A SHEA BUTTER-BASED KETAMINE OINTMENT:
THE AMELIORATIVE EFFECTS OF TRANSDERMAL KETAMINE
ON BEHAVIORAL DESPAIR

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DECLARATION OF ORIGINALITY

I, Merve Akan, certify that

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ABSTRACT

A Shea Butter-Based Ketamine Ointment:

The Ameliorative Effects of Transdermal Ketamine on Behavioral Despair

After a decade of monoaminergic antidepressants, repurposing ketamine in depression launched a new era with its rapid-onset nature and unique action mechanism based on glutamatergic neuroplasticity. Ketamine and its enantiomers were delivered by common enteral and parenteral routes with the disadvantages of first-pass effect, relatively low bioavailability, rapid plasma fluctuations, or inconvenience. The combination of ketamine and transdermal delivery is a worthy choice to overcome most of these disadvantages with steady plasma concentrations and bypassing the gastrointestinal tract. Transdermal ketamine has been used for pain therapy and recently gained attention for its use with different types of depression. In order to offer a user-friendly alternative for depression, the present study aimed to assess the behavioral results of 2-day ketamine ointment treatment in the rodent model of depression, behavioral despair. Naïve14 adult male Wistar rats were grouped as the ketamine and vehicle groups which received a shea butter-based 5% w/w ketamine ointment and drug-free vehicle, respectively for 2 days twice daily on shaved dorsal backs. The ketamine group showed significantly lower immobility scores in the Forced Swim Test (FST) compared to the vehicle group. The locomotor activity and anxiety levels were similar for both groups assessed through the Open Field Test (OFT) and Elevated Plus Maze (EPM). Our results demonstrated that 2-day ketamine ointment treatment produces an antidepressant effect without causing an increase in the general locomotor activity or a change in anxiety levels. Ketamine with its chemically ideal nature for transdermal delivery may offer a potent therapeutic response as an alternative for traditional antidepressants.

ÖZET

Shea Yağı Bazlı Ketamin Merhemi:
Transdermal Ketaminin Öğrenilmiş Çaresizlik Üzerindeki İyileştirici Etkisi

Glutamat sistemi üzerinden hızlı antidepresan etki gösteren ketamin, monoaminerjik antidepresanlardan sonra depresyon tedavisinde yeni bir dönem başlatmıştır. Bugüne dek ketamin ve enantiyomerleri birçok enteral ve parenteral yöntemle verilmiş olup düşük biyoyararlanım, plazma seviyesinde dalgalanmalar ve ilk geçiş etkisi gibi dezavantajlara sahiptir. Dengeli plazma seviyesi sağlayan ve ilk geçiş etkisinden etkilenmeyen bir yöntem olan deriden uygulamanın ketamin tedavisine entegre edilmesi bu noktada önem arz etmektedir. Deriden ketamin uygulaması bugüne dek ağrı tedavisinde kullanılmış olup depresyon için literatüre yeni dahil olmaktadır. Depresyon hastaları için kullanıcı dostu bir yöntem geliştirmek amacıyla, bu çalışma 2 günlük ketamin merhemi tedavisinin sıçanlardaki davranışsal etkisini incelemeyi amaçlamaktadır. Bu doğrultuda, ketamin ve kontrol grubu olarak ikiye ayrılan 14 naif erkek Wistar sıçanına 2 gün boyunca günde 2 doz olmak üzere shea yağı total ağırlığına göre %5 ketamin içeren ve ilaçsız taşıyıcı merhem uygulanmıştır. Ketamin grubu kemirgen depresyon paradigması Zorunlu Yüzme Testi'nde (ZYT) kontrol grubuna göre anlamlı derecede düşük immobilite skoru göstermiştir. İki grubun lokomotor aktivite ölçen Açık Alan Testi (AAT) ve kaygı benzeri davranış ölçen Yükseltilmiş Artı Labirent Testi (YALT) sonuçları birbirine yakın çıkmıştır. Bu sonuçlar, 2 günlük ketamin merhemi uygulamasının anksiyete düzeyini ve lokomotor aktiviteyi etkilemeden, antidepresan etki gösterdiğine işaret etmektedir. Glutamaterjik sistem üzerinden çalışan ketaminin deri yoluyla depresyon tedavisinde kullanılması klasik antidepresanlara karşı önemli bir alternatif oluşturmaktadır.

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All is One, One is All…

to the highest good of All…

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ABBREVIATIONS

AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor

EPM: Elevated Plus Maze

FST: Forced Swim Test

GABA: Gamma-aminobutyric acid

GI: Gastrointestinal

IM: Intramuscular

IV: Intravenous

LH: Learned helplessness

MAO: Monoamine Oxidase Inhibitor

MDD: Major Depressive Disorder

NaSSA: Noradrenaline and Serotonin Selective Antagonist

NDRI: Noradrenaline and Dopamine Reuptake Inhibitor

NMDAR: N-methyl-D-Aspartate Receptor

OFT: Open Field Test

SC: Stratum Corneum

SARI: Serotonin Antagonist Reuptake Inhibitor

SNRI: Selective Norepinephrin Reuptake Inhibitor

SSRI: Selective Serotonin Reuptake Inhibitor

TCA: Tricyclic Antidepressant

Tmax: Time to reach maximum concentration

TRD: Treatment-resistant Depression

CHAPTER 1

INTRODUCTION

Depression have been a complex phenomenon of human mind and body with a history of 2500 years (Horwitz, Wakefield & Lorenzo-Luaces, 2016). In the 5th century, Hippocrates (a Greek physician) coined the term of ‘’melancholia'', in other words, ‘’nervous breakdown’’, to describe depression as a disordered balance of bodily fluids like blood, phlegm or bile with a notion of health, as an internal equilibrium of the body (Hippocrates, 1923). He stated that melancholia is a long-term fear of sadness which can be linked to black bile affecting the whole body state, rather than causing a localized disturbance. Thus, their understanding of mental illnesses was beyond the identification and amelioration of symptoms but analyzing them in a contextual manner, on the contrary of modern medicine (Horwitz, Wakefield & Lorenzo-Luaces, 2016). The evolution of melancholia continued with Galen who classified the symptoms as theme-specific fixed ideas similar to delusions, in addition to affective states of fear and despair (Galen, 1952). The combination of the ideas of Galen and Hippocrates created the basis of four temperaments described in the modern psychology consisting of four fundamental personality types of phlegmatic, melancholic, choleric and sanguine personality. These four personality types were related to four bodily humours and four seasons where each one is related to different emotional and cognitive personality characteristics.

After a decade of attempts to identify depression as a complex disorder including neurobiological and affective facets, the first medical identification of depression as a ‘’major depressive disorder’’ (MDD) was described by the

"Diagnostic and Statistical Manual of Mental Disorders'' DSM-3 (3rd ed.; DSM-3; American Psychiatric Association, 1980). The most recent version of the DSM-V manualincludes anhedonia or depressed mood as the cardinal symptoms accompanied by fatigue, guilt, weight loss and psychomotor retardation, and recurrent ideation of death for a MDD diagnosis (5th ed.; DSM-5; American Psychiatric Association, 2013). The diagnosis of depression requires at least 5 symptoms to be present simultaneously for at least 2 weeks. The diagnostic system of DSM-V has a polythetic nature which allows the possible individual differences in the course of disorder and differences in the emerged group of symptoms with a requirement of meeting a specific number of symptoms among all.

Overall, melancholia remains as the first attempt to identify depression despite of its recent use as a sub-feature of depression, described as a depression type with ''melancholic features'' in DSM-V. Over the years, depression have become a compelling health problem experienced by 322 million people globally (World Health Organization [WHO], 2017).

1.1 The discovery of antidepressants and monoamine hypothesis for depression

The discovery of the first antidepressants paved the path for the generation of biological hypotheses on affective disorders during the 1950s (López-Muñoz & Alamo, 2009). The origin of antidepressants lies back in the discovery of monoamine inhibition capacity of hydrazine molecules, which were previously used to treat tuberculosis with the observation of increased vitality in patients (Zeller, Barsky, Fouts, Kirschheimer & Van Orden, 1952). After describing the mood elevating effect of hydrazines as ''psychic energizers'', these findings were followed by a new line of research followed by the discovery of imipramine as an imipraminic or tricyclic

antidepressant (TCA) in 1957 (Kuhn, 1958). Following these findings, the history of antidepressants continue with irreversible monoamine oxidase (MAO) inhibitor phenelzine and the first selective serotonin reuptake inhibitor (SSRI) fluoxetine discovered in 1960s and 1980s, respectively (see for a review, López-Muñoz & Alamo., 2009). During this period of research, the term of ‘’antidepressant’’ was thought to be coined by Salzer and Lurie in 1953.

The exciting possibility of finding a mechanism to treat depression launched the era of ‘’monoamine hypothesis’’ described by Schildkraut in 1965, formerly named ‘’catecholamine hypothesis’’. This hypothesis postulates the therapeutic effect of these agents by a transient increase in the synaptic availability of extracellular monoamine levels by reuptake or degradation inhibition pathways (Bunney & Davis, 1965; Schildkraut, 1965). It is based on the decreased levels or availability of monoamines like serotonin, dopamine or norepinephrin in the extracellular space, as one of the underlying biological reasons for depression (Hirschfeld, 2000). With the growing literature on depression treatment, the recent family of classical antidepressants includes SSRIs, serotonin and noradrenalin reuptake inhibitors (SNRIs), noradrenalin reuptake inhibitors (NRIs), noradrenalin and dopamine reuptake inhibitors (NDRIs), MAO-inhibitors and TCAs which inhibit serotonin and noradrenalin reuptake as well as various receptors and specific antagonists affecting noradrenalin and serotonin mechanisms (NaSSAs), serotonin antagonists (SARIs) which are lesser known types of antidepressants (see for a review, Karamustafalioglu & Yumrukcal, 2011). With this large family of antidepressant agents, the monoamine hypothesis has been used for depression for more than 50 years, with its easy and understandable nature (Schulman, Hermann, & Walker, 2013).

However, the monoamine hypothesis fails to explain at least three important aspects about depression. First, it is known that one-third of MDD patients do not respond to at least two or more medications which is defined as the ‘’treatment-resistant depression’’ (TRD) (Rush et al., 2006). They remain refractory after the treatment which shows a failure of classical agents in the induction of a sustained remission rate (Insel & Wang, 2009; Katz et al., 2004). In relation to this, Ionescu, Rosenbaum and Alpert (2015) emphasized that this stagnation in the treatment of TRD is not a patient-based issue and is caused by a lack of more targeting treatment regimens.

Second, the monoamine hypothesis fails to explain the temporal gap between the immediate increase of extracellular monoamine levels in minutes to hours and delayed clinical response up to 2 to 4 weeks (Hyman & Nestler, 1996; Racagni & Popoli, 2008). One argument for this temporal gap was the time needed for the autoreceptor desensitization of serotonin transporters to achieve the elevated firing rate in dorsal raphe neurons (Blier & Montigny, 1994). Still, this relationship was not enough to clarify all antidepressants’ action mechanisms, such as the rapid-acting agents which represent another question mark regarding the monoamine hypothesis.

Third and the most ground-breaking example is the rapid-acting antidepressants with a much shorter temporal gap between the response and administration which casted doubt on the monoamine hypothesis (Sanacora, Treccani, & Popoli, 2012). As the pivotal finding in 2000, Berman and colleagues showed that a single subanesthetic dose of ketamine exerted therapeutic response in the first 72 hours of infusion. Following this, a pivotal finding for the preclinical antidepressant effect of ketamine was reported by Yilmaz, Schulz, Aksoy and Canbeyli (2002). They investigated the ameliorative effect of a single intraperitoneal

injection of 160 mg/kg ketamine in the rodent behavioral despair model which was controlled by a saline-injected group. In their experiment with 55 male Wistar rats, the ketamine group showed significantly lower scores of immobility in the test day of the FST compared to the saline-treated group and most strikingly, the antidepressant effect was present in the FST 3, 7 or 10 days after the treatment. Additionally, they reported that ketamine treatment did not cause any hyperactivity with non-significant differences in the frequencies of diving, jumping and head shake behaviors between the groups. These results were in concordance with the findings of Berman and colleagues (2000) and represented the pioneering class of studies on the potency of ketamine's antidepressant property.

The findings about ketamine as a glutamatergic N-*methyl*-D-aspartate receptor (NMDAR) antagonist and other fast-acting antidepressant agents launched a new area focusing on its rapid-onset action with sustained efficacy through NMDAR antagonism. Unsurprisingly, the question marks associated with the monoamine hypothesis triggered the researchers to search for alternative or complementary mechanisms expanding beyond the monoamine hypothesis (Sanacora, Treccani, & Popoli, 2012).

1.2 The glutamate-based neuroplasticity hypothesis

''Glutamate hypothesis'' is one of the most valid group of ideas which explains the biological basis of damaged neuroplasticity pathways associated with the primary excitatory neurotransmitter of our brain, glutamate. Long before the clear investigations about this hypothesis, Hirschfeld (2000) suggested alternative mechanisms as a potential ''crosstalk'' between the neurotransmitter systems. Additionally, a more recent paper of Sanacora, Treccani and Popoli (2012)

highlighted the importance of the glutamatergic system as the primary biological underpinning of depression and the need of a shift from monoamine deficits to glutamate-based neuroplasticity.

Glutamate is one of the most abundant amino acid in the central nervous system with inhibitory γ-aminobutyric acid (GABA) and it is the major neurotransmitter for neuronal excitation in tripartite synapses. The brain mass is made up of 85% neocortex tissue and glutamate is primarily found in neocortex neurons and synapses (Douglas & Martin, 2007). Sanacora and colleagues (2012) focused on this fact and proposed the brain as a ‘’glutamatergic excitatory machine’’ which is governed by a delicate balance between excitation and inhibition.

The glutamatergic system is regulated by three ionotropic glutamatergic receptors which are the NMDARs, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) and kainate receptors. The NMDAR was named after its selective agonist of N-*methyl*-D-aspartate (NMDA) and it was discovered with the synthesis of NMDA in 1981 (Watkins, 1981). The excitotoxicity of excess glutamate was targeted by many psychiatric treatments in terms of an antagonism to ameliorate the dysregulations caused by overexciations of the NMDAR (see for a review, Sanacora & Schatzberg, 2015). The very first treatment of depression which is electroconvulsive shock was also shown to elicit adaptive changes in ligand binding sites of the NMDAR in rat cortex (Paul, Layer, Skolnick & Nowak, 1993). See Figure 1 for the schematic representation of the NMDAR and its binding sites.

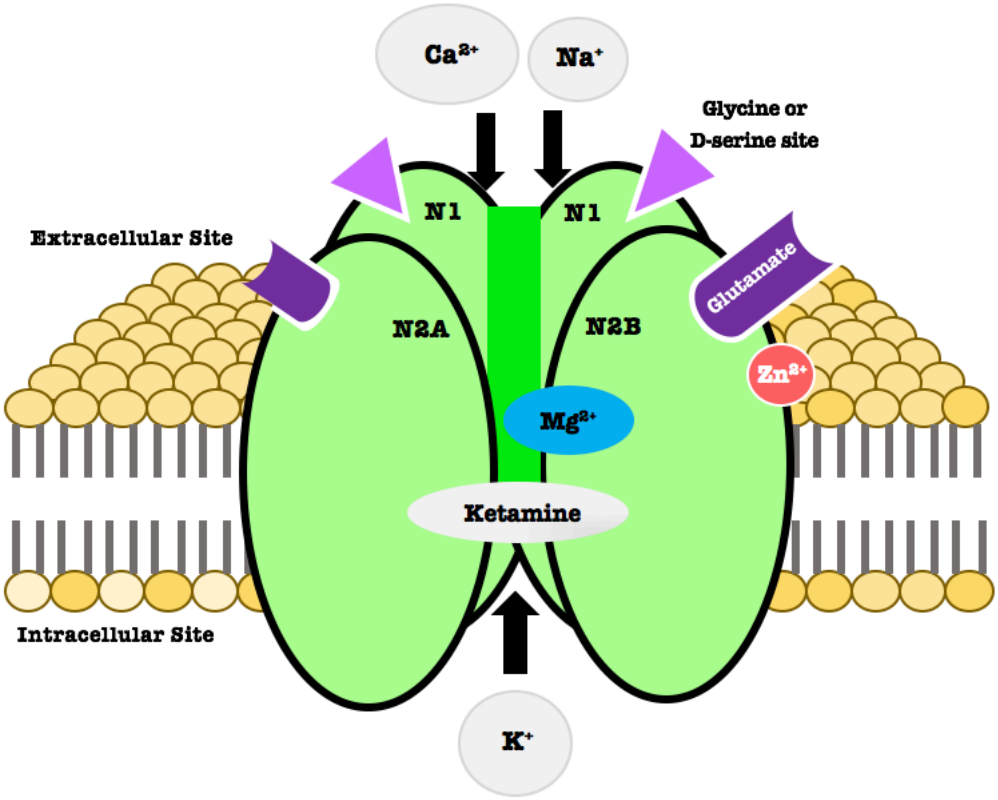

Figure 1. Structure of the NMDAR
Note: The figure is a schematic illustration of the receptor to show potential ligand binding sites and ion flow

Hasler and colleagues (2007) demonstrated that MDD patients have significantly reduced levels of glutamate and GABA levels in the dorsal and ventromedial prefrontal cortex (PFC) when compared to healthy subjects. Similarly, Altamura, Maes and Meltzer (1995) reported that perturbations in the levels of glutamate and an inhibitory amino acid, taurine in the plasma of depressive patients. Glutamate was also elevated in the post-mortem tissues from the frontal cortices of MDD and bipolar depression patients, controlled for their time period of being post-mortem (Hashimoto et al., 2007). In an extensive review, Schmaal and colleagues (2019) put forth that PFC is another brain region which undergoes a volumetric reduction in patients with suicidal MDD patients. They underlined that the

identification of regional alterations can be future biomarkers of depressive patients with a higher risk of suicide.

Neuroplasticity is another candidate, being a biomarker for depression in the brains of depressive patients which is mainly processed through glutamatergic receptors (Duman, Aghajanian, Sanacora & Krystal, 2016). Neuroplasticity is a functionally and morphologically adaptive process which refers to neurogenesis, synaptogenesis, dendritic branching and lengthening and the increase in spine density. It is the critical mechanism of the brain to adapt to internal and external stimuli like stress (Zilles, 1992). Chronic stress models in rodents induced atrophy of apical dendrites in the hippocampus CA3 pyramidal cells (Watanabe, Gould & McEwen, 1992). In a chronic mild stress model of rodent depression, decreased adult hippocampal cytogenesis was shown to correlate with a recovery from anhedonia following antidepressant treatment (Jayatissa, Bisgaard, Tingstrom, Papp & Wiborg, 2006).

The earlier studies described hippocampal volumetric reduction as a sign of depression (Magarinos & McEwen, 1995). Magarinos and McEwen (1995) underlined the involvement of glutamatergic NMDAR antagonists and corticosterone secretion in restraint stress-induced apical dendrite atrophy reversal in the hippocampus. Five different agents from monoaminergic and non-monoaminergic class reversed the dendritic atrophy and decrease in spine density in the hippocampus of rats induced by chronic mild stress (Bessa et al., 2009).

The opposite structural alterations were reported in the amygdala where stress exposure promoted dendritic lengthening with an anxiolytic effect (Vyas et al., 2002). Interestingly, they concluded a differentiation between the chronic stress type affecting specifically basolateral amygdala pyramidal neurons as a potent candidate

for affective diseases. In a complementary manner to all the aforementioned findings, the classical antidepressants failed to exert therapeutic impact when the adult neurogenesis was inhibited through radiation (Santarelli et al., 2003).

The relationship between the excitatory system and structural alterations is an undeniable part of depression treatment considering the glutamate receptors which regulate the neuroplasticity processes like neurite growth or apoptosis (Mattson, 1996). Therefore, NMDAR antagonists are the best examples to see the synergism between glutamatergic system and neuroplasticity, like ketamine. The rapid-acting antidepressant ketamine uses a glutamate-mediated pathway to promote new synaptic connections and reset the damaged neuronal network in the brain (Deyama & Duman, 2020; Li et al., 2010). Deyama and Duman (2020) suggested that the neurogenesis can be related to the sustained therapeutic impact of ketamine, which requires a time delay to complete the hippocampal neurogenesis. Surprisingly, Yamada and Jinno (2019) observed a neuronal progenitor density increase and new granule cell in ventral hippocampus of mice, 2 days after the single dose ketamine treatment.

The class of fast-onset antidepressants is mainly dominated by two types of molecules which are the NMDAR antagonists, NMDAR partial agonists and gamma-aminobutyric acid (GABA) allosteric regulators (Li, 2020). Esketamine nasal spray and Brexanolone were the first two non-monoaminergic antidepressants for TRD and post-partum depression, respectively (Federal Administration of Food and Drug [FDA], 2019).

Altogether, glutamate-based neuroplasticity hypothesis provided a more valid answer for the question marks of the monoamine hypothesis. These agents represent the beginning of a new era for the treatment of depression with a fast-acting action

mechanism.

1.3 Clinical and preclinical studies on NMDAR antagonism

Trullas and Skolnick (1990) were the first researchers who demonstrated the antidepressant action of the glutamatergic NMDAR antagonists in their extensive preclinical study. They showed that the NMDAR antagonists (2-amino-7-phosphonoheptanoic acid) AP-7, a non-competitive antagonist (dizocilpine) MK-801 and a partial antagonist (1-aminocyclopropanecarboxylic acid) ACPC induce an antidepressant effect in behavioral despair of rodents (Trullas & Skolnick, 1990).

