INVESTIGATION OF THE MECHANISMS OF ACTION OF KETAMINE ON THE MONOAMINE SYSTEMS. ELECTROPHYSIOLOGICAL STUDIES ON THE RAT BRAIN

Chidiebere Michael Iro

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[Master of Science]

[Department of Neuroscience]
[Faculty of Cellular and Molecular Medicine]
University of Ottawa

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Abstract

Background: A single infusion of ketamine has rapid antidepressant properties, although the drawback is a lack of sustained effect. A previous study showed a rapid enhancement (within 2 hours) in ventral tegmental area (VTA) dopamine (DA) neuron population and locus coeruleus (LC) norepinephrine (NE) firing and bursting activity following a single ketamine administration. The current study investigated whether these changes are present 24 hours after a single administration and if they are maintained with repeated administration. Additionally, we examined dorsal raphe nucleus (DRN) serotonin (5-HT) neurons to assess the effects of single and repeated ketamine administration on these neurons.

Methods: Ketamine (10 mg/kg, i.p.) was administered to male Sprague Dawley rats once or repeatedly (3 times/week) for 2 weeks. After single and repeated administration of ketamine, electrophysiological recordings were done in the VTA, LC and DRN in anesthetized rats, 24 hrs, 3 or 7 days post-administration. Spike frequency, bursting, and for VTA neurons, spontaneously active neurons/trajectory were assessed.

Results: In the VTA, LC and DRN, 24 hrs after ketamine was injected acutely there was no significant difference between controls and treated animals in all parameters assessed. However, after repeated administration, there was an increase in bursting and number of spontaneously discharging neurons per tract of VTA DA neurons as well as an increase in frequency of discharge of LC NE neurons. While the increased number of spontaneously discharging neurons per tract had dissipated after 3 days, the enhanced bursting was still present but dissipated after 7 days. As for LC NE neurons, the increased frequency of discharge was no longer present after 3 days. No significant differences in the firing of DRN 5-HT neurons were observed between controls and treated animals even after ketamine was administered repeatedly.

Conclusion: These results indicate that repeated but not acute administration of ketamine maintained the increase in population activity of DA neurons and firing activity of NE neurons.

List of Abbreviations

5-HT	Serotonin
Akt	Protein kinase B
AMPA	3-hydroxy-5-methyl-4-isoxazoleproprionic acid
ANOVA	Analysis of variance
BDNF	Brain derived neurotrophic factor
cAMP	Cyclic adenosine monophosphate
DA	Dopamine
DAG	Diacyl glycerol
DRN	Dorsal raphe nucleus
ECF	Extracellular fluid
FDA	Food and drug administration
FST	Forced swim test
GABA	γ-Aminobutyric acid
GIRK	G protein-coupled inwardly rectifying current
GPCR	G protein-coupled receptor
GSK-3	Glycogen synthase kinase-3
HPA	Hypothalamic-pituitary-adrenal
LC	Locus coeruleus
L-DOPA	L-dihydroxyphenylalanine
LDTg	Laterodorsal tegmentum
LH	Learned helplessness
LHb	Lateral habenula
LTD	Long term depression
LTP	Long term potentiation
MAO	Monoamine Oxidase
MAO-A	Monoamine oxidase type A
MDD	Major depressive disorder
mGLUR	Metabotropic glutamate receptor
MHPG	3-methoxy-4-hydroxyphenylglycol
mPFC	Medial prefrontal cortex
mTOR	Mechanistic target of rapamycin
NBQX	2, 3-dihidroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline
NE	Norepinephrine
NMDA	N-methyl-D-aspartic acid
PCP	Phencyclidine
PET	Positron emission tomography
PKC	Protein kinase C
RMTg	Rostromedial tegmental nucleus
RT_{50}	Time required for 50 % recovery of firing rate
SEM	Standard error of the mean
SN	Substantia nigra

SSRI	Selective serotonin reuptake inhibitor
TH	Tyrosine hydroxylase
TrkB	Tropomyosin receptor kinase B
VTA	Ventral tegmental area
ρCPA	DL-4-chlorophenylalanine ethyl ester hydrochloride

