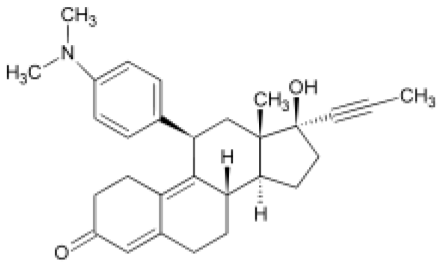

Fig 19: molecular structure of mifepristone.

Mifepristone is a glucocorticoid receptor antagonist that is used as a chemical abortion drug in the first two months of pregnancy. This drug has shown efficacy in the management of MDD with psychotic features, based on the results of open studies. One study analyzed the possible antidepressant effects of this drug and pointed out that it was well-tolerated; however, no significant differences were observed with regard to symptom improvement at the primary endpoint. However, a direct link was found between plasma levels of mifepristone and the improvement of psychotic symptoms. Finally, in 2017, the results of a multicenter, randomized, double-blind clinical trial comparing mifepristone with a placebo in the treatment of psychotic depression were published. An interim analysis was performed and found no statistically significant differences in the primary efficacy endpoint. Recruitment of the group was therefore discontinued. However, a secondary analysis confirmed previous results on the association between mifepristone plasma levels and reactivity, confirming that levels of 1637 ng/ml or higher were associated with a statistically significant reduction in psychotic symptoms compared to placebo [92].

Next, the comparison between the currently described therapy and the one described in this paper is described. In particular, the main defining characteristics of these therapies including therapeutic effects, targets, and adverse effects are compared.

10. Comparison between the two therapies

In this section, I would like to introduce a comparison between the current prescribed therapy and the therapy consisting of rapid-acting antidepressants (RAADs).

An important difference between these two therapies is the period required for the therapeutic effects to appear. In detail, RAADs manifest their effects quickly compared to the current drugs which manifest their effects after a long period. On the subject of effects, another difference is the appearance of side effects that occur in both therapies. RAADs can cause even serious adverse effects such as neurotoxicity (MK-801) together with a possible appearance of signs of abuse (ketamine), whereas the current drugs show adverse effects in a shorter time than their therapeutic effects.

One difference between the two therapies is the molecular targets of the drugs. The molecular targets of currently prescribed drugs include receptors involved in the metabolism of monoamine neurotransmitters, especially serotonin. In contrast, RAADs act directly or indirectly in the glutamatergic synapse mechanism by decreasing excitotoxicity caused by excessive stimulation of the glutamatergic receptor.

A property related to RAADs is the rapid efficacy in patients with major depressive disorder associated with high suicide risk, this efficacy is evident after administration of a single dose.

According to the comparison, RAADs would present very promising compared to current drugs, but the patient's pathological situation must always be taken into account, i.e. the causes of the depression must be understood, as the prescription of such drugs is limited and for extreme cases (intranasal Esketamine). There are currently alternative drugs to ketamine that can be part of antidepressant therapy.

11. Conclusion and future prospects

This work aimed to describe a new drug therapy strategy for major depressive disorder, a complex disease to treat as the currently prescribed drugs take a long time to show their effects. Moreover, depressed patients can develop resistance to therapy, making it difficult to prescribe a correct and effective treatment. In recent decades, many studies have investigated a possible molecular target in the NMDA receptor, an ion channel permeable to the Ca2+ cation, as the administration of a single dose of ketamine, a non-selective NMDA receptor antagonist, induced a rapid antidepressant effect by decreasing the risk of suicide in depressed patients. Still, the exact structure of this receptor needs to be analyzed although there are developments thanks to new technologies including neuroimaging, a technique that provides robust and reproducible models of the functional neuroanatomy of the brain's network architecture. In addition, the structure of the receptor was recently updated thank to CRYO-EM technique [144]; particularly was identified the change in conformation when an (*S*)-ketamine is present in it as shown in Fig 20. One possible antidepressant mechanism is the activation of a TrkB receptor via binding to Brain-Derived Neurotrophic Factor (BDNF).