Following this finding, a large group of studies investigated various NMDAR antagonists like eliprodil, dizocilpine, amantadine, memantine and bifemelane on their antidepressant efficacy in preclinical depression models (Layer, Popik, Olds & Skolnick, 1995; Meloni et al., 1993; Moryl, Danysz, Quack, 1993). A key study by Skolnick and colleagues (1996) showed that chronic but not acute administration of seventeen different antidepressants takes place in the NMDAR structural adaptations.

Papp and Moryl (1994) demonstrated the antidepressant role of MK-801, CG-37849 and its enantiomer in anhedonia induced by chronic mild stress model. They reported that these NMDAR antagonists promoted a gradual increase in sucrose uptake which is the reversal of depressive symptom, anhedonia. Interestingly, these agents exerted a comparable antidepressant activity with the chronic treatment of imipramine as a TCA.

Complementarily, an adaptive function of several antidepressants was reported on the NMDAR structure after chronic treatment and they emphasized ligand-gated ion channels as the overlapping pathway of various antidepressant drugs (Paul, Nowak, Layer, Popik & Skolnick, 1994).

There are also micronutrients among NMDAR antagonists like zinc and magnesium with extracellular and intrachannel bindings site on the receptor with antidepressant properties (Pochwat et al., 2014; Szewczyk, Szopa, Serefko, Poleszak, & Nowak, 2018). See Figure 1 for binding sites of zinc and magnesium micronutrients.

In the scope of clinical findings, lanicemine is another example for NMDAR antagonists which was administered by repeated intravenous (IV) infusions for MDD patients and achieved depressive symptom improvements after 3 weeks (Sanacora, Smith & Pathak, 2014). Another NMDAR antagonist is nitrous oxide showed significant reduction in the Hamilton Depression Scale (HAMD) scores in depressive patients when administered via inhalation (Nagele et al., 2015). Amantadine is another example which showed synergistic antidepressant effect with imipramine in the Hamilton Depression Rating Scale (HDRS), while imipramine alone did not exert any therapeutic effect (Rogoz et al., 2007).

Since the critical finding of Trullas and Skolnick in 1990, these findings point out the large body of studies have continued to increase for more than 30 years for research on NMDAR and depression.

1.4 Ketamine

1.4.1 The origin of ketamine as a ‘’dissociative anesthetic’’

The discovery of ketamine is based on the phencyclidine piperidine (PCP or ‘’angel dust’’) molecule which was an ‘’cataleptic anesthetic’’ and analgesic agent without the common side-effect of cardiorespiratory depression, in contrast to other functionally similar agents (Domino, 1980; Maddox, Godefroi & Parcell, 1965). PCP

was first determined as a safe clinical anesthetic but later it was shown to induce a state of sensory deprivation which causes to a loss of feeling in limbs (Greifenstein, DeVault, Yoshitake & Gajewski, 1958). It was introduced to the medical world in the name of ‘’Sernyl’’ rooted from the serenity of the patients after its administration. Its street names are ‘’peace pill’’, ‘’angel dust’’, ‘’elephant tranquilizier’’ and it has many other street names in relation with its peculiar side effects. Soon after the observations of its adverse side effects, it was restricted for veterinary use with a name of ‘’Sernylan’’ during 1950s.

As the derivatives of PCP can serve for this purpose, Calvin Stevens as a chemist at Parke Davis synthesized a molecule from arylcyclohexyl class named as ‘’CI-581’’ in 1956 (Domino, 1980). This molecule was a combination of amine and ketone functional groups and defined as ‘’ketamine’’ soon after its discovery (See Figure 2).

Ketamine was similar to PCP in terms of not triggering respiratory and cardiovascular depression and superior with its rapid onset nature (Domino, Chodoff & Corssen, 1965). Still, it was inducing a peculiar state of consciousness where the patients were seemed to be disconnected from their environment. Ultimately, Domino and colleagues (1965) coined the term ‘’dissociative anesthetic’’ for ketamine to express its specific type of anesthesia. This state was defined as an electrophysiological and functional dissociation between the neocortex, hippocampus and thalamic regions (Corssen & Domino, 1966; Miyasaka & Domino, 1968).

Schroeder and colleagues (2016) showed that corticocortical sensory information transfer is blocked during ketamine anesthesia in the intracranial recordings of monkeys. It causes a structural disconnection between cortico-cortical networks while the sensory network remains functionally normal during anesthesia.

This finding overlaps with the patient descriptions of dissociative anesthesia and explain its network-level neuronal basis.

Corssen and Domino (1966) reported that ketamine can act as a potent analgesic which is suitable for repeated administration as its side-effect of emergence delirium is transient. After a long journey, ‘’Ketalar’’ was the first ketamine formulation approved by the FDA in 1970. Ketamine is now being used as a Schedule III agent and as a human and veterinary anesthetic. Similar to other psychoactive agents, ketamine have also been used as a street drug under the names of ‘’horse tranquilizer’’, ‘’special K’’ or ‘’vitamin K’’.

1.4.2 Chemical structure of ketamine

Ketamine is an arylcycloalkylamine with a chiral center of carbon atom it has two optic enantiomers as *S*(+) ketamine (esketamine) and R(-) ketamine (arketamine). Esketamine has a significantly higher affinity for the NMDAR and it acts as a more potent anesthetic compared to arketamine (White et al., 1985). In terms of depression treatment, arketamine was shown to induce more effective therapeutic effect with less side effects (Zhang, Li, & Hashimoto, 2014). See Figure 2 for chemical structures of enantiomers with chirality.

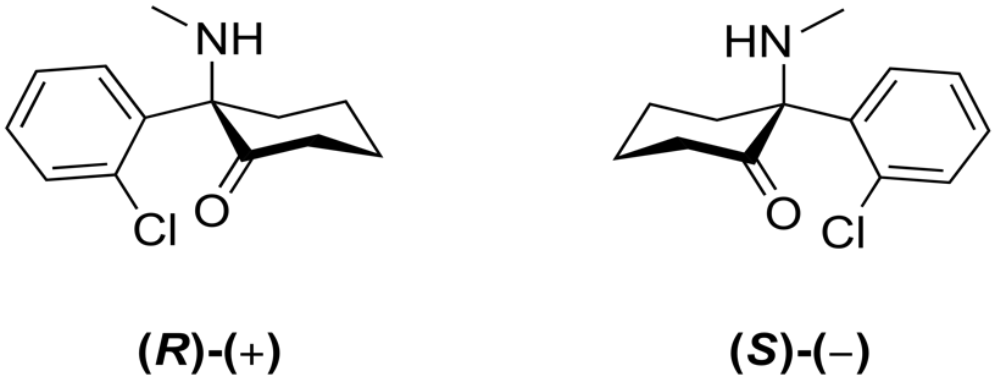

Figure 2. The optic enantiomers of ketamine

1.4.3 Metabolism of ketamine

The biotransformation of ketamine is governed by hepatic cytochrome enzymes CYP3A4 and CYP2B6 to its primary metabolite, norketamine. Following this step, CYP2A6 and CYP2B6 enzymes convert norketamine into hydroxynorketamine (HNK) and by dehydrogenation into dehydronorketamine (DHNK) (Zanos & Gould, 2018). The metabolic pathway of ketamine can be summarized as the primary process of *N*-demethylation of ketamine into norketamine which is followed by major and minor oxidation steps to yield HNK isomers (Adams, Baillie, Trevor & Castagnoli, 1981). See Figure 3 for a stereoselective metabolic chart of ketamine.

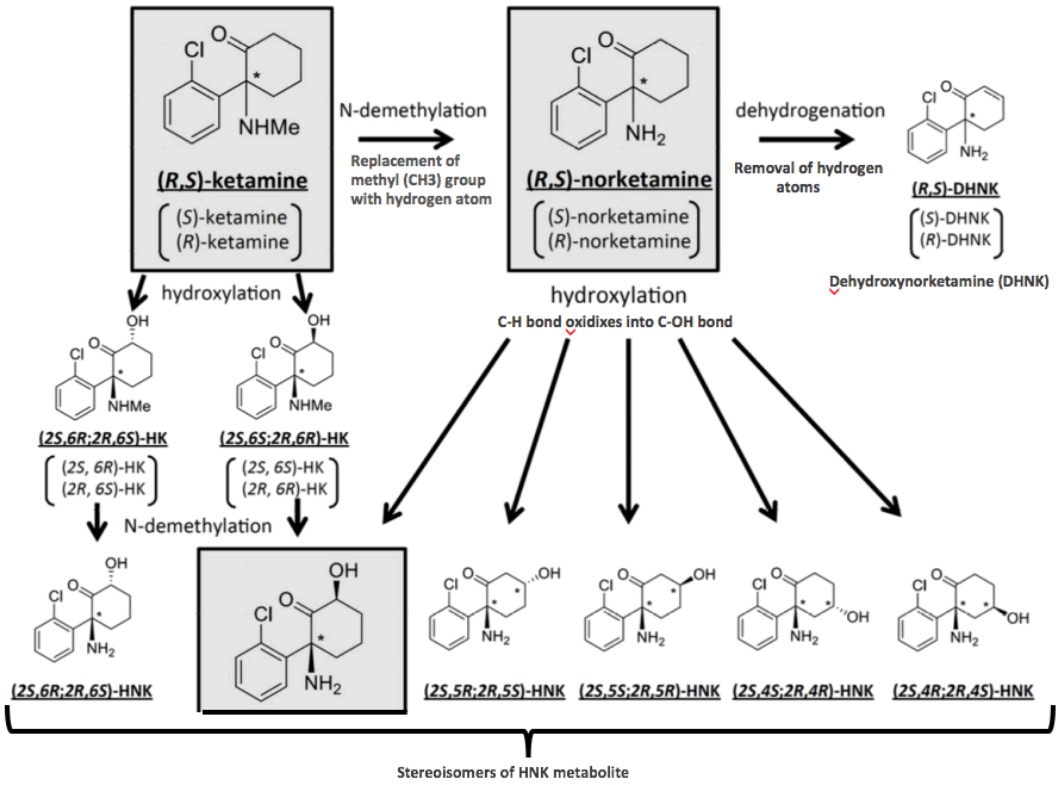

Figure 3. Stereoselective metabolic chart of ketamine
Note: The main chemical processes for ketamine metabolism is methyl and hydrogen replacement, conversion of carbon-hydrogen bond into carbon-hydroxyl bond and removal of hydrogen atoms (modified from Chaki, 2017). The dots indicate chiral carbon centers in the compound

1.4.4 Pharmacokinetics of ketamine

Ketamine is a white colored salt which is highly water and lipid soluble. It is commercially sold as a racemic mixture of two enantiomers in the form of a ketamine salt dissolved in saline in addition to enantiopure forms. Ketamine is accumulated in the brain with its moderately high lipid solubility (Cohen et al., 1973).

Ketamine is found in a free-unbound form or in a plasma protein-bound form in plasma (Dayton, Stiller, Cook & Perel,1983). The plasma protein binding of ketamine was reported as relatively low, ranging from 23% to 47% (Dayton, Stiller, Cook, & Perel, 1983). With its low plasma binding property, ketamine is distributed

in the body rapidly with a distribution half-life of 10 to 15 minutes and with an elimination half-life of 2 to 3 hours following IV administration (Domino et al., 1984). Ketamine is rapidly distributed in the tissues where peak concentrations were found in most tissues only after 15 minutes following IV delivery with highest amounts in kidneys, body fat and liver and lowest concentrations in the plasma (Chang & Glazko, 1974). Thus, the amount of ketamine bound to a receptor can be higher than its unbound amount in the plasma during anesthesia (Orser, Pennefather & MacDonald, 1997). The main elimination pathway of ketamine is renal excretion as urine by 91% and feces by 1 to 3% (Chang & Glazko, 1974).

1.4.5 Ketamine and NMDAR inhibition

The first identification of ketamine as non-competitive NMDAR antagonist by Anis and colleagues in 1983. Orser, Pennefather and MacDonald (1997) proposed two main mechanism of ketamine NMDAR inhibition as decreasing the probability of receptor opening, decreasing the mean open time of receptor and decreasing the channel opening frequency.

Ketamine is a unique ‘’trap-blocking’’ inhibitor of the NMDAR by remaining bound to the intrachannel PCP site with a slow off-rate after the disintegration of agonist. In other words, the receptor is closed upon the disintegration of the agonist while ketamine is bound to its intrachannel site. Following this, the opening probability of receptor depends on the subsequent agonist binding (re-opening of the channel) and ketamine decreases the number of receptors available for opening (Orser, Pennefather & MacDonald, 1997). Macdonald and Nowak (1991) described this mechanism as a transitory modulation between the open and closed state of the NMDAR induced by ketamine. Ketamine traps the receptor by 86% as a high-

trapping antagonist, and as a non-competitive antagonist with a binding ability independent of the agonist's presence (Orser, Pennefather & MacDonald, 1997).

1.5 Potential antidepressant mechanisms of ketamine

The pivotal mechanism of ketamine on analgesia, anesthesia and depression have been its NMDAR blockage (Zanos & Gould, 2018). Ketamine as a non-competitive NMDAR antagonist acts by binding to the PCP-site to block the ion channel (Orser, Pennefather, & MacDonald, 1997). Ketamine's antidepressant action mechanism has been investigated regarding its direct inhibition and a disinhibition process on pyramidal neurons in cortex (Miller, Moran & Hall, 2016).

Basically, NMDAR blockade triggers a series of neurochemical processes including the pathways of eukaryotic elongation factor 2 kinase **(**eEF2K), brain-derived neurotrophic factor (BDNF), the mammalian target of rapamycin (mTOR) and AMPAR (Zanos & Gould, 2018). This cascade is associated with the neuroplasticity changes seen during the course of MDD as aforementioned, specifically in hippocampus (Suzuki & Monteggia, 2020).

In addition to its direct activity on NMDAR, the ‘’disinhibition hypothesis’’ postulates the affinity of ketamine to the NMDARs specifically in the regions which are rich in GABAergic interneurons (Zorumski, Izumi & Mennerick, 2016). Ketamine shifts the synaptic transmission balance to excitation by indirectly disinhibiting excitatory pyramidal neurons and an increase in glutamatergic synapses through synaptic plasticity (Autry et al, 2011). It is also defined as the preferential NMDAR antagonism on distinct sub-groups of inhibitory interneurons (Miller, Moran & Hall, 2016).

It should be noted that there are growing in vitro, in vivo, preclinical and clinical evidences for both hypotheses (Miller, Moran & Hall, 2016). The receptor subunit or interneuron subtype preference of ketamine and the neurochemical cascade details were suggested for investigation to have a clear distinction between the significance or priority of direct or indirect inhibition (Miller, Moran & Hall, 2016; Suzuki & Montaggio, 2020).

There are also alternative or complementary mechanisms which were not associated with NMDAR-independent activity of ketamine metabolite (HNK), opioid, serotonin or dopamine receptors (Zanos & Gould, 2018). In addition to these, there are related elements of its mechanism like sleep patterns and clock-genes (Orozco-solis et al., 2017).

Overall, Zanos and Gould (2018) concluded that these mechanisms are likely to be complementary rather than being mutually exclusive which create the unique action mechanism of ketamine for MDD. This raises the possibility of a holistic metabolism of all metabolites of ketamine which results in the antidepressant function of ketamine, rather than the sole impact of single metabolite.

1.5.1 The elements of NMDAR-dependent action mechanism

1.5.1.1 AMPAR potentiation

AMPARs mediate the depolarization step required for the NMDAR channel opening which regulates the synaptic changes described as long-term potentiation (LTP) and long-term depression (LTD) (Derkach et al., 2007).

Li and colleagues (2010) found that the administration of an AMPAR antagonist blocked the phosphorylation of the further neurochemical cascades

required for the antidepressant efficacy of ketamine.

In terms of behavioral response, Maeng and colleagues (2008) demonstrated that the pretreatment of an AMPAR antagonist attenuated behavioral response of ketamine measured by the FST in mice.

Additionally, Koike, Iijima and Chaki (2011) treated rodents with an AMPAR antagonist which blocked the antidepressant response both in a learned helplessness (LH) and tail suspension test (TST) paradigms. Additionally, they confirmed both the rapid action and sustained therapeutic response of ketamine in the same paradigms by a single injection of ketamine whose antidepressant impact began in 30 minutes and lasted for 72 hours.

Acute ketamine treatment elevates the AMPAR-dependent neurotransmission in the serotonergic dorsal raphe nucleus which was shown to project to the PFC (Llamosas et al., 2018). They also showed that another NMDAR antagonist (D-AP5) failed to exert the same AMPAR-dependent neurotransmission which underlines that this process can be unique for ketamine's mechanism.

It is important to underline that AMPAR-dependent processes are required for both direct and indirect inhibition as it is involved in the LTP-like synaptic plasticity changes and the maintenance of recently formed or strengthened synapses following ketamine treatment (Miller, Moran & Hall, 2016).

1.5.1.2 Eukaryotic elongation factor 2 kinase (eEF2K) dephosphorylation

The other name of eEF2K is Ca^{+2}/calmodulin dependent protein kinase III (CaMK-III) as an extract of mammalian cells (Nairn et al., 1985). It is involved in the dendritic protein translation and takes place in distinct types of plasticity (Park & Poo, 2013).

Ketamine inhibits the glutamate release by the inhibition of the NMDAR which deactivates the phosphorylation of eEF2K in the hippocampus and subsequently the BDNF protein translation is triggered (Autry et al., 2011; Gideons, Kavalali & Monteggia, 2014).

Strikingly, Gideons, Kavalali and Monteggia (2014) presented the differences between the ketamine and mematine (NMDAR antagonist) originate from their differences in the downstream intracellular pathways that are regulated upon the receptor inhibition. They reported that memantine does not block the phosphorylation of the eEF2K which also affects the other downstream pathways like BDNF synthesis and mTOR signaling. In line with this, Suzuki and Monteggia (2020) emphasized eEF2K signaling as one of the key determinant of ketamine's antagonism of NMDAR.

Altogether, eEF2K acts as a primary substrate between the rapid antidepressant effect and synaptic potentiation including previous and further steps of NMDAR inhibition related neurochemical processes.

1.5.1.3 Brain-derived neurotrophic factor (BDNF) synthesis

BDNF is a neurotrophic protein found abundantly in the brain, specifically in the hippocampus and cortical tissues and regulates synaptic plasticity, synapse formation, neuronal growth and neurotransmission (Altar, 1999; Binder & Scharfman, 2004). It became the most investigated neurotrophin for depression (Sen, Duman & Sanacora, 2008).

Autry and colleagues (2011) showed that ketamine had no antidepressant effect on BDNF knock-out mice where the wild-type group showed significant reduction in the FST in the first 30 minutes of treatment. Interestingly, the

ameliorative impact of ketamine was still detectable after 24 hours in the wild-type group indicating the requirement of BDNF pathway for the sustained effect of ketamine.

The same group of researchers demonstrated the requirement of eEF2K deactivation to increase BDNF expression in mice. The administration of eEF2K inhibitors was correlated with an increase in hippocampal BDNF levels and antidepressant responses were observed in mice (Autry et al., 2011). It was also shown that tropomycin receptor kinase B (TrkB) is needed for the fast-acting antidepressant properties of ketamine with the absence of therapeutic effect in TrkB knockout mice (Autry et al., 2011). Together, these findings suggest that BDNF-dependent modulations are essential for the therapeutic functioning of ketamine in MDD.

1.5.1.4 The mammalian target of rapamycin (mTOR) signaling activation

The activation of the mammalian target of rapamycin (mTOR) pathway is associated with the synaptic protein synthesis which are essential for new spine synapse formation and growth (Hoeffer & Klann, 2010). mTOR signaling is also responsible from its downstream pathways including BDNF protein translation and AMPAR activity which are driven by the NMDAR overactivation and results in neuronal atrophy (Tang et al., 2015).

Llamosas and colleagues (2018) observed that ketamine's antidepressant functioning was blocked by the failure of AMPAR-mediated synaptic transmission under the inhibition of mTOR signaling. In line with this finding, Li and colleagues (2010) reported that mTOR inhibitor rapamycin inhibited the electrophysiological impact of ketamine in rodent PFC.

In relation with the BDNF activation, mTOR was characterized as an upstream element which regulates surface AMPAR receptors (Nosyreva et al., 2013). To sum up, mTOR signaling is one of the significant elements in the cascade of NMDAR-inhibition of ketamine with its synaptic plasticity and activator role for upstream and downstream molecular pathways.

1.5.1.5 Summary of NMDAR-dependent action mechanism

It should be noted that all of the neurochemical elements mentioned in the previous part work together in the antidepressant mechanism of ketamine dependent on the NMDAR inhibition (Zanos et al., 2018). In short, when the AMPARs are activated, the ion channel of the NMDAR is inhibited, evoking the postsynaptic neuroplasticity-related cellular cascades: suppression of eEF2K and triggering of BDNF protein and other dendritic protein translations (Kavalali & Monteggia, 2012). The upregulated BDNF synthesis triggers downstream pathways of mTOR signaling and results in synaptogenesis seen in the MDD patients under ketamine treatment (see for extensive reviews: Kavalali & Monteggia, 2012; Zanos et al., 2018).

1.5.2 The elements of NMDAR-independent action mechanism

1.5.2.1 Metabolites of ketamine

As previously mentioned, ketamine undergoes an extensive metabolism which produces norketamine, hydroxyketamine, dehydronorketamine and hydroxynorketamine metabolites (Adams, Baillie, Trevor and Castagnoli, 1981).