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Introduction

As a leading cause of disability worldwide, major depressive disorder (MDD) elicits a significant social, psychological and financial burden on individuals diagnosed with it, as well as their family and friends (Ferrari et al., 2013; Rush et al., 2006). In order to be diagnosed with MDD, a person must present with at least five of nine symptoms over an unbroken two-week period. Additionally, at least one of these symptoms must be either depressed mood, or significant loss of interest or pleasure, otherwise known as anhedonia (DSM-5, 2015). The rest of these symptoms include sleeping too much or too little, significant weight loss, gain, or change in appetite, slowing down or speeding up of thought, fatigue or loss of energy, feelings of worthlessness or excessive inappropriate guilt, diminished ability to think or concentrate and lastly, recurrent thoughts of death or suicide (DSM-5, 2015). As can be extrapolated from this list of symptoms, the population of patients diagnosed with MDD is very heterogeneous. It is no surprise then, that treatment response to the disorder is also variable (Rush et al., 2006). Treatment of MDD is effectuated using psychotherapy, pharmacotherapy, or a combination of both. There are several forms of psychotherapy which are effective for the treatment of MDD. However, our knowledge of the brain regions involved in the disorder has been informed in large part by sites of action of antidepressant medications which produce an alleviation of depressive symptoms (Cuijpers et al., 2013; Leichsenring, Steinert, & Hoyer, 2016). For example, monoamine oxidase (MAO) inhibitors and tricyclic reuptake inhibitors, which were discovered in the early 1950s and became popular in the 60s (Cutler & Heiser, 1978). These medications act on the monoamine systems to increase levels of corresponding neurotransmitters, subsequently resulting in a decrease in depressive symptoms in a significant proportion of depressed patients (Rush et al., 2006). The monoamine systems are comprised of serotonin (5-HT), dopamine (DA) and norepinephrine (NE), and the finding that tricyclic reuptake inhibitors effectively relieve

depressive symptoms in some patients led to the monoamine hypothesis of depression (Bunney & Davis, 1965; Delgado, 2000; Hirschfeld, 2000; Schildkraut, 1965).

Since the efficacious use of tricyclic reuptake inhibitors in the treatment of MDD, various studies have established the involvement of these systems in the therapeutics of the disorder (Delgado, 2000; Dunlop & Nemeroff, 2007; Guiard, El Mansari, Merali, & Blier, 2008; Rush et al., 2006; Schatzberg et al., 2004). Further evidence for the involvement of the monoamine systems in the pharmacotherapy of depression came from a study showing that reserpine, an extract of the Rauwolfia serpentine plant, and used for the treatment of hypertensive vascular disease in the 1950s, worsened depressive symptoms in patients (Muller, Pryor, Gibbons, & Orgain, 1955). It was shown that reserpine inhibits the vesicular monoamine transporter, thereby depleting brain monoamine levels (Muller et al., 1955). As such, the involvement of the various monoaminergic systems has been, and continues to be explored for their relevance in the treatment of MDD.

5-HT system

It is impossible to discuss the treatment of MDD without taking the 5-HT system into consideration. Evidence implicating 5-HT in MDD came, amongst other research, from a postmortem study showing that 5-HT concentrations were depleted in depressive patients who died by suicide (Shaw D. M., Camps F. E., 1967). Also, shortly after the widespread use of tricyclic reuptake inhibitors in the treatment of MDD, several studies were conducted to assess the regions of the brain targeted by these medications and how they produce an antidepressant effect. A study by Bradshaw and colleagues (1973), in cats, found that when applied iontophoretically, tricyclic reuptake inhibitors are able to modify the response of cortical neurons to 5-HT and NE neurons (Bradshaw, Roberts, & Szabadi, 1973). Subsequently, de Montigny and Aghajanian showed that chronic administration of clinically effective tricyclic reuptake inhibitors