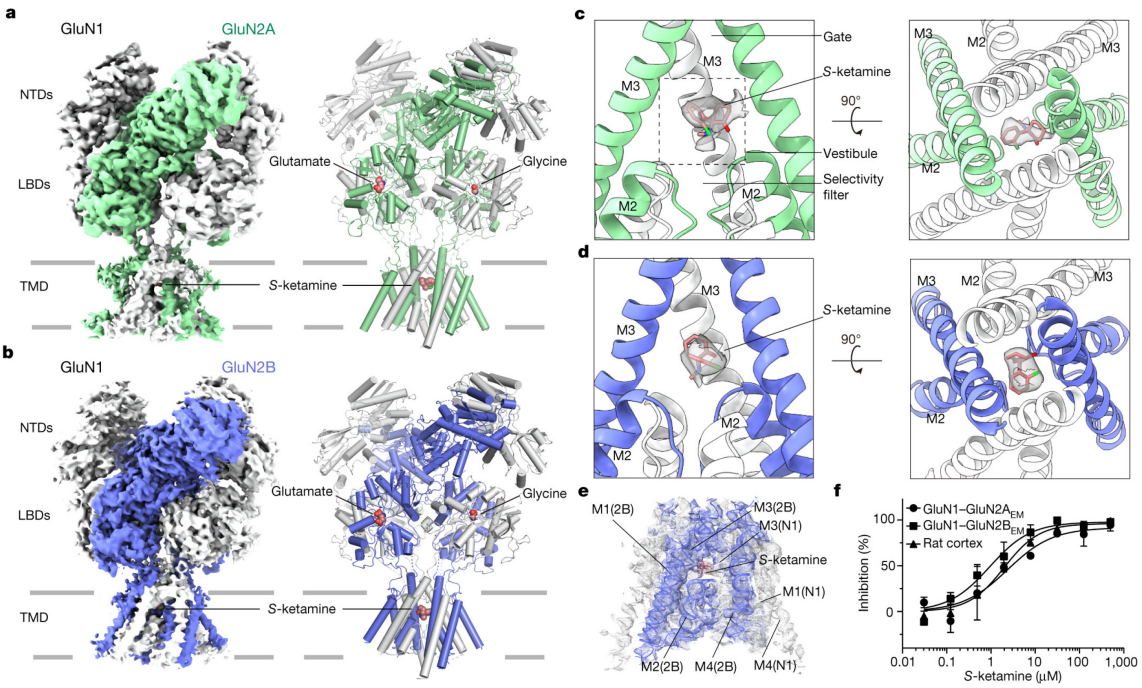

Fig. 20: Cryo-EM densities (left) and structural models (right) of human GluN1–GluN2B receptor. GluN1 subunits are represented in grey with glycine captured in the LBDs; GluN2B is represented in blue, with glutamate presented in the clefts. S-ketamine was captured within the TMD in both receptors.

Ketamine is considered the progenitor of this new class of therapeutics as it has shown many beneficial effects, as well as being in association with other proteins such as MOR opioid

receptors; and with the microbiota, which may influence the course of the disease. In addition, ketamine can interact with other drugs leading to synergy or being an obstacle between them. Therefore, the FDA has approved the nasal spray of the enantiomer S-ketamine for marketing, but, due to its abuse potential and the requirement of hospitalization of the patient due to the occurrence of dissociative side effects, it is restricted for severely impaired patients. For this reason, research has shifted in search of other compounds capable of manifesting antidepressant effects without serious side effects. Memantine, a drug prescribed for Alzheimer's disease, has shown antidepressant effects but is not as potent as ketamine, so attention has shifted to one of its derivatives, nitromemantine, which shows beneficial effects and could be an excellent candidate for antidepressant therapy. Other non-selective antagonists are MK-801, lanicemine, dextromethorphan, and norketamine where only the first named drug has been abandoned due to its neurotoxic effects. In addition to antagonism, other compounds have been investigated, notably rapastinel, an NMDA receptor partial agonist that is still being studied but promises well-tolerated antidepressant effects.

Recently, researchers have hypothesized that the glutamatergic system is not the only one involved in depression, indeed many other systems may play a key role in it. The systems involved are the opioid system, the serotoninergic system, endogenous neuromodulators, the GABAergic system, the cholinergic system and the hypothalamic-pituitary-adrenal axis. Each system has one or more candidate compounds that have shown antidepressant action and are currently being studied.

In conclusion, further studies concerning RAADs and, above all, studies aimed at identifying a rapid and valid therapy against major depressive disorder are needed. The introduction of new drugs with rapid antidepressant action is a breakthrough in the treatment of this complex disease. However, more in-depth studies are needed to assess the efficacy and safety of the drugs in patients with this disorder.

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12.1 Figures

Fig. 1: *Chia-Hsueh Lee, Wei Lu¨, Jennifer Carlisle Michel1, April Goehring, Juan Du, Xianqiang Song & Eric Gouaux; NMDA receptor structures reveal subunit arrangement and pore architecture; doi:10.1038/nature13548*

Fig. 2: Pitt Medical Neuroscience, Neurophylogy and Synaptic Transmission. Honors Human Physiology; BIOSC 1070, NROSCI 1070, MSNBIO 2070; Fall Semester 2020; *Neurophysiology Modules;* Module 5: Glutamate

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Fig. 5: www.biopharmaservices.com/blog/clinical-research-spotlight-ketamine-norketamine-and-hydroxynorketamine, @2021 BioPharma Services Inc.

Fig 20: Zhang, Y., Ye, F., Zhang, T. et al. Structural basis of ketamine action on human NMDA receptors. Nature 596*, 301–305 (2021). https://doi.org/10.1038/s41586-021-03769-9*