One of the most extensive studies among ketamine metabolites on depression belongs to Zanos and colleagues (2016) where they investigated (2S,6S;2R,6R)-

HNK antidepression mechanism at a behavioral, cellular, electrophysiological and electroencephalogramic level. They argued that the metabolite ameliorates depressive symptoms in an NMDAR-independent manner but through a sustained AMPAR activation and most importantly, without the side effects of ketamine.

Similarly, Pham and colleagues (2018) also reported antidepressant properties of (*2R,6R*)-HNK in the FST after intraperitoneal administration and direct injection to medial PFC. However, a null result was obtained with the same metabolite in a model of chronic social defeat stress and LH paradigm in mice (Yang et al., 2017).

In an extensive clinical study conducted by Zarate and colleagues (2012) reported that there is a clear distinction of specific metabolite concentrations between the bipolar depression and MDD patients after a 40-minute infusion of subanesthetic dose ketamine. Interestingly, three different metabolites were negatively correlated with the dissociative symptoms.

The distinctive classification of different ketamine metabolites raised the possibility of a downstream metabolism changing with the type of depression. Thus, they suggested that selective delivery of metabolites can provide depression type specific treatments.

1.5.2.2 Serotonergic system

Considering classical antidepressants, serotonergic system is a critical component for MDD treatments. For instance, Dujardin and colleagues (2016) reported the possible involvement of 5-HT receptors for the glutamate-driven intracellular cascade in ketamine's action mechanism.

Kadriu and colleagues (2021) emphasized a highly overlapping pathway between serotonergic psychedelics and ketamine where both rewire the brain circuits

by glutamate-dependent pathways of neuroplasticity. They note that both serotonergic psychedelics and ketamine exert both an acute and a delayed electrophysiological activity in the brain and share common neuroimaging markers like the rapid functional network disintegration (Carhart-Harris et al., 2016; Zacharias et al., 2020).

Overall, these findings point out an overlapping mechanism between serotonergic psychedelics, serotonergic antidepressants and ketamine which can illuminate both the therapeutic impact and side-effects of ketamine in the light of the serotonergic system literature.

1.5.2.3 Dopaminergic system

Dopaminergic system is characterized as the reward-system of the brain and the target of drugs with abuse potential (Sulzer, 2011). Unsurprisingly, Kapur and Seeman (2002) reported that ketamine shows strong affinity to the dopamine D2 receptors in addition to the excitatory and inhibitory systems.

Previous studies reported that single dose of ketamine triggers dopamine neurotransmission as a further process of AMPAR activation which determines the overall behavioral response (Moghaddam, Adams, Verma & Daly, 1997). Interestingly, they mentioned an alternative approach to glutamate hypothesis as a possibility of hyper-activation to the non-NMDAR receptors which increases the dopamine levels in the PFC. A more recent preclinical study conducted by Tan and colleagues (2012), long-term use of ketamine resulted in a more persistent activation of subcortical dopaminergic system which may cause cognitive dysfunctions.

In a comprehensive systematic review and meta-analysis, significantly elevated dopamine levels in cortex were detected in striatum, nucleus accumbens and

frontal cortex following an acute, subanesthetic dose ketamine administration (Kokkinou, Ashok & Howes, 2017). Additionally, they report an enhancement in the firing of dopaminergic neurons in the same regions when compared to control conditions. However, they note that there is a need of extra studies to conclude information on dopamine levels of other regions like hippocampus, ventral pallidum and cerebellum and in the clinical samples.

1.5.2.4 Opioid receptors

Not only the glutamatergic receptors but also mu, delta and kappa opioid receptors, monoaminergic receptors and cholinergic receptors (muscarinic and nicotinic) are involved in the ketamine's antidepressant functioning (Mion & Villevieille, 2013). For instance, the pretreatment of naltrexone as an opioid receptor antagonist diminished the antidepressant effects of ketamine in a clinical TRD sample (Williams, Heifets & Bentzley, 2019). They reported that specifically mu opioid receptors can be necessary for the acute therapeutic impact of ketamine.

However, a recent study showed that opioid receptors are insufficient for ketamine to induce antidepressant effects and underlined the need of a coordinated mechanism with glutamatergic receptors (Klein, Chandar, Sheriff & Malidov, 2020).

1.5.2.5 Sleep patterns and clock genes

Slow-wave activity enhancement was correlated with the synaptic plasticity enhancement and BDNF levels in rodent models (Huber, Tononi & Cirelli, 2007). Unsurprisingly, ketamine's antidepressant effect was associated with SWS activity, total sleep increase and a decrease observed in the first two nights following the infusion (Duncan & Zarate, 2013). The same researchers emphasized the possibility

of persistent changes occurring through sleep consolidation periods to ameliorate depressive symptoms.

Orozco-solis and colleagues (2017) analyzed the clock gene expressions of ketamine and sleep deprivation for their rapid-acting antidepressants. Interestingly, ketamine and sleep deprivation were shown to share common transcriptional profiles about circadian rhythm and neuroplasticity. Similarly, low dose ketamine was shown to reset dysregulated clock which govern the circadian rhythm (Bunney et al., 2015). In conclusion, sleep pattern and bodily rhythms are other potential biomarkers for ketamine's role in MDD.

1.5.2.6 Anti-inflammatory properties of ketamine

In the systematic review of Dale, Somogyi, Li, Sullivan and Shavit (2012), it was shown that ketamine acts as an anti-inflammatory agent by decreasing the early response inflammation molecules like interleukin. Related to depression, Tan and colleagues (2017) reported a reversal of behavioral and immunological pre-inflammatory response in a mice depression model of chronic restraint stress after ketamine treatment.

Another study of single subanesthetic dose ketamine treatment in the elderly reported postoperative delirium after surgeries which can be associated with a neuro-inflammatory state (Avidan et al., 2017). Another study also reported a non-correlated cytokine levels with the treatment response after ketamine administration to TRD patients (Kiraly et al., 2017).

Overall, there is a strong possibility for the inflammation-based activity of ketamine on its antidepressant function in line with the inflammatory profile of MDD patients. However, there is a need of more clinical and preclinical data which directly

binds depression and ketamine's anti-inflammatory nature.

1.5.2.7 Different views on the antidepressant action mechanism of ketamine

Kavalali and Monteggia (2012) argued that the challenging part of solving the ketamine's action mechanism is to imagine the transient inhibition of the neuronal pathways which result in long-term benefits. They underlined the short half-life of ketamine and emphasized the possibility of ketamine to exert a transient NMDAR blockade which reset the downstream neurochemical pathways which lead to its antidepressant effect. Zanos and Gould (2018) also made a similar summary saying that ketamine strengthens the excitatory synapses in a sustained manner which result in synaptic plasticity changes.

Kavalali and Monteggia (2012) also criticized the disinhibition hypothesis with the findings of a preclinical study which failed to mimic the rapid antidepressant effects by a pharmacological suppression of inhibition (Autry et al., 2011). Together, they underlined the critical role of miniature molecular processes in the rapid antidepressant mechanism with synaptic-level consequences in the brain.

Lastly, the logic between the NMDAR blockade and stress-induced elevation in glutamate levels is counterintuitive from a classical neuroscience point of view. Normally, NMDARs promote neuroplasticity and their blockade causes vice versa of the desired effect which is the normalization of dysregulated neuroplasticity. The NR2A-containing receptors accumulated in the synapse where the NR2A and NR2B-containing ones in the extrasynaptic space in hippocampus (Thomas, Miller & Westbrook, 2006). It is known that synaptic and extrasynaptic NMDAR activity cause opposite effects on neuroplasticity and their blockade protects brain towards glutamate toxicity (Hardingham, Fukunaga & Bading, 2002). Thus, it is important to

underline that the NMDAR blockade is a selective process on extrasynaptic receptors.

1.6 Clinical and preclinical studies on ketamine's antidepressant effect

Khorramzadeh and Lotfy (1973) conducted the first clinical study of ketamine on 100 psychiatric disorder patients with a bolus IV administration of 0.2 mg to 1 mg/kg ketamine. They reported that patients experienced an emotional discharge, recall of suppressed childhood memories, even in some patients they went back to the time of events close to the time of their illnesses began. They concluded ketamine as a safe psychotomimetic agent with mind expanding and detaching effects from the environment which is different than being asleep. They named the side effects of ketamine as ''abreactive'' including its mind expanding property.

Sofia and Hanakal (1975) conducted the first preclinical study showing the antidepressant effect of 26.7 mg/kg and 40 mg/kg oral ketamine in ptosis and hypothermia in mice which are the rodent phenotypes of depression reversed by classical antidepressants. They reported that ketamine ameliorated these phenotypes as a candidate antidepressant molecule.

The breakthrough of ketamine launched with the clinical findings of Berman and colleagues in 2000. As the first clinical study of ketamine and its antidepressant effect, they used a single subanesthetic 0.5 mg/kg dose of ketamine infusion on 8 patients and achieved a significant reduction of 50% in the HAMD scores of treated patients compared to control saline group (Berman et al., 2000). However, this study had a limited follow-up covering 3 days after the treatment in which patients did not obtain baseline scores in the HAMD.

To analyze the duration of antidepressant effects of ketamine, Zarate and his group (2006) administered the same subanesthetic dose of ketamine 0.5 mg/kg in TRD patients which resulted in a significant reduction in HDRS emerging at the 110 minutes after the treatment and lasted for 7 days. It is important to note that psychotomimetic effects of ketamine was diminished in the first 2 hours of administration.

In a clinical study, ketamine showed significant reduction in Montgomery-Asberg Depression Rating Scale (MADRS) within first 40 minutes of IV infusion and the peak improvements were observed on the second day where the total therapeutic effect lasted for 3 days in 16 TRD patients (Diazgranados et al., 2010). A subsequent study also reported antidepressant effect in the first 24 hours of treatment with mild psychotomimetic effects in a larger sample size of 147 patients (Newport et al., 2015). Following these, Murrough and colleagues (2013) conducted a study with single ketamine infusion and midazolam anesthetic as the control compound. They reported that ketamine TRD group experienced significantly greater amelioration in their depressive symptoms measured by MADRS in the first 24 hour of the treatment.

Lastly, Lapidus and colleagues (2014) utilized a 50 mg intranasal delivery for ketamine and 8 out of 18 patients showed significant reduction in MADRS scores in 24 hours after treatment with minimal psychotomimetic effects. For extensive reviews see Hillhouse & Porter, 2015; Jelen & Stone, 2021.

A preclinical pioneering finding was reported by Yilmaz and colleagues (2002) in a rodent behavioral despair model where 160 mg/kg intraperitoneal ketamine injection showed prolonged antidepressant effect lasted after 3, 7, or 10 days of the treatment compared to the saline-treated group of Wistar rats. As a false

positive control, the ketamine treatment did not cause any increase in the locomotor activity in terms of head shake, diving and jumping behavior.

Another preclinical study was conducted by Engin, Treit and Dickinson (2009) which showed that single injections of 10 mg/kg and 50 mg/kg ketamine reduces immobility time in the FST while not affecting locomotor activity levels in the OFT. Additionally, they showed anxiolytic activity of the agent with rats spending more time in the open arm in the EPM. They concluded that ketamine was unique in its antidepressant effect which can be controlled by the OFT, in contrast to a similar antidepressant MK-801 which reduces the immobility while increasing general locomotion in the OFT.

Another key study was conducted by Li and colleagues (2011) which found that ketamine ameliorates chronic unpredictable stress-induced depressive symptoms and shows an anxiolytic effect in rats. They reported that ketamine reverses the synaptic level damage of long-term chronic stress on a behavioral and neuronal level. Oral subanesthetic dose of ketamine was shown to induce antidepressant effect on behavioral despair without inducing any adverse effect on cognitive abilities (Ecevitoglu, Canbeyli & Unal, 2019).

In an extensive behavioral and molecular study on ketamine, Autry and colleagues (2011) showed both the fast-onset and sustained therapeutic properties of ketamine in specific neurochemical cascades triggered by the NMDAR inhibition. They crosschecked the false positive of a locomotor activity increase in the OFT and analyzed depressive symptom progress with LH, fear conditioning, FST and novelty suppressed feeding paradigms.

1.7 Routes of administration for ketamine

The physiochemical nature of ketamine enables a wide-range of delivery methods like oral, IV, intramuscular (IM), intranasal, sublingual and rectal delivery.

IV administration is the most common delivery for ketamine with 100% bioavailability which reaches maximum plasma concentrations rapidly (Clements, Nimmo, & Grant, 1982). IM injection has 93% bioavailability and rapid onset of 15 to 60 minutes which is the time needed to reach maximum concentration (Tmax) after the administration (Clements et al., 1982). IM injection is usually preferred for patients with limited IV access. However, injections need frequent dosing due to short half-life of ketamine and have the risk of abuse and disease transmission.

Another common technique is oral administration of ketamine as a more convenient method than IV or IM injection. Despite its user-friendly nature, oral administration suffers from extensive metabolism of ketamine which decreases the oral bioavailability to 17% to 23% with a maximum plasma concentration achieved approximately in 30 minutes (Clements et al., 1982; Grant, Nimmo, & Clements, 1981). The reason for this relatively low bioavailability is the extensive gastrointestinal (GI) metabolism which is open to drug-drug interaction specifically with the cytochrome enzyme inhibitor drugs.

Sublingual formulations are also available for ketamine with similar pharmacokinetic properties of low bioavailability ranging from 24% to 29% and reaching to maximum concentration in 5 to 18 minutes (Chong, Schug, Page-Sharp, Jenkins, & Ilett, 2009; Rolan, Lim, Sunderland, Liu, & Molnar, 2014). Rectal delivery is another option for ketamine again with a low bioavailability 11% to 25% and the concentration of ketamine reaches maximum in 30 to 45 minutes (Idvall, Aronsen, Stenberg, & Paalzow, 1983; Malinovsky, Servin, Cozian, Lepage, &

Pinaud, 1996). These specific routes can be preferred for patients with health conditions limiting classical routes with higher bioavailability.

The most recent route is intranasal delivery of esketamine with the approval of nasal spray ‘’Spravato’’ in 2019 (FDA, 2019). This formulation is used for TRD as an adjunct therapy with oral antidepressants. This delivery route has advantages over classical methods like the direct delivery to brain without interacting with blood-brain barrier and bypassing the first-pass metabolic processes (Erdő, Bors, Farkas, Bajza, & Gizurarson, 2018). Intranasal route has 45% bioavailability depending on the quality of application (Yanagihara et al., 2003). Despite of its important advantages, it suffers from low drug permeability without adding permeation enhancers to the formulation due to nasal secretion problems and risk of abuse without additional chemicals (Erdő et al., 2018). In addition to these, Galvez and colleagues (2018) reported that 100 mg self-administered nasal esketamine caused acute psychotomimetic side effects, two-fold variability between patients’ plasma ketamine and norketamine levels and problems during self-administration due to incoordination. See Table 1 for a summary of time to reach maximum concentration, bioavailability and route of delivery.

Overall, these classical routes with fast-acting properties are successful preferences for emergency cases like acute pain. Similarly, routes with low bioavailability can also be suitable for patients with GI tract or swallowing problems. However, there is an unmet need of alternative delivery methods which provide sustained plasma concentrations without interacting with GI tract and liver metabolism which also offers convenience to the patients with lower risk of abuse. Transdermal drug delivery represents one of the most effective method which has these properties overcoming the common disadvantages of classical routes.

Table 1. Pharmacokinetic properties of ketamine with different routes of delivery

References	Route of Delivery	Bioavailability	Tmax
Clements et al., 1982; Loo et al., 2016	IV	100%	0-5 min
Clements et al., 1982; Grant et al., 1981; Loo et al., 2016	IM	92-93%	15-22 min
Glue, 2020; Loo et al., 2016	Subcutaneous	91%	15-30 min
Clements et al.,1982; Grant et al., 1981; Yanagihara et al., 2003	Oral	17%-20%	30-33min
Chong et al., 2009; Rolan et al., 2014; Yanagihara et al., 2003	Sublingual	29%-33%	15-45 min
Kaube et al., 2000; Malinovsky et al., 1996; Yanagihara et al., 2003	Nasal	45%-50%	15-20 min
Idvall, 1983; Malinovsky et al., 1996; Yanagihara et al., 2003	Rectal	25%-29%	23-41 min

1.8 Transdermal drug delivery

Skin is one of the most impressive element of evolution as it is both a physical encapsulation of an organism and biochemical barrier between the internal and external world of a body. It is the largest organ with a weight of 4 to 9 kilograms and 2 m^2 surface area. Overcoming this protective layer is a purpose of transdermal drug delivery, for skin to act as a drug-carrier to the systemic absorption.

The outer-most layer of the skin is called ‘’stratum corneum’’ (SC) and under this layer there are epidermis, dermis layers and subdermal tissue, respectively (see Figure 4). SC layer of the skin is extremely thin (approximately 20 micrometers) and the least permeable one composed of keratinocytes staggered in a lipophilic matrix which was described as ‘’brick and mortar assembly’’ (Elias, 1981). It is the first mechanical barrier of skin with dead corneocytes which exert resistance to any mechanical stimuli. It is interesting that SC is only 10-15 cells inside the structure but represents the most challenging barrier for a drug with its intracellular space composed of both lipophilic and hydrophilic domains (Elias, 1981).

Overall, the drug passes through non-viable SC, viable epidermis and finally dermis which includes capillaries to carry the drug to systemic absorption, respectively. See Figure 4 for skin layers.

First, the drug released from the formulation or transdermal patch is absorbed via sweat glands or hair follicles on the SC or via transcellular or intercellular pathways and gets diffused across the lipid barrier to epidermal and dermal tissue which ultimately drained into the capillaries to reach systemic absorption (Godin, 2007).

The compound partitioned into the skin layers is gradually released over an extended period of time which provides sustained plasma concentrations (see for a review, Tanner & Marks, 2008). This gradual release represents the most powerful medical advantages of drug delivery through skin to avoid plasma fluctuations. See Figure 4 for a detailed illustration of permeation pathways, human skin layer structure and permeation mechanism of topical and transdermal delivery.

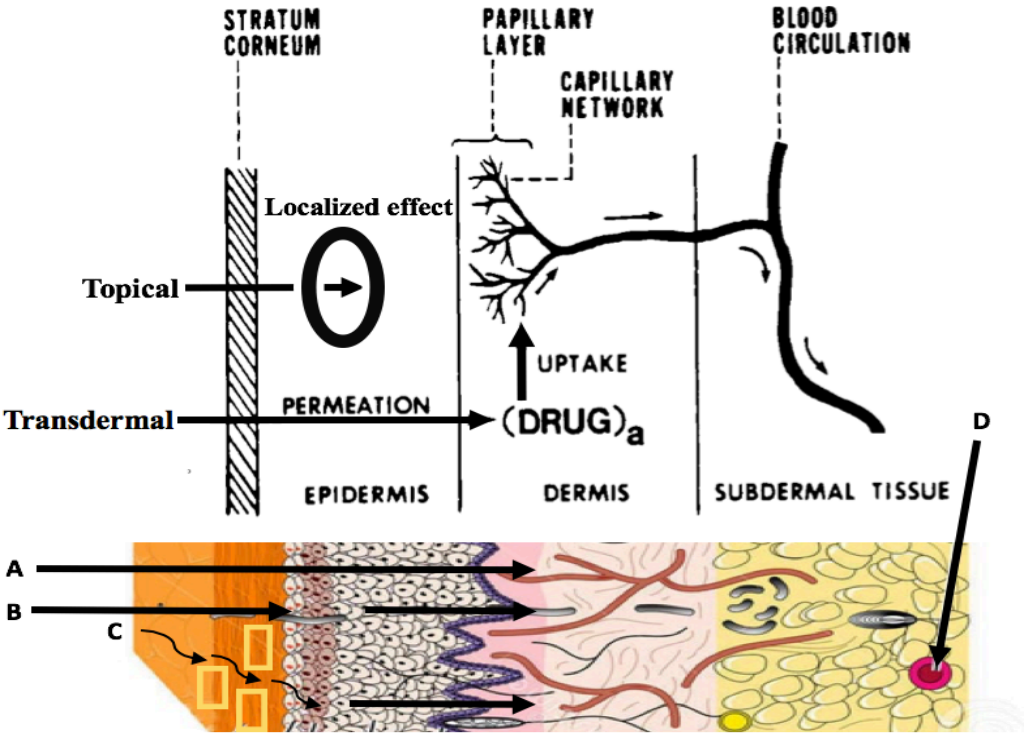

Figure 4. Skin layers and diffusion pathways
Note: Intracellular absorption (A), transcellular (intracellular) route (B) through the absorption by sweat glands (gray eliptic shapes following arrow B) or hair follicles and intercellular route (C) through the brick-mortar structure of corneocytes in the SC (yellow rectangles). The systemic absorption occurs when the drug reaches to the arteries in the subdermal tissue by transdermal permeation (D) (modified from Chien, 1987)

Percutaneous absorption occurs via passive diffusion across skin layers in multiple steps. The applied drug have three alternative routes across skin as transfollicular (transappendageal), intracellular and intercellular pathways.

The first alternative for drug permeation is the transepidermal route which consists of transcellular route as a more direct passage through corneocytes for ideally non-polar molecules and intercellular route as a more common passage occurs with the interaction between drug and membrane proteins and lipids of ideally polar molecules (Roberts, 1997; Trommer & Neubert, 2006). Transcellular route includes the passage of an ideally both lipophilic and hydrophilic molecule through

the cytoplasm of dead keratinocytes and lipid layer of SC. The third transfollicular route provides permeation via pores, cavitations, hair follicles or sweat glands which is less common due to their relatively rare presence on skin (Trommer & Neubert, 2006). This route is ideal for polar or hydrophilic agents which can be transferred via sweat. Still, the diffusion pathways can work together depending on the physiochemical state of the drug which makes these diffusions not mutually exclusive (Roberts, 1997).