produced a selective increase in the inhibitory response of forebrain neurons to 5-HT applied by microiontophoresis (de Montigny & Aghajanian, 1978). This effect took one to two weeks to develop- a time course consistent with the delayed onset of therapeutic effect in humans. Soon after this discovery, there was a breakthrough in our understanding of the therapeutics of MDD. This was the finding that a subset of 5-HT receptors, which have a high affinity for 5-HT, act as auto-receptors, thus providing inhibitory input unto the cell in the presence of 5-HT in the synapse (de Montigny & Blier, 1983). As such, when selective serotonin reuptake inhibitors (SSRIs) are administered over time, these receptors become desensitized and the inhibition is alleviated, leading to overall increased 5-HT availability (de Montigny & Blier, 1983). This key discovery further clarified the time lag to remission experienced by patients on these medications and helped establish the importance of the 5-HT system in treatment response. These autoreceptors were subsequently characterized as belonging to a specific subtype, the 5-HT_{1A} receptor (Marcinkiewicz, Verge, Gozlan, Pichat, & Hamon, 1984).

Distribution of 5-HT neurons

Majority of 5-HT neurons are localized in the raphe nucleus and send projections to various brain regions. Moore et al (1978) showed that projections from the raphe bundle project to the ventral tegmental area (VTA), the habenular complex, thalamus, amygdala, hippocampal complex, and posterior cortex amongst other areas (Moore, Halaris, & Jones, 1978). These are all regions of the brain that have been shown to be involved in emotional processing, sleep, feeding behavior and memory which are dysregulated in MDD (Nichols & Nichols, 2008). In addition to projecting to a variety of regions all over the brain, it has been shown that there are seven families of 5-HT receptors, each with several receptor subtypes. We will explore these receptors and their implications in the treatment of MDD.

5-HT$_1$ receptor

The 5-HT$_1$ receptor was classified based on its high affinity for 5-HT (Peroutka, Lebovitz, & Snyder, 1981). It is further subdivided into 5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$ and 5HT$_{1F}$ receptor subtypes, all of which are $G_{i/o}$ coupled receptors, because their activation results in decreased production of cyclic adenosine monophosphate (cAMP) and inhibition of adenylyl cyclase (Nichols & Nichols, 2008). The main receptor subtypes implicated in MDD are the 5HT$_{1A}$ and 5-HT$_{1B}$ receptors, with limited evidence for the 5-HT$_{1E}$ receptor. While there is always new evidence arising for the involvement of other receptor subtypes, focus will be dedicated to studies which have explored the above-mentioned receptors in the treatment of MDD.

5-HT$_{1A}$ receptor

The 5-HT$_{1A}$ receptor was the first of the 5-HT receptor subtypes to be characterized, and it has been studied extensively (Blier & De Montigny, 1987; Palacios, 2016; Pazos & Palacios, 1985; Peroutka, 1986). 5-HT$_{1A}$ receptors are located both pre- and post-synaptically throughout the brain. Their activation results in hyperpolarization and reduction in the firing rate of the cell (Nichols & Nichols, 2008). As was previously mentioned, experiments by Blier and de Montigny showed that 5-HT$_{1A}$ receptors expressed in the raphe act as somatodendritic autoreceptors, inhibiting 5-HT cells there through G protein-coupled inwardly rectifying potassium channels (GIRKs) (Blier & De Montigny, 1987; Luscher, Jan, Markus, Malenka, & Nicoll, 1997). There is a high density of postsynaptic 5-HT$_{1A}$ receptors in the hippocampus, localized in the CA1, CA2 and dentate gyrus fields (Luscher et al., 1997). Additionally, it has been shown that while SSRI administration often results in the desensitization of the 5-HT$_{1A}$ receptor, tricicylic re-uptake inhibitors produce an enhancement of serotonergic transmission by facilitating the activation of G proteins by the post-synaptic 5-HT$_{1A}$ receptor (Blier & Bouchard, 1994; Chaput, De Montigny,