If the drug permeation remains insufficient for a therapeutic outcome, there are various techniques to enhance the drug permeation via physical enhancement methods like ultrasound waves (pushing the drug with oscillating sound waves), laser, heat, physical ablation (creating extra pores on skin), iontophoresis (small electrical current-driven permeation) and chemical permeation enhancers (disturbing the highly ordered structure of skin layers) (see for a review, Prausnitz & Langer, 2008). Roberts (1997) argued that the drug diffusion occurs with the logic of ‘’like dissolves like’’ in basic chemistry and suggested chemicals with the most similar physiochemical profile to the SC can represent the high efficacy permeation enhancers. However, the performance of chemical permeation enhancers are correlated with skin irritability with a small class of exceptional enhancers which triggered the scientific arena to search for improved combinations of different enhancement methods both physical and chemical (Prausnitz & Langer, 2008).

Unsurprisingly, this complex nature of skin has some requirements for drugs for an ideal skin penetration like molecular size, skin hydration, skin permeability, ph of the drug, lipid and water solubility, drug metabolism and formulation stability (Tanner & Marks, 2008). The optimal drug for this route was suggested to have a molecular weight under 500 Dalton, lipophilicity constant between 1 to 4, solubility

in water higher than 1 mg/ml, melting point under 200°C, pH in a range of 5 to 9, partition coefficient greater than 2 and relatively low dose delivered per day by 10 mg. It should be noted that even if the agent passes all these criteria does not necessarily have an ideal profile in terms of treatment efficacy (Naik, Kalia & Guy, 2000). See Table 2 for the ideal physiochemical properties and corresponding values of ketamine which are fully satisfied except the dose per day. This requirement is only a recommendation and most drugs used in transdermal formulations do not meet this criteria by being above 20 mg per day (see for a review of transdermal formulations exceeding the recommended dosing, Naik, Kalia & Guy, 2000).

One of the most critical properties of a drug to permeate skin is an optimal combination of being both lipophilic and hydrophilic (Naik, Kalia & Guy, 2000). A very lipophilic drug can be stuck in the SC and cannot be transferred to systemic circulation by binding to other lipid molecules in the layer. Similarly, a very water-soluble drug has a lower possibility of passing through SC due to the lipid matrix. Thus, ketamine has a powerful profile regarding lipid and water solubility for transdermal delivery. Ketamine fulfills most of the criteria for being an ideal compound for transdermal delivery with high lipid and water solubility, molecular weight of 237.72, pH of 3.5 (in 10% solution for injection) and a melting point of 92.5 and partition coefficient of 3.12 and log value of oil to water partition coefficient of 2.18 which enable ketamine to diffuse efficiently through SC. See Table 1 for a summary of physiochemical properties of ketamine and recommended values.

Table 2. Summary of physiochemical properties for skin permeating drugs and ketamine salt

	Ideal Physiochemical Properties for Skin Permeating Drugs	Physiochemical Properties of Ketamine Salt
Molecular weight	< 500 Dalton	237.72
Melting point	< 200°C	92.5
pH (saturated solution)	5 to 9	3.5 to 5.5
Water solubility	> 1mg/ml	200 mg/ml
LogKo/w (oil/water partition coefficient)	1 to 4	2.18
LogP (partition coeffcient)	2 to 5	3.12
Oral bioavailability	Low	17% - 23%
Dose per day	< 20 mg/day	0.5 to 100 mg/kg (according to therapeutic aim)

1.8.1 Advantages and disadvantages of transdermal delivery

Transdermal delivery has multiple advantageous in terms of sustained plasma levels, abuse deterrence, drug delivery extended in time and its convenience of use and treatment adherence.

First, transdermal delivery provides a steady plasma concentration as an important property to achieve optimal therapeutic response without unwanted side effects, plasma fluctuations or the need for frequent dosing (Naik, Kalia, & Guy, 2000). This property is critical for drugs like ketamine with short half-life.

Second, skin acts as a cellular reservoir for the drug and provides a drug release extending over a period of time through the absorption by capillaries in dermis layer (Mathias & Hussain, 2010). This sustained distribution to systemic circulation provides a window of therapeutic action in a synergism with the absence of first-pass effect due to GI metabolism.

Third, transdermal formulations are highly suitable for adding extra chemicals like permeation enhancers or emulsifiers to avoid abuse. These chemicals

make the isolation of ketamine salt much more challenging with easily accessible house-hold chemicals like bleachers or bicarbonate and minimizes the possibility of direct swallowing with additional chemicals which are designed for external use only.

Lastly, this route offers a user-friendly, non-invasive method for patients which may help them to adhere the treatment for the required period of time. Also, it is suitable for the patients in special conditions like challenges in swallowing or injections and with GI tract problems. Also, patients can stop the dosing or treatment by removing or stopping the application. Overall, these advantages are worth to develop formulations for transdermal ketamine delivery.

Still, transdermal delivery should be carefully formulized to avoid skin irritation, physical instability of the formula, application easiness for the patient which can be worn for a period of time depending on the dose and treatment. Most importantly, the drug incorporated to the formulation should be selected carefully for the aforementioned physiochemical properties and probable chemical reactions with other ingredients in the mixture. Lastly, the drug release and permeation rate should be recorded to obtain a quantitative data of the delivery formula by techniques like diffusion cells and in vitro skin samples.

1.9 Transdermal delivery for ketamine

Ketamine was employed in the form of cream, gel and ointment for the treatment of pain by numerous studies (Gammaitoni, Gallagher, & Welz-Bosna, 2000; Lynch, Clark, & Sawynok, 2003; Lynch, Clark, Sawynok & Sullivan, 2005; Pöyhiä & Vainio, 2006; Quan, Wellish, & Gilden, 2003; Lauretti, Amaral, Dias, Lanchote, & Mattos, 2014).

For the studies utilizing ketamine in the cream form, Russo and Santarelli (2016) formulated a combination analgesic cream with 10% ketamine which was applied 3 times daily and achieved 60% relief in complex pain syndrome patients. Poyhia and Vainio (2006) tried 5, 10, 30 and 50 mg/ml ketamine gels to relieve capsaicin-evoked pain in 9 patients with pain syndrome and achieved significantly reduced scores by 50 mg/ml ketamine gel, in Visual Analog Scale (VAS). They also argued that their dose was relatively low for pain relief. In another placebo-controlled study, Lynch and colleagues (2005) used 4 ml of 1% ketamine cream or 2% amitriptyline and 1% ketamine combination cream to 80 patients with neuropathic pain, 3 times per day for 3 weeks and reported the need of higher doses to obtain therapeutic effect like 4% of active drug. They also previously demonstrated a combination cream with 0.5% ketamine and 1% amitriptyline showed analgesic properties starting on the 3rd day of treatment and continued to increase for 7 days in their randomized, placebo-controlled study on neuropathic pain (Lynch et al., 2003).

For ointment form of ketamine, Ushida and colleagues (2002) applied 0.25% to 1.5% ketamine ointments 3 times per day to 7 patients and all cases achieved rapid pain relief for complex regional pain syndrome, measured by VAS. Another case study of Crowley, Flores, Hughes and Iacono (1998) reported that 10 to 20 mg/ml ketamine ointment provided pain relief in seconds to minutes for the patients with neuropathic and sympathetic pain syndromes. Their results were successfully replicated with same dose and sample size by Gammaitoni, Gallagher, & Welz-Bosna, 2000.

In terms of a transdermal patch with ketamine, Azevedo, Lauretti, Pereira and Reis (2000) achieved a delayed need for an additional analgesic administration up to

4 hours by applying 25 mg/day ketamine patch for 3 days after a gynecology surgery in their placebo-controlled experiment.

There are also studies investigating the pharmacodynamic properties of transdermal and topical application of ketamine (Bassani & Banov, 2016; Kubota et al., 2018; Wang et al., 2020). For instance, Bassani and Banov (2015) formulated a 5% w/w ketamine cream with two different vehicle bases of Lipoderm, and achieved maximum concentrations 6 to 10 hours after the application in vitro. In the same study, they used other analgesic agents like gabapentin, clonidine and baclofen and reported that ketamine resulted in the highest absorption rate compared to other agents with a peak concentration in 6 to 10 hours by being diffused to all layers of skin measured by diffusion cell system and chromatography analysis. They pointed out the availability of these agents to be delivered via transdermal systems with successful absorption rates.

Overall, ketamine has been used in pain therapy for a long time in transdermal and topical formulations in the forms of cream, gel, patch or ointment. Some of the studies reported that the effect of dermal administration of ketamine can act on the NMDAR to exert pain relief with both a central and peripheral effect (Azevedo et al., 2000; Poyhia et al., 2006).

In addition to the use of ketamine in pain therapy, potential administration of ketamine through skin have only gained attention recently which will be discussed in the next section.

1.10 Transdermal delivery in depression

The only Food and Drug Administration (FDA) approved antidepressant with a transdermal delivery system is selegiline (STS; Emsan). Bodkin and Amsterdam

(2002) conducted the first clinical trial with transdermal selegiline system with 177 adult MDD patients who were applied 20 mg daily selegiline patches for 6 weeks, on their upper arm each morning. Significant differences on depression scales HDRS and MADRS were achieved with 46% and 79% improvements, respectively. The side effects were application site reactions like redness, itching and rash. Recently, Lu and colleagues (2020) achieved a clinical improvement for TRD by utilizing a combination of IV ketamine and selegiline patch. There are also successful transdermal systems for neurological disorders like Parkinson's and Alzheimer's Disease (Giladi et al., 2007; Winblad et al., 2007).

The approval of intranasal spray in 2019 launched a new period for ketamine to be delivered via different routes like transdermal delivery with two recent studies (Courtney et al., 2020; Bhattaccharjee et al., 2020). For the first time, Courtenay and colleagues (2020) constructed a transdermal hydrogel system of esketamine and achieved a 24-hour sustained delivery of 30 to 100 mg drug for TRD treatment. They utilized microporation as the physical enhancement which form tiny pores on the skin to increase the efficacy and speed of drug delivery. They measured plasma concentration levels which were maintained at a detectable level from 1.5 to 26 hours following the patch application.

Bhattaccharjee and colleagues (2020) replicated the microporation-based transdermal delivery which works with the same logic of IV ketamine infusion of 0.5 mg/kg subanesthetic dose for TRD treatment. They also included a transdermal syem for brexanolone which is a glutamate-based antidepressant for post-partum depression. These two studies were the first attempts of delivering ketamine transdermally for depression treatment. Their success emphasizes the suitable nature of ketamine for for this route of delivery and applicability of this delivery.

1.11 Present study

The common disadvantages of classical antidepressants like their delayed onset and patients without therapeutic response directed the attention to agents with different action mechanisms for pathophysiology of depression. Ketamine became one of these agents which gained attention of the literature with its glutamatergic action mechanism, rapid onset nature and suitable molecular chemistry for many alternative routes of delivery. However, the chemically ideal nature of ketamine became both an advantage and a disadvantage as it demands serious effort for finding the best one.

Transdermal or topical delivery of ketamine is the most recent administration route after the use of intranasal spray launched in 2019 for depression, by being more user-friendly and with relatively better metabolic processing through skin permeation. Up to my knowledge, there are no studies except two studies which formulated transdermal esketamine for TRD with physical enhancement methods in terms of investigating transdermal ketamine on MDD (Courtney et al., 2020; Bhattaccharjee et al., 2020).

Considering the growing literature on this alternative method of delivery, the present study aims to show the preclinical performance of a shea butter-based ketamine ointment in behavioral despair, which is the rodent model of clinical depression. Here, it is hypothesized that the repeated application 5% shea butter-based ketamine ointment for 2 days twice per day ameliorates behavioral despair in rats compared to the vehicle group without causing a measurable change in locomotor activity. For the EPM as an anxiety assessment, a two-tailed hypothesis was generated to see the relationship between transdermal ketamine and its anxiety-related effect on rats due to the controversial findings of previous studies and the absence of literature

employing ketamine in a transdermal formulation to assess its effect on anxiety. The previous findings will be discussed in the last chapter.

Behavioral despair has the common symptom of physical activity reduction with clinical depression which was measured by the Forced Swim Test (FST) conceptualized by an immobility behavior (Unal & Canbeyli, 2019). To eliminate the possibility of an increase in general locomotor activity caused by ketamine, the Open Field Test (OFT) is employed which was hypothesized to be the same between the experimental groups. Contributing to the transdermal antidepressant potential of ketamine can deepen the knowledge on glutamatergic pathway of depression with ketamine's unique action mechanism through skin and offer more convenient methods for depressive patients with different conditions of health and depression types.

CHAPTER 2

MATERIALS AND METHODS

2.1 Subjects

Adult male Wistar rats (4 to 8-month-old, 313±60 g; n = 14) were housed in individual cages (21 ± 1 °C; ~50% humidity; 12:12 day/night cycle with lights on at 8:00) and had *ad libitum* access to food and water. Six animals were used for pilot ointment application studies and fourteen animals were used for the experimental study. All procedures were carried out by licensed experimenters with the approval of the Boğaziçi University Ethics Committee for the Local Use of Animals in Experiments.

2.2 Ointment Preparation

An original ketamine ointment was formulized by utilizing shea butter (Nu-Ka, Antalya, Turkey) as the oily phase and ketamine hydrochloride salt solution (Arion Pharmaceuticals, Istanbul, Turkey) as the aqueous phase. Shea butter is a natural fat extracted from the kernels of an African tree *Butyrospermum Parkii*. The melting point of shea butter is around 36.6 °C which is near to the adult body temperature. It has natural emulsifying and moisturizing properties with its wide-range fatty acid and antioxidant vitamin content. In addition to these, shea butter acted as a permeation enhancer in different formulations including methotrexate and metoclopramide as the active drugs (Augustina, Okhuelegbe and Ikhuoria, 2020; Sapino, 2017). Thus, shea butter was selected as a multi-functional and biocompatible drug-carrier phase for the ointment. See Figure 5.

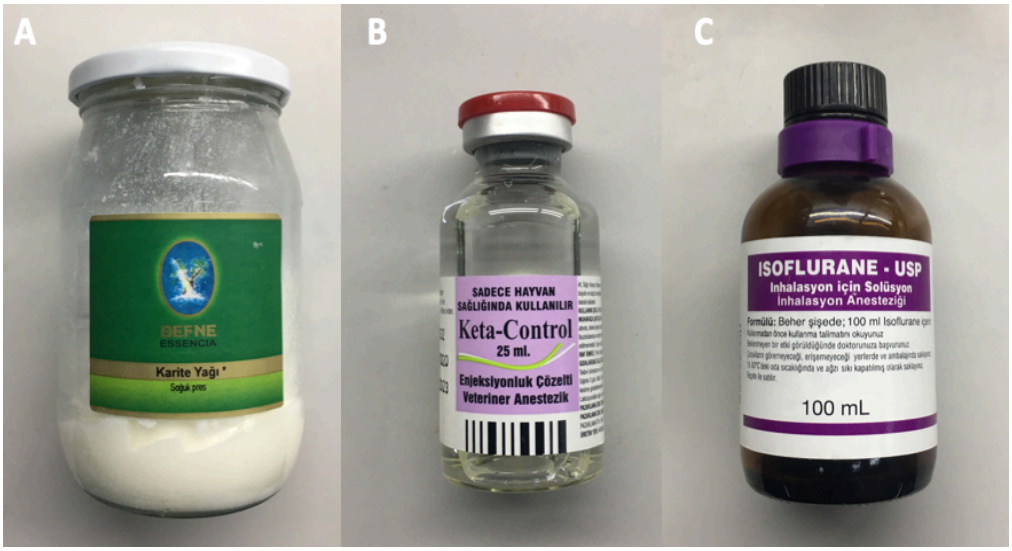

Figure 5. Chemicals used in the present study
Note: Shea butter used as the oily base of the ointment (A). Ketamine hydrochloride solution was used as the drug in the ointment (B). Isoflurane was used to induce short-term anesthesia (C)

Shea butter exists as a greasy solid at room temperature and it was melted by double-boil method. The temperature of the double-boil was kept under 50 °C to avoid any chemical alteration. As the first step of ointment preparation, a large sum of 30 gram shea butter was melted by double-boil method on a heating plate. Melted shea butter was casted onto aluminum foils into individual ointment amount of 2.8 grams to make them ready for ketamine or shea butter addition to complete the formulation, according to the experimental group. After achieving the semi-solid texture of casted shea butter, the aqueous phase of ketamine hydrochloride solution was added slowly. The final active drug concentration was 5% weight to weight of total oily phase of shea butter for the ketamine ointments. Final formulations of ketamine and vehicle ointments weighed as 4.2 grams either including only shea butter or shea butter with ketamine hydrochloride. See Figure 6. The two phases were mixed thoroughly on a magnetic stirrer for 20 minutes until a virtually homogeneous mixture is achieved.

Figure 6. Double-boil method

2.3 Ointment Application

Animals in the ketamine group received 5% ketamine ointment prepared in 2.8 grams of oily base shea butter and the vehicle group received the same total amount of vehicle ointment including only shea butter (Figure 7). Ointment applications were done 2 times per day (morning, 9:00-10:00 and noon, 13:00-14:00) for 2 days. For the first ointment application, animals were briefly anesthetized by putting the animals in a box with an isoflurane soaked gauze patch to shave their mid-dorsal region with a trimmer. The time window of anesthesia was 15 to 20 minutes maximum per animal. Subsequent applications were done without anesthesia. The application site was covered with an adhesive plaster to avoid leakage until the next dose of ointment (see Figure 8).

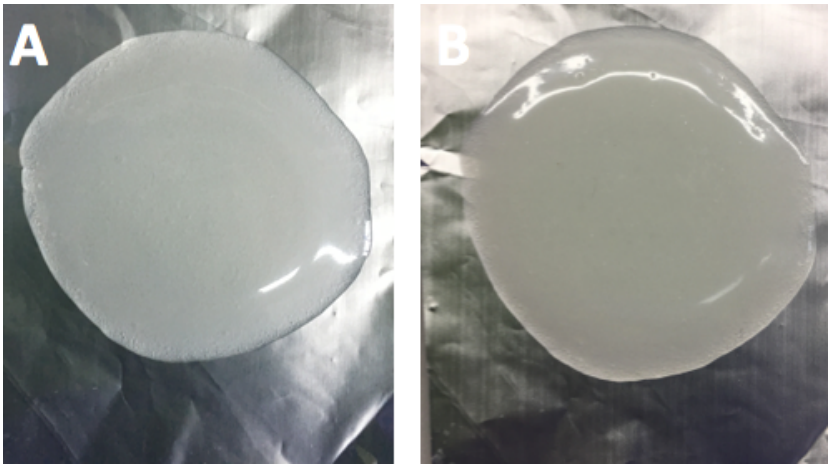

Figure 7. Ketamine and vehicle ointments
Note: The doses of 5% Ketamine ointment (A) and vehicle ointment (B) ready to be applied to the dorsal back of rats

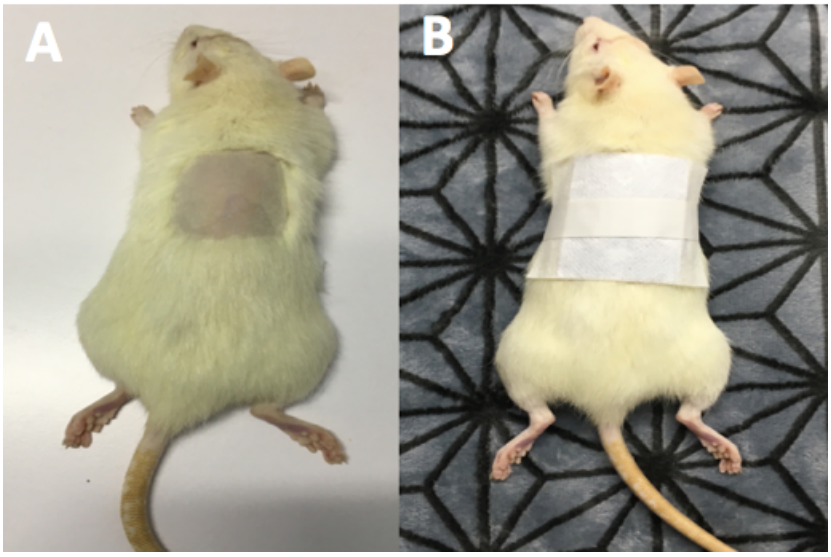

Figure 8. The trimmed dorsal back of the rat and flaster application
Note: The dorsal back of the rat was shaved for ointment application (A). To avoid any leakage adhesive plaster was applied to the application area (B)

2.4 Behavioral experiments

2.4.1 The Forced Swim Test (FST)

Behavioral despair is a rodent depression model which is assessed by the classical protocol of the FST (Porsolt, Le Pichon, & Jalfre, 1977; Slattery & Cryan, 2012).

This measurement is based on ‘’immobility’’ behavior which is defined as the absolute lack of movement except of keeping the nostrils above the water for breathing. For this purpose, each animal was subjected to two consecutive tests with an interval of 24 hours with 15 and 5 minutes long, respectively.

In each test, animals were placed individually into a Plexiglas cylinder with 45 cm of height and 30 cm of diameter filled with 23°C± 1 °C water up to 25 cm. See Figure 9. Each subject was familiarized to the testing room for 5 minutes before each trial in their own cages. The temperature of water was refreshed and kept within the 23°C± 1 °C range, for each trial.

The preclinical model of depression is ‘’behavioral despair’’ in rodents. The immobility behavior in this model represents the physical activity reduction in clinical depression, as one of the most parallel symptom between these models (Unal & Canbeyli, 2019). Thus, an increase in the immobility score in the second test compared to the first test is an indicator of behavioral despair. The naïve rats are expected to show an immobility (not showing any coping behavior to escape from water like swimming or struggling) after an inescapable stress condition with the first test session of the FST.

For this purposes, experimenters who were blind to the experimental conditions were present in the room in each session. At the end of each test, the animal was taken into a single cage to dry under an heating lamp located 30 cm above the cage after towel drying. Each session was recorded by a video camera (HDR-CX240 Handycam, SONY, CA) for the offline analyses of immobility, swimming and struggling behaviors. Swimming was coded as the behavior for horizontal movement in the water and struggling was coded as the behavior of a

vigorous attempt to climb and escape vertically from the water. The coding was done with identical chronometers for each experimenter (ZSD808 Water-resistant chronometers).

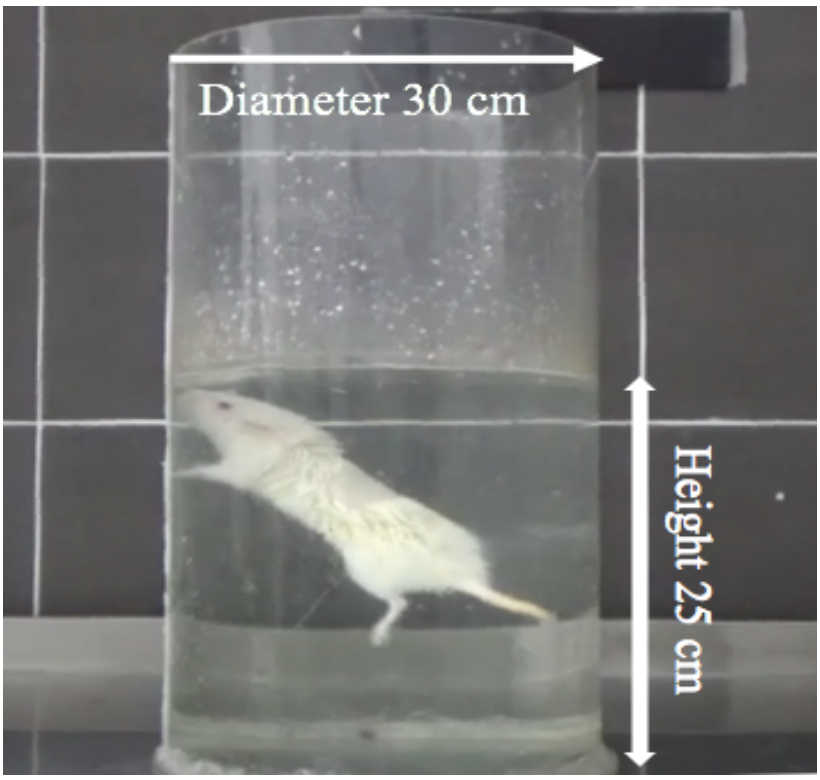

Figure 9. FST setting

2.4.2 The Open Field Test (OFT)

This test is a well-recognized paradigm for exploratory behavior, anxiety and locomotor activity in rodents and it is also used for the confirmation of other behavioral tests to minimize the ‘’false positive’’ results due to mobility behavior except from the aim of experimental manipulation (Hall & Ballachey, 1932; Walsh & Cummins, 1976).

The test is based on the phylogenic tendency of rodents to display fear or anxiety-mediated responses towards stimuli like unknown open area or bright light exposure. For this purpose, a square-shaped maze is divided into squares and coded

for theoretical regions as center/internal and the periphery 10 cm inside the walls. The height of the dark walls of the maze were 30 cm (see Figure 10).

The index of anxiety is based on the total time spent along the walls and in the corners which was named as ‘’thigmotaxis’’. In terms of exploratory behavior and anxiety, rodents stand temporarily on their hind legs named as ‘’rearing’’ (Lever, Burton, & O’Keefe, 2006). To measure these concepts, the number of rearing was coded during the task. Additionally, fecal boli as a physiological sign of anxiety was recorded after each session.

This test was done the next day of last FST session with a counterbalanced order. Each animal was dropped to the center of the maze and recorded by video for 5 minutes. Before each trial, animals were familiarized to the testing room for 5 minutes in their own cages. At the end of each session, maze was wiped with 70% alcohol solution to avoid any odor left from the last subject and left for drying.

Figure 10. OFT setting

2.4.3 The Elevated Plus Maze (EPM)

The EPM is a validated anxiety-related behavior measurement test in rodents (Campos, Fogaça, Aguiar, & Guimarães, 2013; Walf & Frye, 2007). It is based on the anxiety-inducing effect of being in a new environment which is elevated (Wall & Messier, 2001). For this purposes, a plus-shaped maze with open and closed arms was used. The maze has two open arms with 10 cm of width and 50 cm of length. The arms are 50 cm above the ground. The opens arms are also enclosed by a wire fence where the perpendicular closed arms are enclosed by non-transparent walls with the height of 40 cm. Each rat was dropped to the center of maze by facing an open arm for 5 minutes. The reason of the placement towards open arm is that they innately fear open and elevated spaces (Montgomery, 1955; Pellow, Chopin, File, & Briley, 1985). Before each trial, animals were familiarized to the testing room for 5

minutes in their own cages. After each use, maze was wiped with 70% alcohol solution and left for drying.

The indicators of anxiety are the amount of time spent on closed-arm, increased freezing behavior, reduction in the entry to open-arm, decreased exploration near the center and the increase in the number and time of smelling by standing. Another physiological sign of anxiety is defecation as an autonomous nervous system reaction to fear, anxiety or intense excitement (Ramos, Pereira, Martins, Wehrmeister & Izidio, 2008). The time spent in open and closed arms and the frequency of entries to the arms are recorded. The measurement is based on the positive correlation between anxiety levels and the time spent in closed arms as well as the frequency of closed arm entrances. Two experimenters who were blind to the experimental conditions coded the time spent in each arm and fecal boli.

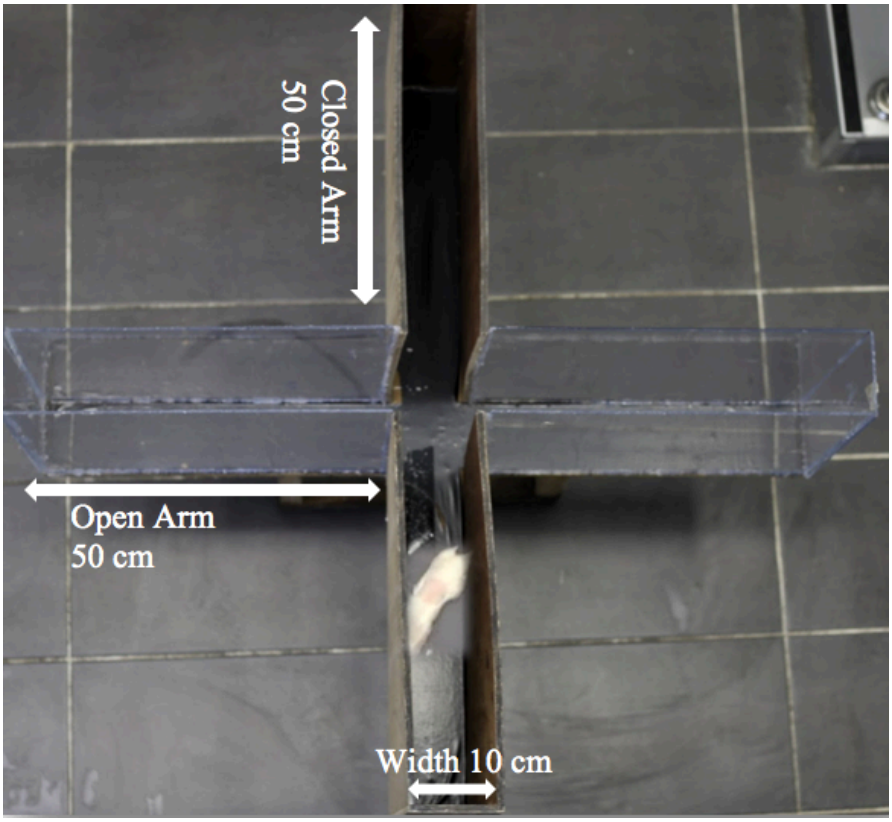

Figure 11. EPM setting

2.5 Experimental Procedure

On the first day, the animals were randomly divided into two experimental groups as vehicle and ketamine which received drug-free shea butter ointment and 5% ketamine ointment, respectively. Both groups were applied 4 doses of ointment in the morning (09:00) and noon (13:00) for 2 consecutive days on shaved dorsal area. Approximately 2 hour after the last dose of ointment, the first session of FST was conducted which was followed by the second test after 24 hours. On the fourth day of the experiment, OFT and EPM were completed respectively. Animals were weighed in the first and last day of the experiment. See Figure 12 for the timescale.

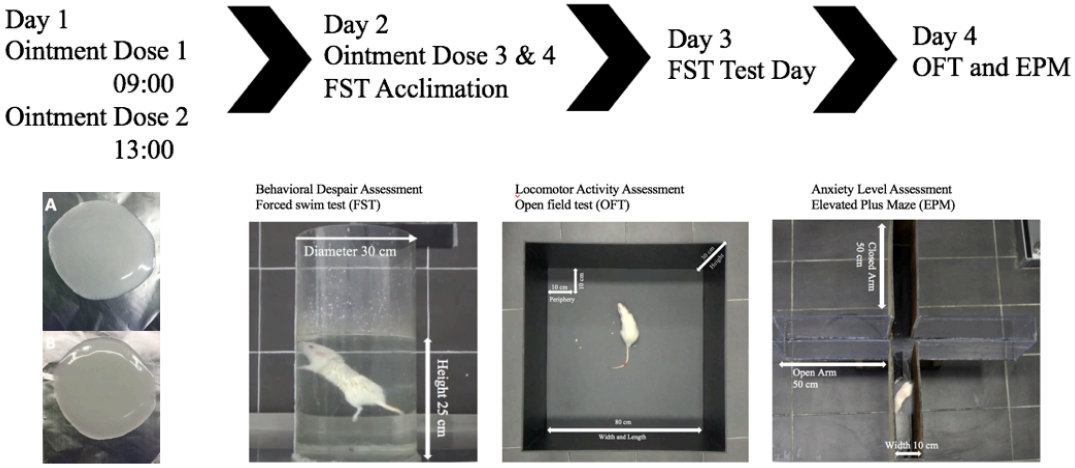

Figure 12. Timescale of the present study
Note: The ketamine ointment (A) and vehicle ointments (B) were applied at the same times on the first and second day.

CHAPTER 3

RESULTS

3.1 Statistical analyses

Independent samples t-test was employed for behavioral test comparisons as the parametric statistical analysis. Paired samples t-test was used for the analysis of weight differences of each group on the first and the last day of the experiment. To control the age as a covariate, 1-way ANCOVA (Analysis of Covariance) was employed for all behavioral tests. All analyses were performed in the Statistical Package for Social Sciences (SPSS, 24.0) and the graphs were designed in Excel (Microsoft Office, 2016) and Adobe Illustrator CC (21.01).

3.2 The FST (Behavioral Despair Assessment)

In the assessment of behavioral despair in the FST, there is a significant difference between the means of immobility of ketamine (M = 22.90, SD = 8.53) and vehicle group (M = 64.53, SD = 25.84), $t(12) = 4.04$, $p = 0.002$. The results show that the 5% ketamine ointment treatment showed an antidepressant effect on the ketamine group, compared to the vehicle group who received drug-free vehicle ointment (see Figure 13). As the sample consisted of 4-months old and 8-months old rats, age was controlled as a covariate in an analysis of covariance. The effect of age was not significant for the immobility scores of the ketamine and vehicle group, $F(1,11) = 2.86$, $p = 0.12$. Additionally, fecal boli counts were not significantly different between the ketamine (M = 4.29, SD = 1.80) and vehicle group (M = 4.14, SD = 1.8457), $t(12) = -0.16$, $p = 0.88$.

Swimming and struggling behavior were coded for the second test of the FST to compare vehicle and ketamine groups as coping mechanisms. Consistent with the significantly different immobility scores, ketamine group showed significantly higher scores of swimming (M = 221.29, SD = 19.70) when compared to vehicle group (M = 183.57, SD = 27.32), $t(12) = -2.96$, $p = 0.01$. The difference between the struggling scores were not significantly different between ketamine (M = 39.86, SD = 11.89) and vehicle group (M = 33.14, SD = 9.62), $t(12) = 1.161$, $p = 0.268$.

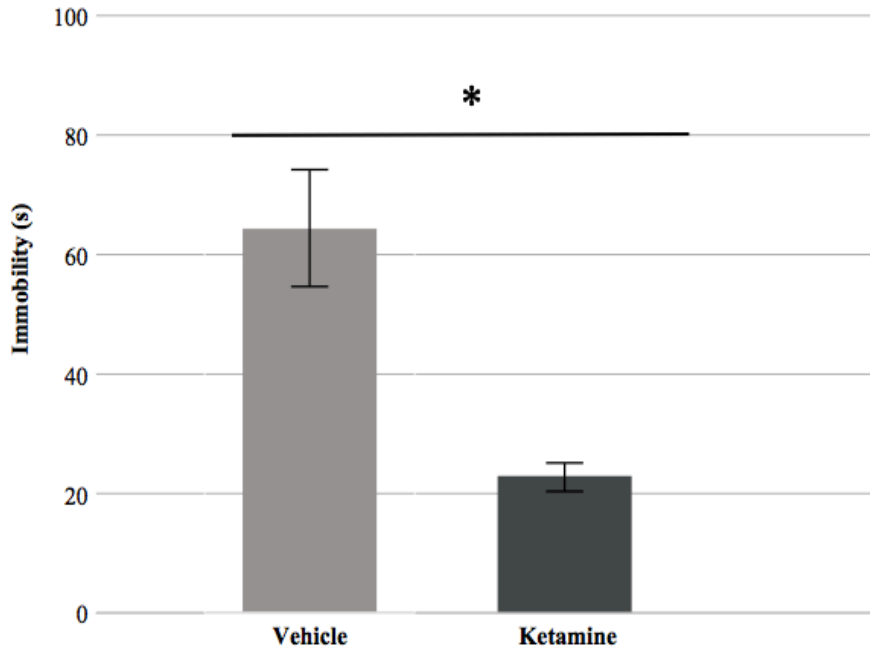

Figure 13. Immobility scores of ketamine and vehicle group in the FST
Note: The significantly lower immobility score of ketamine group shows the antidepressant activity of the ketamine ointment. Asterisk indicates a statistical significance at the level of α = 0.05. Error bars denote the standard error of the mean (SEM)

3.3 The OFT (Locomotor Activity Assessment)

Locomotor activity was assessed by the comparison of mean locomotor activity in the OFT. There was no difference between the means of locomotor activity of

ketamine (M = 65.00, SD = 22.49) and vehicle group (M = 54.14, SD = 21.84), $t(12)$ = -0.92, p = 0.38. OFT is also used as a control task to analyze whether the antidepressant induces a general increase in locomotor activity and a subsequent ‘’false’’ positive result in the FST (Borsini & Meli, 1988). The non-significant difference in locomotor activity minimizes this possibility and confirms the antidepressant function of ketamine ointment. See Figure 14.

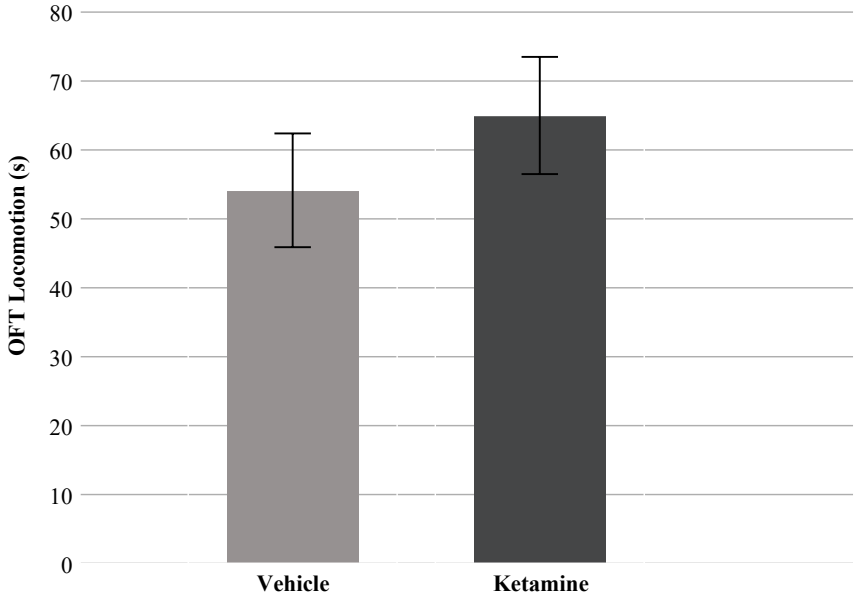

Figure 14. Locomotor activity of ketamine and vehicle groups in the OFT
Note: The non-significant difference between the locomotor activity of two groups shows that the ketamine ointment did not cause an increase in the general mobility in ketamine group. Error bars indicate SEM

There was no difference in the time spent in center area between the ketamine (M = 20.86, SD = 14.16) and vehicle group (M = 23.14, SD = 17.91), $t(12)$ = 0.26, p = 0.80. The time spent in the periphery was also non-significant between the ketamine (M = 580.14, SD = 14.60) and vehicle group (M = 578.57, SD = 19.31), $t(12)$ = -0.17, p = 0.87. The time spent in the center area was significantly different for two groups when age was controlled, $F(1, 11)$ = 1.610, p = 0.231.

The number of exploratory rearing behavior between the groups was not different between ketamine (M = 15.43, SD = 4.04) and vehicle group (M = 17.57, SD = 6.83), $t(12) = 0.71$, $p = 0.49$. Fecal boli is another indication of anxiety in rodents which were counted after each session. In the OFT, the difference in the number of boli was non-significant between the ketamine (M = 4.86, SD = 3.18) and vehicle group (M = 5.71, SD = 3.15), $t(12) = 0.51$, $p = 0.62$.

3.4 The EPM (Anxiety Level Assessment)

The anxiety-like behavior in ketamine and vehicle group was assessed by the OFT and EPM tests which measure anxiety levels with thigmotaxis and arm preference, respectively. In EPM, the percentage of closed arm preference was not significantly different between the ketamine (M = 48.14, SD = 45.58) and vehicle group (M = 24.86, SD = 36.91), $t(12) = -1.05$, $p = 0.31$. Ketamine (M = 157.00, SD = 134.42) and vehicle group (M = 215.57, SD = 117.18) did not spend significantly different amount of time in the open arm, $t(12) = 0.87$, $p = 0.40$. See Figure 15. Similarly, there was no difference between the ketamine (M = 144.71, SD = 137.10) and vehicle group (M = 74.71, SD = 110.92) for the time spent in closed arm, $t(12) = -1.05$, $p = 0.31$. See Figure 15.

The difference in the time spent in open arm was non-significant for both groups when controlled for the age of subjects, $F(1, 11) = 0.163$, $p = 0.694$. Similarly, the time spent in closed arm was non-significant for both groups controlling for age, $F(1, 11) = 0.326$, $p = 0.579$.

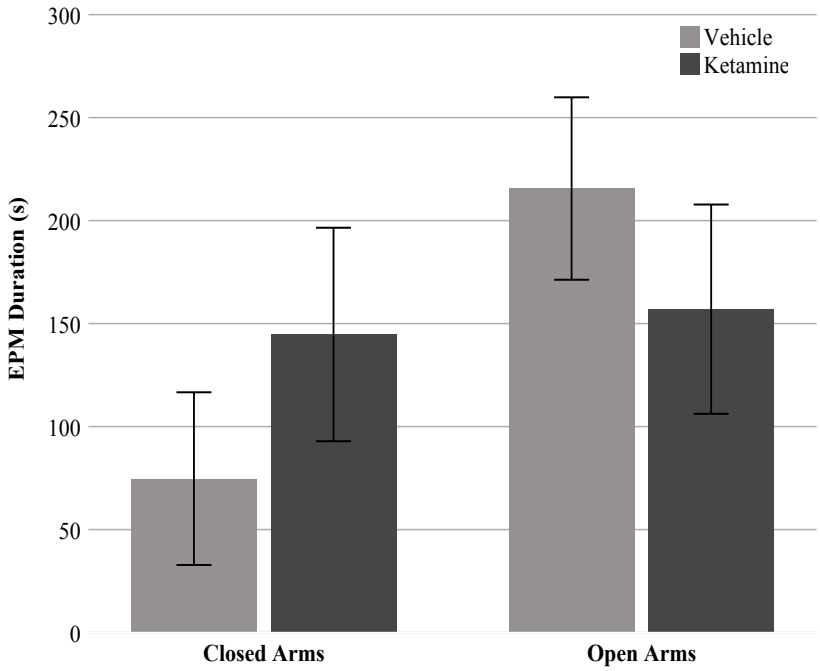

Figure 15. Time spent in closed and open arm of the EPM
Note: The difference in the duration of time spent in open and closed arms of the EPM was not significant between the two experimental groups. This result shows that ketamine and vehicle groups have no difference in their levels of anxiety

The tendency to spend more time in the closed arms indicates a higher level of anxiety in rodents and the results of EPM show that the two groups did not differ on their levels of anxiety with no difference on their arm preference. The results of the EPM is consistent with the aforementioned results of the OFT where the time spent in the periphery of the maze was not different between the groups. Thus, 5% ketamine ointment did not have an anxiolytic effect in rats but it alleviated behavioral despair as an antidepressant agent. The fecal boli count as a physiological nervous system sign to anxiety was not significantly different for the ketamine (M = 1.43, SD = 1.62) and vehicle group (M = 2.57, SD = 2.23), $t(12) = 1.10$, $p = 0.29$.