& Blier, 1991; Gravel & De Montigny, 1987). This increased sensitivity has been shown to occur in the hippocampus following repeated administration of electroconvulsive shocks (Hayakawa, Shimizu, Nishida, Motohashi, & Yamawaki, 1994; Ishihara, Taku, Hiroshi, Shigeto, & Masashi, 1999; Szabo and Blier 2001). Use of immediate release 5-HT_{1A} agonists for depression is limited by the finding that full agonists produce light headedness and gastrointestinal side effects in humans, thus restricting clinical use. (Savitz, Lucki, & Drevets, 2009). However, their relevance in the therapy of depression is established (Blier & Ward, 2003; Savitz et al., 2009). Indeed, in 2016 the food and drug administration (FDA) ruled favorably on the efficacy of the extended release formulation of the 5-HT_{1A} agonist gepirone in the treatment of MDD.

5-HT_{1B} receptor

The 5-HT_{1B} receptor was initially believed to be absent in humans. However, the 5-$HT_{1D\beta}$ receptor was later discovered to be an ortholog of the rat 5-HT_{1B} receptor and renamed accordingly (Adham, Romanienko, Hartig, Weinshank, & Branchek, 1992; Hamblin, Metcalf, McGuffin, & Karpells, 1992). These receptors have been shown to be involved in the constriction of human cerebral arteries, and are implicated in migraine therapy (Nichols & Nichols, 2008). They are mostly localized on axon terminals present in the globus pallidus and substantia nigra as well as in the superior colliculus, enteropenduncular nuclei, periaqueductal gray, hypothalamus and amygdala (Nichols & Nichols, 2008).

Important to their implication in the therapy of depression, 5-HT_{1B} receptors function as presynaptic heteroreceptors on non-serotonergic neurons including γ-Aminobutyric acid (GABA) and glutamate neurons, and as autoreceptors to modulate serotonin release in the raphe (Martin, Hannon, Phillips, & Heal, 1992; Sari, 2004). Results from animal studies have also shown that they are involved in aggression and impulsivity (Groenink, Van Bogaert, Van Der Gugten, Oosting, & Olivier, 2003; Martin et al., 1992). Additionally, similar to 5-HT_{1A}, studies

suggest that the co-administration of a 5-HT_{1B} antagonist with SSRIs could decrease the lag to onset of antidepressant effects (Nichols & Nichols, 2008).

5-HT_{1E} receptor

The literature on the 5-HT_{1E} receptor is limited. This is due to two main factors; the first is that to our knowledge, there is no 5-HT_{1E} receptor in rats or mice (Bai et al., 2004) — the two primary species employed in animal studies. Secondly, there is a lack of ligands specific for this receptor (Nichols & Nichols, 2008). As such, it has been difficult to properly investigate the $5HT_{1E}$ receptor and its implication in various disorders. However, a study by Palacios and colleagues showed that mRNA for the 5-HT_{1E} receptor is localized in the caudate, putamen and amygdala which may have implications for MDD (Bruinvels et al., 1994).

5-HT_2 receptor

The 5-HT_2 receptors are $G_{q/11}$- coupled receptors. Activation of these receptors causes hydrolysis of membrane phosphoinositides which results in the formation of diacyl glycerol (DAG) and inositol phosphates (Nichols & Nichols, 2008).These molecules can then act as secondary messengers to phosphorylate protein kinase C (PKC) or increase intracellular calcium leading to developmental and cell migration processes (Nichols & Nichols, 2008). There is substantial evidence for the involvement of the 5-HT_{2A} and 5-HT_{2C} receptors in the treatment of MDD with increasing evidence showing that the 5-HT_{2B} receptor is also important for the actions of certain antidepressant medications.