Ketamine group weight difference was not significant on the first (M = 312.00, SD = 61.61) and last day (M = 302.00, SD = 51.91) of the experiment, $t(6) = 1.08$, $p = 0.32$. Similarly, vehicle group's weight difference was not significant

between the first (M = 313, SD = 60.04) and the last day (M = 303, SD = 51.70) of the experiment, $t(6) = 1.15$, $p = 0.29$.

The overall results showed that an acute 2-day 5% ketamine ointment treatment ameliorates behavioral despair in rodents measured by the FST by controlling their locomotor activity. Additionally, the agent had no impact on anxiety levels measured by two different experimental designs focusing on thigmotaxis and preferences of elevated open or closed arm.

CHAPTER 4

DISCUSSION

The present study showed that a 2-day application of a shea butter-based 5% ketamine ointment two times per day provides a therapeutic response in the rodent model of depression, behavioral despair. The behavioral results were assessed by the FST, OFT and EPM in terms of immobility, locomotor activity and anxiety levels respectively. The ketamine group showed significantly less scores in immobility compared to vehicle group in the FST. Two groups did not differ in their general locomotor activity in the OFT, which shows that the ketamine ointment did not induce an increase in locomotor activity as a potential bias for immobility scores of each group. In the EPM, both experimental groups showed similar levels of anxiety assessed by the times spent in the open and closed arms of the maze. Overall, this findings illuminate the potential of ketamine's use in transdermal formulations to act as a rapid-acting antidepressant in acute treatments.

The FST was emphasized as a gold standard for the measurement of behavioral despair based on the physical activity reduction as a common symptom of clinical depression (Unal & Canbeyli, 2019). However, it has been criticized as a potential learning paradigm rather than behavioral despair for rodents (De Pablo, Parra, Segovia, & Guillamón, 1989; West, 1990). Keeping the possibility of a behavioral acquirement during the FST, it should be noted that this test is primarily utilized for its reliability to measure an antidepressant's efficacy (Unal & Canbeyli, 2019). This test can differentiate the mobility-inducing agents which indicates its power to measure ''psychomotor retardation'' as a physical symptom rooted from cognitive deficits like depression. It is also important to assess bottom-up patterns of

sensorimotor system in depression, in addition to its well-known top-down processes in the fronto-limbic region of the human brain. Thus, FST was employed to measure the result of ointment as one of the most reliable behavioral paradigm of recent neuroscience literature.

Rodents are innately social animals and social isolation causes stress-related behavioral and neurobiological responses (see for a review, Mumtaz, Khan, Zubair & Dehpour, 2018). In the present study, 7-days single cage housing of the subjects represented an additional stressor to the water exposure in the FST and intense handling during ointment application. To avoid any confound, each ointment application session was recorded with a chronometer to standardize the handling time for each subject. The present study did not employ any specific stress models like chronic mild stress or chronic unpredictable stress to induce depression, in addition to the aforementioned stressors and FST. Thus, future studies can illuminate the impact of transdermal ketamine in different types of stress models to assess different aspects of ketamine's antidepressant efficacy.

The present study employed an acute treatment of transdermal ketamine with 4 doses applied in 2 days. To analyze the cognitive side effects and antidepressant efficacy of ketamine, future studies can investigate its effect in chronic treatments and with different dose regimens. In addition to this, future studies can investigate the depression symptom of anhedonia by employing a transdermal ketamine formulation as an additional behavioral test battery.

The assess the effect of transdermal ketamine on anxiety, the OFT and EPM were employed in the present study. The results of these tests showed that ketamine and vehicle group were not significantly different in their time of thigmotaxis in the OFT and in their time spent in the closed arm of the EPM. As mentioned previously,

two-tailed hypothesis was generated for the results of these tests due to the following controversial findings and absence of any reference literature on transdermal application of ketamine.

There are studies that report the anxiolytic function of ketamine in clinical studies with 0.5 mg/kg ketamine infusions and single oral administration (Ibrahim et al., 2012; Irwin & Iglewicz, 2010; Irwin et al., 2013). Similarly, a preclinical study of Engin, Treit and Dickinson (2009) reported that ketamine significantly increases the time spent in the open arm of the EPM when compared to its positive control anxiolytic diazepam without increasing general locomotor activity. In addition to their behavioral finding, they showed that both single intraperitoneal injection of 10 mg/kg and 50 mg/kg of ketamine induce a neurophysiological signature of a frequency decrease in hippocampal theta oscillations similar to the oscillatory profile of diazepam and the remaining class of classical anxiolytics. Fraga and colleagues (2018) also reported the anxiolytic effect of ketamine in mice measured by the EPM, OFT and light/dark preference test. Still, there is no consensus on anxiolytic property of ketamine with multiple null results (Becker et al., 2003; Hayase, Yamamoto, & Yamamoto, 2006).

Interestingly, Silvestre and colleagues (2002) found an anxiogenic effect of 35-days chronic administration oral ketamine measured by the EPM without affecting the activity in the OFT. Another interesting finding was from the pivotal study of Krystal and colleagues (1994) which showed an increase caused by high dose (0.5 mg/kg) ketamine administration on anxiety levels where low dose (0.1 mg/kg) ketamine shows anxiolytic effect in a clinical cohort, compared to the baseline anxiety levels measured by VAS.

To my knowledge, the relationship between ketamine and anxiety has not been investigated via transdermal delivery before. Thus, it is important to note that the aforementioned studies investigated the oral and injection methods as methods of ketamine administration. In the present study, there was no anxiolytic or anxiogenic effect of transdermal ketamine on the ketamine and vehicle group. This can be explained by a sustained bloodstream distribution through transdermal delivery of a relatively high dose, compared to the aforementioned studies. The steady distribution of 100 mg per dose ketamine into bloodstream can be a factor to explain our null result in terms of anxiety levels in the EPM. In conclusion, there is a need of clinical and preclinical findings to reach a consensus on ketamine's effect on anxiety with various doses and administration methods.

The present study utilized a dose of 100 mg ketamine hydrochloride salt in 2.8 grams of shea butter oily base to achieve a 5% w/w ointment. It was mentioned that the ideal dose for a transdermal delivery was under 10 mg per day. The reason utilizing a higher dose was that the permeation rate was not recorded by diffusion cells or any other technical method in the present study. For example, in a transdermal patch study utilized ketamine in the formulation measured the absorbed amount of ketamine as 35% of the loaded total drug (Kubota et al., 2018). Thus, the absorbed amount of ketamine was not expected to exceed 50% of 100 mg total dose.

Supporting this, 100 mg ketamine should have resulted in an anesthetic state for the rodents if 100% skin permeation was achieved. The virtually absence of psychotomimetic side effects or an anesthesia state after multiple applications indicated a moderately suitable dose permeation following the treatment. Additionally, shea butter was used for the first time in a ketamine delivery formulation which limited the previous knowledge of any permeation rates.

Lastly, intranasal esketamine delivery was applied with a dose of 100 mg (applied in separate applications to reach the total dose) despite of its acute side effects and direct delivery to brain with bypassing blood-brain barrier (Galvez et al., 2018). Similarly, there are transdermal patch systems which employed much higher doses like 160 mg nitroglycerin as the systems can be worn for a proper treatment period (see for a review, Pastore et al., 2014). Overall, there are multiple reasons to select a high dose in transdermal delivery which was formulated for the first time by the present study. Still, future studies can enhance the ingredients with plasticizers and record the permeation rates to improve the drug delivery design which remains beyond the scope of this thesis.

Given that depression treatments require a period of time to show optimal efficacy, single and repeated ketamine administration has become a popular topic of discussion recently. Weston, Fitzgerald ad Watson (2021) reviewed 24 rodent studies which used repeated ketamine administration measured by the FST. They concluded the outstanding effect of repeated ketamine administration in preclinical studies when compared to single dose. However, they also underlined the variability in literature showing both immobility increases and decreases in a dose-dependent manner, after repeated treatment. Here, a 2-day repeated ketamine administration was done with 4 doses of ketamine and vehicle ointments. Depending on the non-significant locomotor activity and significant immobility scores, the present study contributed to the part of literature which indicates an immobility decrease following repeated administration. Still, there is an urgent need for consensus on an optimal therapeutic time window for ketamine.

As the presence of hair can hamper the ketamine ointment permeation and classical ketamine-induced anesthesia cannot be used, isoflurane gas anesthesia was

chosen in the present study for a fine shaving of dorsal back hair during the first ointment application. However, Antila and colleagues (2017) demonstrated that a single 30-minute isoflurane treatment shows antidepressant effect 6 days after anesthesia, in a LH paradigm in mice, with an involvement of BDNF receptor and mTOR signaling activation similar to ketamine. Also, Brown and colleagues (2018) showed that 2-hour long isoflurane administration reversed LH depression model supported with electrophysiological recordings. In contrast, Herbst and colleagues (2019) investigated the effect of anesthetics preceding rodent behavioral tests like the FST and EPM. They found that single 20-minute administration of isoflurane or thiopental anesthesia do not affect the anxiolytic effect of diazepam in the EPM and the antidepressant effect of imipramine in the FST 2 hours and 7 days after the administration of anesthesia.

Here, a brief isoflurane anesthesia was used only for the first ointment application which has a temporal gap of 52 hours between the first behavioral test (FST acclimation test). Additionally, the optimal dose to induce isoflurane anesthesia was 4.2 ml isoflurane dropped on gauze patch in a 7 liter box. To minimize the risk of anesthesia and possible behavioral effects, we optimized the anesthesia by using 3 ml isoflurane for a 7 liter box, which is below the recommended dose of isoflurane in the protocols. Lastly, the total duration of anesthesia was a maximum 20 minutes which is shorter than the aforementioned literature. Also, all animals were physically active and awake at the end of the anesthesia.

Considering these factors and controversial findings mentioned above, it was hypothesized that the possible antidepressant effect induced by isoflurane was less likely to occur under these conditions. Still, there is a need for further investigations with more standardized dose and administration method (inhalation anesthesia

equipment or drop-jar method).

The sample of the present study consists of 14 male Wistar rats as mentioned in Chapter 2. There are studies reporting a sex difference on the antidepressant effect of ketamine (Franceschelli, Sens, Herchick, Helen & Pitychoutis, 2015; Thelen et al., 2016). Franceschelli and colleagues (2015) demonstrated that antidepressant properties of ketamine lasts longer for male mice up to 7 days of treatment in a chronic mild stress model of depression, without affecting their locomotor activity in the OFT. They also reported a higher reactivity for the earlier therapeutic effects of ketamine in the first 30 minutes and the 24th hour of injection and showed a reduction in locomotor activity in female mice. Similarly, Thelen and colleagues (2016) showed that repeated 10 mg/kg ketamine treatment for 3 weeks induced anxiogenic and depressive states in female mice, measured by the OFT and FST. Interestingly, the same treatment induced antidepressant effect in male counterparts. The sex-oriented differences were not limited to behavioral results but consistent with neurochemical impact of ketamine in terms of the hippocampal protein and serotonin levels.

Considering that the present study applied ketamine for 2 days (acute) and through dermal application, these sex differences need to be clarified with further studies using the same delivery route by using sex-type homogeneous samples with Wistar rats. The standardization of the sample types can be beneficial for future sample selections of ketamine treatments both in preclinical and clinical levels.

Transdermal administration is a multi-faceted technique due to the factors affecting skin absorption like the physiochemical properties of ingredients in the formula, the type of skin and pre-treatments before drug administration and the

environmental factors (Godin & Touitou, 2007). Therefore, each transdermal formulation and experimental design have specific advantages and disadvantages.

Regarding the toxic and unsustainable nature of artificial chemicals in transdermal formulations, the present study employed natural fat shea butter as the drug-carrier phase of ointment and as an emollient to keep the skin moisture and an emulsifier to keep oily and aqueous phase together. Shea butter showed superior physiochemical properties and drug release profile in comparison to other natural oils like eucalyptus (Augustina, Okhuelegbe, 2020). It was also reported as an ideal vehicle for optimal skin penetration, drug release and stability (Femi-Oyewo et al., 2013). With its unusually high levels of triterpene molecules, it is a natural anti-inflammatory and anti-carcinogenic agent (Akihisa et al., 2010). Depending on multi-functional nature of shea butter, no additional emulsifiers or permeation enhancers were employed in the present study. Still, this formulation can be stabilized to keep its homogeneous semi-solid nature with the addition of emulsifiers and permeation enhancers like soy-lecithin to achieve a more convenient self-administration at clinical level.

Porcine ear skin is the second most similar type of skin compared to humans after the primate skin which is under restriction for experimental use (Gray & Yardley, 1975; Jacobi et al., 2007). Shaved rat skin represents the most structurally similar skin type to human among rodents (Maibach & Wester, 1989). Rodent skin shows 11 times higher drug or chemical permeation compared to human skin (van Ravenzwaay & Leibold, 2004). Based on experimenter observations, the drug was permeated through shaved dorsal rat skin successfully and rapidly in the present study. Future studies can utilize other skin types and diffusion cells to measure the drug delivery quantitatively for further improvements of the formula.

Another important point is the use of pharmaceutical terminology of ‘’transdermal’’ and ‘’topical’’ administration of active drugs which differ in their site of action. These terms are usually used interchangeably in the literature, mostly due to a terminological confusion, mostly. This confusion is rooted in the fact that all formulations which are applied on the skin are generally referred to as ‘’topical’’ in general (Wilbur, 2017). However, there is a difference in their target of action.

‘’Transdermal’’ administration refers to a drug delivery which is initially absorbed by the skin layers and finally reaches to blood circulation for a distribution in the whole body. ‘’Topical’’ administration refers to a localized therapeutic effect which may not necessarily transfer the drug to the layer of dermis which contain capillary vessels of the bloodstream with the aim of therapeutic action on the site of application. See Figure 4 for transdermal and topical permeation through skin layers. Thus, the present study used the term of ‘’transdermal’’ as it is focused on the antidepressant properties of ketamine ointment upon its systematic distribution in the brain. It should be noted that ketamine formulations used in pain therapy used these two terms interchangeably as the conditions of pain can be both site-specific (topical) or affecting the whole body which necessitates a transdermal diffusion. The studies discussed in the Chapter 1 were mentioned to demonstrate the successful skin absorption of ketamine with minimal or no side effects and the potential for its use in other disorders through same route of delivery.

Overall, transdermal delivery holds a prominent and realistic potential for chemically optimal drugs via this route, like ketamine. Ketamine has optimal chemical properties to be delivered through the skin which offers a sustained plasma concentration and minimizes the loss in bioavailability and GI tract irritations. The possibility of overcoming these common shortfalls of classical invasive and non-

invasive delivery methods is an important aspect for all neurological disorder treatments, especially the ones with high rates of discontinuation in treatment.

The present study underlined a novel method for the fast-acting antidepressant ketamine delivery in a natural oily vehicle, shea butter. A basic 5% shea butter-based ketamine ointment successfully ameliorated behavioral despair symptom of immobility in rodents without changing the general locomotor activity and anxiety levels. The absence of a locomotor activity change is a strengthening factor for the antidepressant effect measurement by eliminating the possibility of a false positive result. With all aforementioned limitations, repurposing of a promising antidepressant agent and a user-friendly delivery is valuable for future treatments of depression.

Given the long time with the absence of any novel antidepressants besides intranasal ketamine, glutamate-based antidepressants wait to be therapeutically optimized to launch their own era in depression treatment. Therefore, the synergism of repurposing fast-acting antidepressant ketamine and a highly convenient transdermal delivery can illuminate future studies to focus on novel formulations with higher skin compatibility for a wider range of neuropsychiatric drugs.

REFERENCES

Adams, J. D., Jr, Baillie, T. A., Trevor, A. J., & Castagnoli, N., Jr (1981). Studies on the biotransformation of ketamine. 1-Identification of metabolites produced in vitro from rat liver microsomal preparations. *Biomedical Mass Spectrometry*, *8*(11), 527–538. doi.org/10.1002/bms.1200081103

Akihisa, T., Kojima, N., Kikuchi, T., Yasukawa, K., Tokuda, H., T. Masters, E., … Manosroi, J. (2010). Anti-Inflammatory and Chemopreventive Effects of Triterpene Cinnamates and Acetates from Shea Fat. *Journal of Oleo Science, 59*(6), 273–280.

Altamura, C., Maes, M., Dai, J., & Meltzer, H. Y. (1995). Plasma concentrations of excitatory amino acids, serine, glycine, taurine and histidine in major depression. *European Neuropsychopharmacology*, *5 Suppl*, 71–75.

Altar, C. A. (1999). Neurotrophins and depression. *Trends in Pharmacological Sciences*, *20*(2), 59-62.

American Psychiatric Association. (1980). *Diagnostic and statistical manual of mental disorders* (3rd ed.). Washington, DC: American Psychiatric Publishing.

American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders (*5th ed*)*. Washington, DC: American Psychiatric Publishing.

Antila, H., Ryazantseva, M., Popova, D., Sipilä, P., Guirado, R., Kohtala, S., ... & Rantamäki, T. (2017). Isoflurane produces antidepressant effects and induces TrkB signaling in rodents. *Scientific Reports, 7*(1), 1-12.

Anis, N. A., Berry, S. C., Burton, N. R., & Lodge, D. (1983). The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. *British Journal of Pharmacology*, *79*(2), 565-575.

Augustina, M. O., Okhuelegbe, E. S., & Ikhuoria, A. M. (2020). Transdermal Delivery of Metoclopramide Using Eucalyptus Oil and Shear Butter. *African Journal of Pharmaceutical Research & Development, 12*(2), 208-216.

Autry, A. E., Adachi, M., Nosyreva, E., Na, E. S., Los, M. F., Cheng, P. F., ... & Monteggia, L. M. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, *475*(7354), 91-95.

Avidan, M. S., Maybrier, H. R., Abdallah, A. B., Jacobsohn, E., Vlisides, P. E., Pryor, K. O., ... & Waszynski, C. (2017). Intraoperative ketamine for prevention of postoperative delirium or pain after major surgery in older adults: An international, multicentre, double-blind, randomised clinical trial. *The Lancet*, *390*(10091), 267-275.

Azevedo, V. M., Lauretti, G. R., Pereira, N. L., & Reis, M. P. (2000). Transdermal ketamine as an adjuvant for postoperative analgesia after abdominal gynecological surgery using lidocaine epidural blockade. *Anesthesia & Analgesia*, *91*(6), 1479-1482.

Bassani, A. S., & Banov, D. (2016). Evaluation of the percutaneous absorption of ketamine HCl, gabapentin, clonidine HCl, and baclofen, in compounded transdermal pain formulations, using the Franz finite dose model. *Pain Medicine*, *17*(2), 230-238.

Becker, A., Peters, B., Schroeder, H., Mann, T., Huether, G., & Grecksch, G. (2003). Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*(4), 687–700. doi.org/10.1016/S0278-5846(03)00080-0

Bessa, J. M., Ferreira, D., Melo, I., Marques, F., Cerqueira, J. J., Palha, J. A., Almeida, O. F., & Sousa, N. (2009). The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Molecular Psychiatry*, *14*(8), 764–739. doi.org/10.1038/mp.2008.119

Bhattaccharjee, S. A., Murnane, K. S., & Banga, A. K. (2020). Transdermal delivery of breakthrough therapeutics for the management of treatment-resistant and post-partum depression. *International Journal of Pharmaceutics*, 120007.

Binder, D. K., & Scharfman, H. E. (2004). Brain-derived neurotrophic factor. *Growth Factors*, *22*(3), 123.

Blier, P., & De Montigny, C. (1994). Current advances and trends in the treatment of depression. *Trends in Pharmacological Sciences*, *15*(7), 220-226.

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., & Krystal, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biological Psychiatry*, *47*(4), 351-354.

Bodkin, J. A., & Amsterdam, J. D. (2002). Transdermal selegiline in major depression: a double-blind, placebo-controlled, parallel-group study in outpatients. *American Journal of Psychiatry*, *159*(11), 1869-1875.

Borsini, F., & Meli, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity?. *Psychopharmacology*, *94*(2), 147-160.

Brown, P. L., Zanos, P., Wang, L., Elmer, G. I., Gould, T. D., & Shepard, P. D. (2018). Isoflurane but not halothane prevents and reverses helpless behavior: A role for EEG burst suppression?. *International Journal of Neuropsychopharmacology, 21*(8), 777-785.

Bunney, W. E., & Davis, J. M. (1965). Norepinephrine in depressive reactions: A review. *Archives of General Psychiatry*, *13*(6), 483-494.

Bunney, W. E. (2015). Circadian dysregulation of clock genes: Clues to rapid treatments in major depressive disorder. *Molecular Psychiatry*, *20*(1), 48-55.

Campos, A. C., Fogaça, M. V., Aguiar, D. C., & Guimaraes, F. S. (2013). Animal models of anxiety disorders and stress. *Brazilian Journal of Psychiatry*, *35*, S101-S111.

Carhart-Harris, R. L., Muthukumaraswamy, S., Roseman, L., Kaelen, M., Droog, W., Murphy, K., ... & Nutt, D. J. (2016). Neural correlates of the LSD experience revealed by multimodal neuroimaging. *Proceedings of the National Academy of Sciences*, *113*(17), 4853-4858.

Chaki S. (2017). Beyond Ketamine: New Approaches to the Development of Safer Antidepressants. *Current Neuropharmacology*, *15*(7), 963–976. doi.org/10.2174/1570159X15666170221101054

Chang, T., & Glazko, A. J. (1974). Biotransformation and disposition of ketamine. *International Anesthesiology Clinics, 12*(2), 157–178.

Chien, Y. W. (1987). Development of transdermal drug delivery systems. *Drug Development and Industrial Pharmacy*, *13*(4-5), 589–651.

Chong, C., Schug, S. A., Page-Sharp, M., Jenkins, B., & Ilett, K. F. (2009). Development of a sublingual/oral formulation of ketamine for use in neuropathic pain. *Clinical Drug Investigation, 29*(5), 317–324.

Clements, J. A., Nimmo, W. S., & Grant, I. S. (1982). Bioavailability, pharmacokinetics, and analgesic activity of ketamine in humans. *Journal of Pharmaceutical Sciences*, *71*(5), 539-542.

Cohen, M. L., Chan, S. L., Way, W. L., & Trevor, A. J. (1973). Distribution in the brain and metabolism of ketamine in the rat after intravenous administration. *The Journal of the American Society of Anesthesiologists*, *39*(4), 370-375.

Corssen, G., & Domino, E. F. (1966). Dissociative anesthesia: Further pharmacologic studies and first clinical experience with the phencyclidine derivative CI-581. *Anesthesia and Analgesia, 45*(1), 29–40.

Courtenay, A. J., McAlister, E., McCrudden, M. T., Vora, L., Steiner, L., Levin, G., ... & Donnelly, R. F. (2020). Hydrogel-forming microneedle arrays as a therapeutic option for transdermal esketamine delivery. *Journal of Controlled Release, 322*, 177-186.

Crowley, K. L., Flores, J. A., Hughes, C. N., & Iacono, R. P. (1998). Clinical application of ketamine ointment in the treatment of sympathetically maintained pain. *International Journal of Pharmaceutical Compounding*, *2*(2), 122–127.

Dale, O., Somogyi, A. A., Li, Y., Sullivan, T., & Shavit, Y. (2012). Does intraoperative ketamine attenuate inflammatory reactivity following surgery? A systematic review and meta-analysis. *Anesthesia and Analgesia*, *115*(4), 934–943. doi.org/10.1213/ANE.0b013e3182662e30

Dayton, G., Stiller, R. L., Cook, D. R., & Perel, J. M. (1983). The binding of ketamine to plasma proteins: Emphasis on human plasma. *European Journal of Clinical Pharmacology*, *24*(6), 825-831.

De Pablo, J. M., Parra, A., Segovia, S., & Guillamón, A. (1989). Learned immobility explains the behavior of rats in the forced swimming test. *Physiology & Behavior, 46*(2), 229–237. doi.org/10.1016/0031-9384(89)90261-8

Derkach, V. A., Oh, M. C., Guire, E. S., & Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nature reviews. Neuroscience*, *8*(2), 101–113. doi.org/10.1038/nrn2055

Deyama, S., & Duman, R. S. (2020). Neurotrophic mechanisms underlying the rapid and sustained antidepressant actions of ketamine. *Pharmacology Biochemistry and Behavior*, *188*, 172837.

DiazGranados, N., Ibrahim, L. A., Brutsche, N. E., Ameli, R., Henter, I. D., Luckenbaugh, D. A., ... & Zarate Jr, C. A. (2010). Rapid resolution of suicidal ideation after a single infusion of an N-methyl-D-aspartate antagonist in patients with treatment-resistant major depressive disorder. *The Journal of Clinical Psychiatry*, *71*(12), 1605-1611.

Domino, E. F., Chodoff, P., & Corssen, G. (1965). Pharmacologic effects of CI-581, a new dissociative anesthetic in man. *Clinical Pharmacology & Therapeutics, 6*(3), 279–291.

Domino, E. F. (1980). History and pharmacology of PCP and PCP-related analogs. *Journal of Psychedelic Drugs*, *12*(3-4), 223-227.

Domino, E. F., Domino, S. E., Smith, R. E., Domino, L. E., Goulet, J. R., Domino, K. E., & Zsigmond, E. K. (1984). Ketamine kinetics in unmedicated and diazepam-premedicated subjects. *Clinical Pharmacology and Therapeutics*, *36*(5), 645–653.

Douglas, R. J., & Martin, K. A. C. (2007). Mapping the matrix: The ways of neocortex. *Neuron*, *56*(2), 226–238.

Du Jardin, K. G., Liebenberg, N., Müller, H. K., Elfving, B., Sanchez, C., & Wegener, G. (2016). Differential interaction with the serotonin system by S-ketamine, vortioxetine, and fluoxetine in a genetic rat model of depression. *Psychopharmacology*, *233*(14), 2813-2825.

Duncan, W. C., & Zarate, C. A. (2013). Ketamine, sleep, and depression: Current status and new questions. *Current Psychiatry Reports*, *15*(9), 394.

Duman, R. S., Aghajanian, G. K., Sanacora, G., & Krystal, J. H. (2016). Synaptic plasticity and depression: New insights from stress and rapid-acting antidepressants. *Nature Medicine*, *22*(3), 238–249. doi.org/10.1038/nm.4050

Ecevitoglu, A., Canbeyli, R., & Unal, G. (2019). Oral ketamine alleviates behavioral despair without cognitive impairment in Wistar rats. *Behavioural Brain Research*, *372*, 112058.

Elias, P. M. (1981). Epidermal lipids, membranes, and keratinization. *International Journal of Dermatology*, *20*(1), 1-19.

Engin, E., Treit, D., & Dickson, C. T. (2009). Anxiolytic-and antidepressant-like properties of ketamine in behavioral and neurophysiological animal models. *Neuroscience*, *161*(2), 359-369.

Erdő, F., Bors, L. A., Farkas, D., Bajza, Á., & Gizurarson, S. (2018). Evaluation of intranasal delivery route of drug administration for brain targeting. *Brain Research Bulletin*, *143*, 155-170.

Femi-Oyewo, M. N., Ajala, T. O., & Mabadeje, A. (2013). The evaluation of shea butter from butyrospermum parkii as a vehicle in sulphur ointment formulations. *West African Journal of Pharmacy*, *23*, 58-65.

Fraga, D. B., Olescowicz, G., Moretti, M., Siteneski, A., Tavares, M. K., Azevedo, D., … Rodrigues, A. L. S. (2018). Anxiolytic effects of ascorbic acid and ketamine in mice. *Journal of Psychiatric Research, 100*, 16–23.

Franceschelli, A., Sens, J., Herchick, S., Thelen, C., & Pitychoutis, P. M. (2015). Sex differences in the rapid and the sustained antidepressant-like effects of ketamine in stress-naïve and "depressed" mice exposed to chronic mild stress. *Neuroscience*, *290*, 49–60. doi.org/10.1016/j.neuroscience.2015.01.008

Galen. (1952). *On the Natural Faculties*, trans. A. J. Brock. London: The Loeb Classical Library.

Gálvez, V., Li, A., Huggins, C., Glue, P., Martin, D., Somogyi, A. A., ... & Loo, C. K. (2018). Repeated intranasal ketamine for treatment-resistant depression–the way to go? Results from a pilot randomised controlled trial. *Journal of Psychopharmacology*, *32*(4), 397-407.

Gammaitoni, A., Gallagher, R. M., & Welz-Bosna, M. (2000). Topical ketamine gel: Possible role in treating neuropathic pain. *Pain Medicine*, *1*(1), 97-100.

Gray, G. M., & Yardley, H. J. (1975). Lipid compositions of cells isolated from pig, human, and rat epidermis. *Journal of Lipid Research*, *16*(6), 434-440.

Greifenstein, F. E., DeVault, M., Yoshitake, J., & Gajewski, J. E. (1958). A study of a 1-aryl cyclo hexyl amine for anesthesia. *Anesthesia & Analgesia*, *37*(5), 283-294.

Gideons, E. S., Kavalali, E. T., & Monteggia, L. M. (2014). Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(23), 8649–8654. doi.org/10.1073/pnas.1323920111

Giladi, N., Boroojerdi, B., Korczyn, A. D., Burn, D. J., Clarke, C. E., Schapira, A. H., & SP513 investigators. (2007). Rotigotine transdermal patch in early Parkinson's disease: A randomized, double-blind, controlled study versus placebo and ropinirole. *Movement Disorders*, *22*(16), 2398-2404.

Glue, P., Medlicott, N. J., Neehoff, S., Surman, P., Lam, F., Hung, N., & Hung, C. T. (2020). Safety and efficacy of extended release ketamine tablets in patients with treatment-resistant depression and anxiety: Open label pilot study. *Therapeutic Advances in Psychopharmacology*, *10*, 2045125320922474.

Grant, I. S., Nimmo, W. S., & Clements, J. A. (1981). Pharmacokinetics and analgesic effects of im and oral ketamine. *BJA: British Journal of Anaesthesia*, *53*(8), 805-810.

Godin, B., & Touitou, E. (2007). Transdermal skin delivery: predictions for humans from in vivo, ex vivo and animal models. *Advanced Drug Delivery Reviews*, *59*(11), 1152-1161.

Hall, C., & Ballachey, E. L. (1932). A study of the rat's behavior in a field. A contribution to method in comparative psychology. *University of California Publications in Psychology*, *6*, 1-12.

Hardingham, G. E., Fukunaga, Y., & Bading, H. (2002). Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nature Neuroscience*, *5*(5), 405–414. doi.org/10.1038/nn835

Harrison, N. L., & Simmonds, M. A. (1985). Quantitative studies on some antagonists of N-methyl D-aspartate in slices of rat cerebral cortex. *British Journal of Pharmacology*, *84*(2), 381–391. doi.org/10.1111/j.1476-5381.1985.tb12922.x

Hashimoto, K., Sawa, A., & Iyo, M. (2007). Increased levels of glutamate in brains from patients with mood disorders. *Biological Psychiatry*, *62*(11), 1310-1316.

Hayase, T., Yamamoto, Y., & Yamamoto, K. (2006). Behavioral effects of ketamine and toxic interactions with psychostimulants. *BMC Neuroscience*, *7*, 25. doi.org/10.1186/1471-2202-7-25

Hasler, G., van der Veen, J. W., Tumonis, T., Meyers, N., Shen, J., & Drevets, W. C. (2007). Reduced prefrontal glutamate/glutamine and γ-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy. *Archives of General Psychiatry, 64*(2), 193.

Herbst, L. S., Gaigher, T., Siqueira, A. A., Joca, S., Sampaio, K. N., & Beijamini, V. (2019). New evidence for refinement of anesthetic choice in procedures preceding the forced swimming test and the elevated plus-maze. *Behavioural Brain Research*, *368*, 111897. doi.org/10.1016/j.bbr.2019.04.011

Hippocrates. (1923). *Works of Hippocrates*, transl. W. H. S. Jones and E. T. Withington. Cambridge, MA: Harvard University Press.

Hillhouse, T. M., & Porter, J. H. (2015). A brief history of the development of antidepressant drugs: from monoamines to glutamate. *Experimental and Clinical Psychopharmacology*, *23*(1), 1.

Hirschfeld R. M. (2000). History and evolution of the monoamine hypothesis of depression. *The Journal of Clinical Psychiatry*, *61*(6), 4–6.

Hoeffer, C. A., & Klann, E. (2010). mTOR signaling: At the crossroads of plasticity, memory and disease. *Trends in Neurosciences*, *33*(2), 67-75.

Horwitz, A. V., Wakefield, J. C., & Lorenzo-Luaces, L. (2016). History of Depression. *Oxford Handbooks Online*.

Huber, R., Tononi, G., & Cirelli, C. (2007). Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*, *30*(2), 129-139.

Hyman, S. E., & Nestler, E. J. (1996). Initiation and adaptation: A paradigm for understanding psychotropic drug action. *The American Journal of Psychiatry*, *153*(2), 151-162.

Ibrahim, L., Diazgranados, N., Franco-Chaves, J., Brutsche, N., Henter, I. D., Kronstein, P., Moaddel, R., Wainer, I., Luckenbaugh, D. A., Manji, H. K., & Zarate, C. A., Jr (2012). Course of improvement in depressive symptoms to a single intravenous infusion of ketamine vs add-on riluzole: Results from a 4-week, double-blind, placebo-controlled study. *Neuropsychopharmacology, 37*(6), 1526–1533. doi.org/10.1038/npp.2011.338

Idvall, J. A. K. F., Aronsen, K. F., Stenberg, P., & Paalzow, L. (1983). Pharmacodynamic and pharmacokinetic interactions between ketamine and diazepam. *European Journal of Clinical Pharmacology*, *24*(3), 337-343.

Insel, T. R., & Wang, P. S. (2009). The STAR* D trial: revealing the need for better treatments. *Psychiatric Services*, *60*(11), 1466-1467.

Ionescu, D. F., Rosenbaum, J. F., & Alpert, J. E. (2015). Pharmacological approaches to the challenge of treatment-resistant depression. *Dialogues in Clinical Neuroscience*, *17*(2), 111.

Irwin, S. A., & Iglewicz, A. (2010). Oral ketamine for the rapid treatment of depression and anxiety in patients receiving hospice care. *Journal of Palliative Medicine*, *13*(7), 903–908. doi.org/10.1089/jpm.2010.9808

Irwin, S. A., Iglewicz, A., Nelesen, R. A., Lo, J. Y., Carr, C. H., Romero, S. D., & Lloyd, L. S. (2013). Daily oral ketamine for the treatment of depression and anxiety in patients receiving hospice care: A 28-day open-label proof-of-concept trial. *Journal of Palliative Medicine*, *16*(8), 958–965.

Jacobi, U., Kaiser, M., Toll, R., Mangelsdorf, S., Audring, H., Otberg, N., ... & Lademann, J. (2007). Porcine ear skin: An in vitro model for human skin. *Skin Research and Technology*, *13*(1), 19-24.

Jayatissa, M. N., Bisgaard, C., Tingström, A., Papp, M., & Wiborg, O. (2006). Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology, 31*(11), 2395-2404.

Jelen, L. A., Young, A. H., & Stone, J. M. (2021). Ketamine: A tale of two enantiomers. *Journal of Psychopharmacology*, *35*(2), 109-123.

Kadriu, B., Greenwald, M., Henter, I. D., Gilbert, J. R., Kraus, C., Park, L. T., & Zarate, C. A. (2021). Ketamine and Serotonergic Psychedelics: Common Mechanisms Underlying the Effects of Rapid-Acting Antidepressants. *The International Journal of Neuropsychopharmacology*, *24*(1), 8–21. doi.org/10.1093/ijnp/pyaa087

Kapur, S., & Seeman, P. (2002). NMDA receptor antagonists ketamine and PCP have direct effects on the dopamine D(2) and serotonin 5-HT(2)receptors-implications for models of schizophrenia. *Molecular Psychiatry*, *7*(8), 837–844. doi.org/10.1038/sj.mp.4001093

Karamustafalıoğlu, O., & Yumrukçal, H. (2011). Depresyon ve anksiyete bozuklukları. *Şişli Etfal Hastanesi Tıp Bülteni*, *45*(2), 65-74.

Katz, M. M., Tekell, J. L., Bowden, C. L., Brannan, S., Houston, J. P., Berman, N., & Frazer, A. (2004). Onset and early behavioral effects of pharmacologically different antidepressants and placebo in depression. *Neuropsychopharmacology*, *29*(3), 566-579.

Kaube, H., Herzog, J., Käufer, T., Dichgans, M., & Diener, H. C. (2000). Aura in some patients with familial hemiplegic migraine can be stopped by intranasal ketamine. *Neurology*, *55*(1), 139-141.

Kavalali, E. T., & Monteggia, L. M. (2012). Synaptic mechanisms underlying rapid antidepressant action of ketamine. *American Journal of Psychiatry*, *169*(11), 1150-1156.

Khorramzadeh, E., & Lotfy, A. O. (1973). The use of ketamine in psychiatry. *Psychosomatics*, *14*(6), 344–346. doi.org/10.1016/S0033-3182(73)71306-2

Kiraly, D. D., Horn, S. R., Van Dam, N. T., Costi, S., Schwartz, J., Kim-Schulze, S., ... & Murrough, J. W. (2017). Altered peripheral immune profiles in treatment-resistant depression: Response to ketamine and prediction of treatment outcome. *Translational Psychiatry*, *7*(3), e1065-e1065.

Klein, M. E., Chandra, J., Sheriff, S., & Malinow, R. (2020). Opioid system is necessary but not sufficient for antidepressive actions of ketamine in rodents. *Proceedings of the National Academy of Sciences*, *117*(5), 2656-2662.

Koike, H., Iijima, M., & Chaki, S. (2011). Involvement of AMPA receptor in both the rapid and sustained antidepressant-like effects of ketamine in animal models of depression. *Behavioural Brain Research*, *224*(1), 107-111.

Kokkinou, M., Ashok, A. H., & Howes, O. D. (2018). The effects of ketamine on dopaminergic function: meta-analysis and review of the implications for neuropsychiatric disorders. *Molecular Psychiatry*, *23*(1), 59-69.

Krystal, J. H., Karper, L. P., Seibyl, J. P., Freeman, G. K., Delaney, R., Bremner, J. D., ... & Charney, D. S. (1994). Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Archives of General Psychiatry, 51*(3), 199-214.

Kubota, R., Maruyama, Y., Wada, Y., Okamoto, A., Tsukamoto, A., & Komiyama, T. (2018). The development of transdermal ketamine patch. *Medical Research Archives*, *6*(4).

Kuhn, R. (1958). The treatment of depressive states with G 22355 (imipramine hydrochloride). *American Journal of Psychiatry*, *115*(5), 459-464.

Lauretti, G. R., Amaral, M., Dias, R. D., Lanchote, V. L., & de Mattos, A. L. (2014). Transdermal ketamine and S (+)-Ketamine as adjuvants following orthopaedic surgery under bupivacaine spinal anaesthesia. *Journal of Physical Chemistry & Biophysics*, *4*(4), 1.

Layer, R. T., Popik, P., Olds, T., & Skolnick, P. (1995). Antidepressant-like actions of the polyamine site NMDA antagonist, eliprodil (SL-82.0715). *Pharmacology, Biochemistry, and Behavior*, *52*(3), 621–627. doi.org/10.1016/0091-3057(95)00155-p

Lapidus, K. A., Levitch, C. F., Perez, A. M., Brallier, J. W., Parides, M. K., Soleimani, L., Feder, A., Iosifescu, D. V., Charney, D. S., & Murrough, J. W. (2014). A randomized controlled trial of intranasal ketamine in major depressive disorder. *Biological Psychiatry*, *76*(12), 970–976. doi.org/10.1016/j.biopsych.2014.03.026

Lever, C., Burton, S., & O Keefe, J. (2006). Rearing on hind legs, environmental novelty, and the hippocampal formation. *Reviews in the Neurosciences*, *17*(1/2), 111.

Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., ... & Duman, R. S. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science*, *329*(5994), 959-964.

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., Li, X. Y., Aghajanian, G., & Duman, R. S. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological Psychiatry*, *69*(8), 754–761. doi.org/10.1016/j.biopsych.2010.12.015

Li Y. F. (2020). A hypothesis of monoamine (5-HT) - Glutamate/GABA long neural circuit: Aiming for fast-onset antidepressant discovery. *Pharmacology & Therapeutics*, *208*, 107494. doi.org/10.1016/j.pharmthera.2020.107494

Llamosas, N., Perez-Caballero, L., Berrocoso, E., Bruzos-Cidon, C., Ugedo, L., & Torrecilla, M. (2019). Ketamine promotes rapid and transient activation of AMPA receptor-mediated synaptic transmission in the dorsal raphe nucleus. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *88*, 243-252.

Loo, C. K., Gálvez, V., O'keefe, E., Mitchell, P. B., Hadzi-Pavlovic, D., Leyden, J., ... & Glue, P. (2016). Placebo-controlled pilot trial testing dose titration and intravenous, intramuscular and subcutaneous routes for ketamine in depression. *Acta Psychiatrica Scandinavica*, *134*(1), 48-56.

López-Muñoz, F., & Alamo, C. (2009). Monoaminergic neurotransmission: The history of the discovery of antidepressants from 1950s until today. *Current Pharmaceutical Design*, *15*(14), 1563-1586.

Lu, B. Y., Agapoff, J. R., Olson, D. J., Williams, S. R., Roller, A., & Goebert, D. (2020). Rapid and sustained improvement in treatment-refractory depression through use of acute intravenous ketamine and concurrent transdermal selegiline: A case series. *Journal of Affective Disorders*, *262*, 40-42.

Lynch, M. E., Clark, A. J., & Sawynok, J. (2003). A pilot study examining topical amitriptyline, ketamine, and a combination of both in the treatment of neuropathic pain. *The Clinical Journal of Pain*, *19*(5), 323-328.

Lynch, M. E., Clark, A. J., Sawynok, J., & Sullivan, M. J. (2005). Topical 2% amitriptyline and 1% ketamine in neuropathic pain syndromes: A randomized, double-blind, placebo-controlled trial. *Anesthesiology*, *103*(1), 140–146. doi.org/10.1097/00000542-200507000-00021

Machado-Vieira, R., Salvadore, G., DiazGranados, N., & Zarate Jr, C. A. (2009). Ketamine and the next generation of antidepressants with a rapid onset of action. *Pharmacology & Therapeutics*, *123*(2), 143-150.