5-HT_{2A} receptor

In-situ hybridization studies have revealed that there is an abundance of 5-HT_{2A} receptor mRNA in the cerebral cortex including the anterior cingulate cortex, motor cranial nerves, oculomotor nuclei and other regions of the brainstem, with less in the hippocampus and midbrain

(Pompeiano, Palacios, & Mengod, 1994). The authors also confirmed the presence of 5-HT_{2A} receptors in the basal ganglia, specifically in the nucleus accumbens, fundus striati and substantia nigra (pars compacta and pars lateralis) (Pompeiano et al., 1994). There were also high levels of this receptor in the amygdala, while intermediate levels were reported in the thalamus and hypothalamus (Pompeiano et al., 1994). Additionally, a study by Roth and colleagues confirmed that in the cortex, 5-HT_{2A} receptors are expressed on pyramidal cells and some interneurons (Willins, Deutch, Ariel, & Roth, 1997).

While some studies have suggested that the distribution of the 5-HT_{2A} receptor is sparse in the brain, there is abundant evidence for the involvement of the receptor in therapeutic response to certain antidepressant medications, especially as an adjunct (Marek, Carpenter, McDougle, & Price, 2003; Morilak, Garlow, & Ciaranello, 1993; Morilak, Somogyi, LujanMiras, & Ciaranello, 1994). Antidepressant medications with antagonistic action at the 5-HT_{2A} receptor include risperidone, olanzapine, mirtazapine, mianserin as well as aripriprazole (DeLeon, Patel, & Crismon, 2004; Marek et al., 2003).

Interestingly, Electrophysiological studies by our lab in rats have shown that augmentation of the SSRI escitalopram with aripripazole is able to rapidly restore 5-HT and DA neuron firing activity (Olga Chernoloz, El Mansari, & Blier, 2009). While aripripazole acts on several other 5-HT and DA receptors, it can be postulated that its activity as an adjunct is also due to the 5-HT_{2A} receptor.

5-HT_{2C} receptor

5-HT_{2C} receptors have been shown to be involved in the modulation of monoaminergic transmission, mood, appetite and endocrine secretion as well as other functional states that are dysregulated in depression (Millan, 2005). There is a widespread distribution of these receptors in the brain, with mRNA for the receptor present in abundance in the choroid plexus as well as

intermediate levels in the caudate-putamen, nucleus accumbens, fundus striati, claustrum, substantia nigra pars compacta, bed nucleus of the stria terminalis and subthalamic nucleus (Pompeiano et al., 1994). Using knock-out mice for the 5-HT_{2C} receptor, Heisler and colleagues showed that mice with this phenotype exhibit heightened anxiety behaviours (Heisler, Zhou, Bajwa, Hsu, & Tecott, 2007). This was evidenced by their avoidance of the open arms in the elevated zero maze, avoidance of the center region in the open field test, as well as avoidance of novel objects (Heisler et al., 2007). The investigators showed that there was activation of extended amygdala corticotropin-releasing hormone neurons which required the activity of $5HT_{2C}$ receptors (Heisler et al., 2007).

The evidence for 5-HT_{2C} receptor activity in reward-related behavior is not clear-cut however. While a study by Bailey and colleagues showed that a 5-HT_{2C} receptor agonist produced enhanced activity of DA neurons and increased incentive motivation, two other studies did not report significant increase in incentive motivation. (Bailey et al., 2016; Bezzina et al., 2015; Fletcher, Sinyard, & Higgins, 2010). Despite the disagreement of increased or decreased incentive motivation, it is clear that the 5-HT_{2C} receptor is involved in motivation behavior and treatment response to various medications. Evidence in support of this can be found in the melatonergic agonist and $5HT_{2C}$ receptor antagonist, agomelatine which produces antidepressant effects (Hale et al., 2010). An electrophysiological study by our lab showed that the administration of agomelatine results in an increase in 5-HT, DA and NE neuron activity thus confirming that it is a potent monoaminergic modulator, and offering insight into the antidepressant activity of the drug (Chenu, El Mansari, & Blier, 2013).