MacDonald, J. F., & Nowak, L. M. (1990). Mechanisms of blockade of excitatory amino acid receptor channels. *Trends in Pharmacological Sciences*, *11*(4), 167–172. doi.org/10.1016/0165-6147(90)90070-O

Maddox, V. H., Godefroi, E. F., & Parcell, R. F. (1965). The synthesis of phencyclidine and other 1-Arylcyclohexylamines. *Journal of Medicinal Chemistry*, *8*, 230–235. doi.org/10.1021/jm00326a019

Maeng, S., Zarate, C. A., Jr, Du, J., Schloesser, R. J., McCammon, J., Chen, G., & Manji, H. K. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: Role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological Psychiatry*, *63*(4), 349–352. doi.org/10.1016/j.biopsych.2007.05.028

Maibach, H. I., & Wester, R. C. (1989). Percutaneous absorption: In vivo methods in humans and animals. *Journal of the American College of Toxicology*, *8*(5), 803-813.

Malinovsky, J. M., Servin, F., Cozian, A., Lepage, J. Y., & Pinaud, M. (1996). Ketamine and norketamine plasma concentrations after iv, nasal and rectal administration in children. *British Journal of Anaesthesia*, *77*(2), 203-207.

Mathias, N. R., & Hussain, M. A. (2010). Non-invasive systemic drug delivery: Developability considerations for alternate routes of administration. *Journal of Pharmaceutical Sciences*, *99*(1), 1–20.

Mattson, M. P. (1996). Calcium and Free Radicals: Mediators of neurotrophic factor and excitatory transmitter-regulated developmental plasticity and cell death. *Perspectives on Developmental Neurobiology*, *3*(2), 79-91.

Mayer, M. L., Westbrook, G. L., & Guthrie, P. B. (1984). Voltage-dependent block by Mg 2+ of NMDA responses in spinal cord neurones. *Nature, 309*(5965), 261-263.

Meloni, D., Gambarana, C., De Montis, M. G., Dal Prá, P., Taddei, I., & Tagliamonte, A. (1993). Dizocilpine antagonizes the effect of chronic imipramine on learned helplessness in rats. *Pharmacology, Biochemistry, and Behavior*, *46*(2), 423–426. doi.org/10.1016/0091-3057(93)90374-3

Miller, O. H., Moran, J. T., & Hall, B. J. (2016). Two cellular hypotheses explaining the initiation of ketamine's antidepressant actions: Direct inhibition and disinhibition. *Neuropharmacology*, *100*, 17-26.

Mion, G., & Villevieille, T. (2013). Ketamine pharmacology: An update (pharmacodynamics and molecular aspects, recent findings). *CNS Neuroscience & Therapeutics*, *19*(6), 370–380. doi.org/10.1111/cns.12099

Miyasaka, M., & Domino, E. F. (1968). Neuronal mechanisms of ketamine-induced anesthesia. *International Journal of Neuropharmacology, 7*(6), 557–573.

Moghaddam, B., Adams, B., Verma, A., & Daly, D. (1997). Activation of glutamatergic neurotransmission by ketamine: A novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex. *Journal of Neuroscience*, *17*(8), 2921-2927.

Montgomery, K. C. (1955). The relation between fear induced by novel stimulation and exploratory drive. *Journal of Comparative and Physiological Psychology*, *48*(4), 254.

Moryl, E., Danysz, W., & Quack, G. (1993). Potential antidepressive properties of amantadine, memantine and bifemelane. *Pharmacology & Toxicology*, *72*(6), 394–397. doi.org/10.1111/j.1600-0773.1993.tb01351.x

Mumtaz, F., Khan, M. I., Zubair, M., & Dehpour, A. R. (2018). Neurobiology and consequences of social isolation stress in animal model—A comprehensive review. *Biomedicine & Pharmacotherapy*, *105*, 1205–1222.

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., Iqbal, S., Pillemer, S., Foulkes, A., Shah, A., Charney, D. S., & Mathew, S. J. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: A two-site randomized controlled trial. *The American Journal of Psychiatry*, *170*(10), 1134–1142. doi.org/10.1176/appi.ajp.2013.13030392

Nagele, P., Duma, A., Kopec, M., Gebara, M. A., Parsoei, A., Walker, M., Janski, A., Panagopoulos, V. N., Cristancho, P., Miller, J. P., Zorumski, C. F., & Conway, C. R. (2015). Nitrous oxide for treatment-resistant major depression: A proof-of-concept trial. *Biological Psychiatry*, *78*(1), 10–18. doi.org/10.1016/j.biopsych.2014.11.016

Naik, A., Kalia, Y. N., & Guy, R. H. (2000). Transdermal drug delivery: Overcoming the skin's barrier function. *Pharmaceutical Science & Technology Today*, *3*(9), 318-326.

Nairn, A. C., Bhagat, B., & Palfrey, H. C. (1985). Identification of calmodulin-dependent protein kinase III and its major Mr 100,000 substrate in mammalian tissues. *Proceedings of the National Academy of Sciences*, *82*(23), 7939-7943.

Newport, D. J., Carpenter, L. L., McDonald, W. M., Potash, J. B., Tohen, M., & Nemeroff, C. B. (2015). Ketamine and other NMDA antagonists: Early clinical trials and possible mechanisms in depression. *American Journal of Psychiatry, 172*(10), 950–966.

Nosyreva, E., Szabla, K., Autry, A. E., Ryazanov, A. G., Monteggia, L. M., & Kavalali, E. T. (2013). Acute suppression of spontaneous neurotransmission drives synaptic potentiation. *Journal of Neuroscience*, *33*(16), 6990-7002.

Nowak, G., Trullas, R., Layer, R. T., Skolnick, P. H. I. L., & Paul, I. A. (1993). Adaptive changes in the N-methyl-D-aspartate receptor complex after chronic treatment with imipramine and 1-aminocyclopropanecarboxylic acid. *Journal of Pharmacology and Experimental Therapeutics*, *265*(3), 1380-1386.

Orozco-Solis, R., Montellier, E., Aguilar-Arnal, L., Sato, S., Vawter, M. P., Bunney, B. G., ... & Sassone-Corsi, P. (2017). A circadian genomic signature common to ketamine and sleep deprivation in the anterior cingulate cortex. *Biological Psychiatry*, *82*(5), 351-360.

Orser, B. A., Pennefather, P. S., & MacDonald, J. F. (1997). Multiple mechanisms of ketamine blockade of N-methyl-D-aspartate receptors. *The Journal of the American Society of Anesthesiologists*, *86*(4), 903-917.

Papp, M., & Moryl, E. (1994). Antidepressant activity of non-competitive and competitive NMDA receptor antagonists in a chronic mild stress model of depression. *European Journal of Pharmacology*, *263*(1-2), 1-7.

Pastore, M. N., Kalia, Y. N., Horstmann, M., & Roberts, M. S. (2015). Transdermal patches: history, development and pharmacology. *British Journal of Pharmacology*, *172*(9), 2179-2209.

Paul, I. A., Layer, R. T., Skolnick, P., & Nowak, G. (1993). Adaptation of the NMDA receptor in rat cortex following chronic electroconvulsive shock or imipramine. *European Journal of Pharmacology*, *247*(3), 305–311. doi.org/10.1016/0922-4106(93)90199-j

Paul, I. A., Nowak, G., Layer, R. T., Popik, P., & Skolnick, P. (1994). Adaptation of the N-methyl-D-aspartate receptor complex following chronic antidepressant treatments. *The Journal of Pharmacology and Experimental Therapeutics*, *269*(1), 95–102.

Park, H., & Poo, M. M. (2013). Neurotrophin regulation of neural circuit development and function. *Nature Reviews Neuroscience*, *14*(1), 7-23.

Pellow, S., Chopin, P., File, S. E., & Briley, M. (1985). Validation of open: Closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *Journal of Neuroscience Methods*, *14*(3), 149-167.

Pham, T. H., Defaix, C., Xu, X., Deng, S. X., Fabresse, N., Alvarez, J. C., ... & Gardier, A. M. (2018). Common neurotransmission recruited in (R, S)-ketamine and (2R, 6R)-hydroxynorketamine–induced sustained antidepressant-like effects. *Biological Psychiatry*, *84*(1), e3-e6.

Pochwat, B., Szewczyk, B., Sowa-Kucma, M., Siwek, A., Doboszewska, U., Piekoszewski, W., ... & Nowak, G. (2014). Antidepressant-like activity of magnesium in the chronic mild stress model in rats: Alterations in the NMDA receptor subunits. *International Journal of Neuropsychopharmacology*, *17*(3), 393-405.

Porsolt, R. D., Le Pichon, M., & Jalfre, M. L. (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature*, *266*(5604), 730-732.

Pöyhiä, R., & Vainio, A. (2006). Topically administered ketamine reduces capsaicin-evoked mechanical hyperalgesia. *The Clinical Journal of Pain*, *22*(1), 32-36.

Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. *Nature Biotechnology*, *26*(11), 1261-1268.

Racagni, G., & Popoli, M. (2008). Cellular and molecular mechanisms in the long-term action of antidepressants. *Dialogues in Clinical Neuroscience, 10*(4), 385.

Ramos, A., Pereira, E., Martins, G. C., Wehrmeister, T. D., & Izídio, G. S. (2008). Integrating the open field, elevated plus maze and light/dark box to assess different types of emotional behaviors in one single trial. *Behavioural Brain Research*, *193*(2), 277-288.

Roberts, M. S. (1997). Targeted drug delivery to the skin and deeper tissues: Role of physiology, solute structure and disease. *Clinical and Experimental Pharmacology and Physiology*, *24*(11), 874-879.

Rogóz, Z., Skuza, G., & Legutko, B. (2007). Repeated co-treatment with imipramine and amantadine induces hippocampal brain-derived neurotrophic factor gene expression in rats. *Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society*, *58*(2), 219–234.

Quan, D., Wellish, M., & Gilden, D. H. (2003). Topical ketamine treatment of postherpetic neuralgia. *Neurology*, *60*(8), 1391-1392.

Rolan, P., Lim, S., Sunderland, V., Liu, Y., & Molnar, V. (2014). The absolute bioavailability of racemic ketamine from a novel sublingual formulation. *British Journal of Clinical Pharmacology*, *77*(6), 1011-1016.

Rush, A. J., Trivedi, M. H., Wisniewski, S. R., Nierenberg, A. A., Stewart, J. W., Warden, D., ... & Fava, M. (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: A STAR* D report. *American Journal of Psychiatry*, *163*(11), 1905-1917.

Russo, M. A., & Santarelli, D. M. (2016). A novel compound analgesic cream (ketamine, pentoxifylline, clonidine, DMSO) for complex regional pain syndrome patients. *Pain Practice*, *16*(1), E14-E20.

Sanacora, G., Treccani, G., & Popoli, M. (2012). Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, *62*(1), 63-77.

Sanacora, G., Smith, M. A., Pathak, S., Su, H. L., Boeijinga, P. H., McCarthy, D. J., & Quirk, M. C. (2014). Lanicemine: a low-trapping NMDA channel blocker produces sustained antidepressant efficacy with minimal psychotomimetic adverse effects. *Molecular Psychiatry*, *19*(9), 978–985.

Sanacora, G., & Schatzberg, A. F. (2015). Ketamine: Promising path or false prophecy in the development of novel therapeutics for mood disorders?. *Neuropsychopharmacology*, *40*(2), 259–267. doi.org/10.1038/npp.2014.261

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., & Hen, R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science*, *301*(5634), 805–809. doi.org/10.1126/science.1083328

Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: A review of supporting evidence. *American Journal of Psychiatry*, *122*(5), 509-522.

Schmaal, L., van Harmelen, A. L., Chatzi, V., Lippard, E. T., Toenders, Y. J., Averill, L. A., ... & Blumberg, H. P. (2020). Imaging suicidal thoughts and behaviors: a comprehensive review of 2 decades of neuroimaging studies. *Molecular Psychiatry*, *25*(2), 408-427.

Schroeder, K. E., Irwin, Z. T., Gaidica, M., Bentley, J. N., Patil, P. G., Mashour, G. A., et al. (2016). Disruption of corticocortical information transfer during ketamine anesthesia in the primate brain. *Neuroimage, 134*, 459–465.

Sen, S., Duman, R., & Sanacora, G. (2008). Serum brain-derived neurotrophic factor, depression, and antidepressant medications: Meta-analyses and implications. *Biological Psychiatry*, *64*(6), 527–532. doi.org/10.1016/j.biopsych.2008.05.005

Skolnick, P., Layer, R. T., Popik, P., Nowak, G., Paul, I. A., & Trullas, R. (1996). Adaptation of *N*-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: Implications for the pharmacotherapy of depression. *Pharmacopsychiatry*, *29*(01), 23-26.

Silvestre, J. S., Pallarés, M., Nadal, R., & Ferré, N. (2002). Opposite effects of ethanol and ketamine in the elevated plus-maze test in Wistar rats undergoing a chronic oral voluntary consumption procedure. *Journal of Psychopharmacology*, *16*(4), 305–312. doi.org/10.1177/026988110201600404

Skolnick, P., Layer, R. T., Popik, P., Nowak, G., Paul, I. A., & Trullas, R. (1996). Adaptation of *N*-methyl-D-aspartate (NMDA) receptors following antidepressant treatment: Implications for the pharmacotherapy of depression. *Pharmacopsychiatry*, *29*(1), 23-26.

Slattery, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols*, *7*(6), 1009-1014.

Sofia, R. D., & Harakal, J. J. (1975). Evaluation of ketamine HCl for anti-depressant activity. *Archives Internationales de Pharmacodynamie et de Therapie*, *214*(1), 68-74.

Sulzer, D. (2011). How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron*, *69*(4), 628-649.

Szewczyk, B., Szopa, A., Serefko, A., Poleszak, E., & Nowak, G. (2018). The role of magnesium and zinc in depression: Similarities and differences. *Magnesium Research*, *31*(3), 78-89.

Tan, S., Wang, Y., Chen, K., Long, Z., & Zou, J. (2017). Ketamine alleviates depressive-like behaviors via down-regulating inflammatory cytokines induced by chronic restraint stress in mice. *Biological and Pharmaceutical Bulletin*, *40*(8), 1260-1267.

Tang, J., Xue, W., Xia, B., Ren, L., Tao, W., Chen, C., ... & Chen, G. (2015). Involvement of normalized NMDA receptor and mTOR-related signaling in rapid antidepressant effects of Yueju and ketamine on chronically stressed mice. *Scientific Reports*, *5*(1), 1-13.

Thelen, C., Sens, J., Mauch, J., Pandit, R., & Pitychoutis, P. M. (2016). Repeated ketamine treatment induces sex-specific behavioral and neurochemical effects in mice. *Behavioural Brain Research*, *312*, 305-312.

Thomas, C. G., Miller, A. J., & Westbrook, G. L. (2006). Synaptic and extrasynaptic NMDA receptor NR2 subunits in cultured hippocampal neurons. *Journal of Neurophysiology*, *95*(3), 1727–1734. doi.org/10.1152/jn.00771.2005

Trommer, H., & Neubert, R. H. (2006). Overcoming the stratum corneum: The modulation of skin penetration. A review. *Skin Pharmacology and Physiology*, *19*(2), 106–121. doi.org/10.1159/000091978

Trullas, R., & Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *European Journal of Pharmacology*, *185*(1), 1-10.

Unal, G., & Canbeyli, R. (2019). Psychomotor retardation in depression: A critical measure of the forced swim test. *Behavioural Brain Research*, *372*, 112047.

Ushida, T., Tani, T., Kanbara, T., Zinchuk, V. S., Kawasaki, M., & Yamamoto, H. (2002). Analgesic effects of ketamine ointment in patients with complex regional pain syndrome type 1. *Regional Anesthesia & Pain Medicine*, *27*(5), 524-528.

Van Ravenzwaay, B., & Leibold, E. (2004). The significance of in vitro rat skin absorption studies to human risk assessment. *Toxicology In Vitro*, *18*(2), 219-225.

Vyas, A., Mitra, R., Rao, B. S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *Journal of Neuroscience*, *22*(15), 6810-6818.

Walf, A. A., & Frye, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nature Protocols*, *2*(2), 322-328.

Wall, P. M., & Messier, C. (2001). Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. *Neuroscience & Biobehavioral Reviews*, *25*(3), 275-286.

Walsh, R. N., & Cummins, R. A. (1976). The open-field test: A critical review. *Psychological Bulletin*, *83*(3), 482.

Wang, R., Gan, J., Li, R., Duan, J., Zhou, J., Lv, M., & Qi, R. (2020). Controlled delivery of ketamine from reduced graphene oxide hydrogel for neuropathic pain: In vitro and in vivo studies. *Journal of Drug Delivery Science and Technology*, *60*, 101964.

Watanabe, Y., Gould, E., & McEwen, B. S. (1992). Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Research, 588*(2), 341-345.

Watkins, J. C., & Evans, R. H. (1981). Excitatory amino acid transmitters. *Annual Review of Pharmacology and Toxicology*, *21*, 165–204. doi.org/10.1146/annurev.pa.21.040181.001121

West, A. P. (1990). Neurobehavioral studies of forced swimming: The role of learning and memory in the forced swim test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 14*(6), 863–IN4.

Weston, R. G., Fitzgerald, P. J., & Watson, B. O. (2021). Repeated dosing of ketamine in the forced swim test: Are multiple shots better than one?. *Frontiers in Psychiatry*, *12*.

White, P. F., Schüttler, J., Shafer, A., Stanski, D. R., Horai, Y., & Trevor, A. J. (1985). Comparative pharmacology of the ketamine isomers: Studies in volunteers. *BJA: British Journal of Anaesthesia*, *57*(2), 197-203.

Wilbur, R. L. (2017). The Difference Between Topical and Transdermal Medications. *Gensco Pharma, May*, 1-2.

Williams, N. R., Heifets, B. D., Bentzley, B. S., Blasey, C., Sudheimer, K. D., Hawkins, J., Lyons, D. M., & Schatzberg, A. F. (2019). Attenuation of antidepressant and antisuicidal effects of ketamine by opioid receptor antagonism. *Molecular Psychiatry*, *24*(12), 1779–1786.

Winblad, B., Cummings, J., Andreasen, N., Grossberg, G., Onofrj, M., Sadowsky, C., ... & Lane, R. (2007). A six-month double-blind, randomized, placebo-controlled study of a transdermal patch in Alzheimer's disease—rivastigmine patch versus capsule. *International Journal of Geriatric Psychiatry, 22*(5), 456-467.

World Health Organization. (2017). Depression and other common mental disorders: Global health estimates. Geneva: *World Health Organization*, 1-24.

Yamada, J., & Jinno, S. (2019). Potential link between antidepressant-like effects of ketamine and promotion of adult neurogenesis in the ventral hippocampus of mice. *Neuropharmacology*, *158*, 107710.

Yanagihara, Y., Ohtani, M., Kariya, S., Uchino, K., Hiraishi, T., Ashizawa, N., ... & Iga, T. (2003). Plasma concentration profiles of ketamine and norketamine after administration of various ketamine preparations to healthy Japanese volunteers. *Biopharmaceutics & Drug Disposition*, *24*(1), 37-43.

Yilmaz, A., Schulz, D., Aksoy, A., & Canbeyli, R. (2002). Prolonged effect of an anesthetic dose of ketamine on behavioral despair. *Pharmacology Biochemistry and Behavior*, *71*(1-2), 341-344.

Zanos, P., Moaddel, R., Morris, P. J., Georgiou, P., Fischell, J., Elmer, G. I., Alkondon, M., Yuan, P., Pribut, H. J., Singh, N. S., Dossou, K. S., Fang, Y., Huang, X. P., Mayo, C. L., Wainer, I. W., Albuquerque, E. X., Thompson, S. M., Thomas, C. J., Zarate, C. A., Jr, & Gould, T. D. (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481–486.

Zanos, P., & Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Molecular Psychiatry*, *23*(4), 801-811.

Zanos, P., Thompson, S. M., Duman, R. S., Zarate, C. A., Jr, & Gould, T. D. (2018). Convergent mechanisms underlying rapid antidepressant action. *CNS Drugs*, *32*(3), 197–227.

Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E., Albuquerque, E. X., Thomas, C. J., Zarate, C. A., Jr, & Gould, T. D. (2018). Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms. *Pharmacological Reviews*, *70*(3), 621–660.

Zarate, C. A., Jr, Brutsche, N., Laje, G., Luckenbaugh, D. A., Venkata, S. L. V., Ramamoorthy, A., ... & Wainer, I. W. (2012). Relationship of ketamine's plasma metabolites with response, diagnosis, and side effects in major depression. *Biological Psychiatry*, *72*(4), 331-338.

Zarate, C. A., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., … Manji, H. K. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Archives of General Psychiatry*, *63*(8), 856.

Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, W. F., & Van Orden, L. S. (1952). Influence of isonicotinic acid hydrazide (INH) and 1-isonicotinyl-2-isopropyl hydrazide (IIH) on bacterial and mammalian enzymes. *Experientia*, *8*(9), 349-350.

Zhang, J. C., Li, S. X., & Hashimoto, K. (2014). R (-)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology, Biochemistry, and Behavior*, *116*, 137–141.

Zorumski, C. F., Izumi, Y., & Mennerick, S. (2016). Ketamine: NMDA receptors and beyond. *Journal of Neuroscience*, *36*(44), 11158-11164.

Zilles, K. (1992). Neuronal plasticity as an adaptive property of the central nervous system. *Annals of Anatomy - Anatomischer Anzeiger, 174*(5), 383–391.