5-HT_{2B} receptor

Compared to the 5-HT_{2A} and 5-HT_{2C} receptors, much less is known about the 5-HT_{2B} receptor. While an in-situ hybridization study by Pompeiano and colleagues failed to find mRNA

for this receptor in the rat brain, it has been implicated in several 5-HT dependent phenotypes including impulsivity, aggressivity and suicidality (Bevilacqua et al., 2010; Pompeiano et al., 1994). Additionally, it was shown that in mice lacking the 5-HT_{2B} receptor, acute response to SSRIs is absent (Diaz & Maroteaux, 2011). Their experiments also revealed that administration of a 5-HT_{2B} receptor agonist induced an antidepressant-like action in the forced swimming test. More recently, it was demonstrated that mice with this 5-HT_{2B} deficient phenotype exhibit schizophrenia-like behaviours as well as a decrease in DA and glutamate concentrations in the dorsal striatum (Pitychoutis, Belmer, Moutkine, Adrien, & Maroteaux, 2015). Interestingly, the adjunct medication aripripazole shows the highest affinity for this receptor, and it has been demonstrated that a selective 5-HT_{2B} antagonist was able to rescue the escitalopram induced decrease in DA neuron firing (Hamati, El Mansari, & Blier, 2019). While it remains to be confirmed in humans, the evidence suggests that 5-HT_{2B} antagonists may have an application in the treatment of depression.

5-HT_3 receptor

5-HT_3 receptors have been discovered to be present in the cortex, amygdala, hippocampus, nucleus accumbens, hypothalamus and other regions of the brain known to be involved in MDD (Kilpatrick, Jones, & Tyers, 1987). There are two known subtypes of the receptor: 5-HT_{3A} and 5-HT_{3B}. Unlike other 5-HT receptors which are G-protein coupled, the $5HT_3$ receptors are transmembrane ligand-gated ion channels (Nichols & Nichols, 2008). Evidence suggests that only the 5-HT_{3A} receptors are present in the central nervous system (van Hooft & Yakel, 2003). Although 5-HT_3 receptors present in the area postrema, which are outside of the blood brain barrier (BBB) mediate nausea produced by circulating levels of 5-HT due to SSRIs or chemotherapy, an antagonist for the receptor has been shown to attenuate increased extracellular dopamine levels induced by direct microinfusion of 5-HT into the nucleus

accumbens in rats (J. Chen, van Praag, & Gardner, 1991). Interestingly, an SSRI with 5-HT_3 antagonizing properties (litoxetine) had been developed and shown not to produce the elevated early nausea produced by SSRIs. (Angel, Schoemaker, Prouteau, Garreau, & Langer, 1993). Also, the tetracyclic medication mirtazapine used to treat depression is an antagonist for this receptor (Berendsen, Broekkamp, & Pinder, 1998).

5-HT_4 receptor

The highest 5-HT_4 receptor densities in the brain are found in the limbic system, as well as the cortico-striatal-tectal pathway and the septo-hippocampal-habenulo-interpenduncular pathway (Eglen, Wong, Dumuis, & Bockaert, 1995). There are at least eight subtypes of this receptor (5-HT_{4A-H}), and in addition to being implicated in memory and learning, there is considerable evidence that they are involved in mood and anxiety (Nichols & Nichols, 2008). Due to their expression in the hippocampus, electrophysiological studies have examined and confirmed that the 5-HT_4 receptors are involved in long term potentiation (LTP) and long term depression (LTD) in the CA1 region (Kemp & Manahan-Vaughan, 2004). Additionally, they have been found to modulate GABA and DA release which is of significant importance in the treatment of MDD (Bockaert, Claeysen, Compan, & Dumuis, 2004).

Indeed, it was shown that selective antagonists for the 5-HT_4 receptor produce anxiolyticlike effects in rats (Kennett, Bright, Blackburn, & Sanger, 1997). More recently, it was shown that treatment with a 5-HT_4 receptor agonist in rats for three days produced behavioural and biochemical responses similar to chronic administration of SSRIs (Lucas et al., 2007). It remains to be seen whether or not this will translate to the production of a 5-HT_4 receptor-specific medications for the treatment of MDD.

5-HT$_5$ receptor

There are two known subtypes of the 5-HT$_5$ receptor (5-HT$_{5A,B}$) however, only the 5HT$_{5A}$ receptor has been cloned in humans (Rees et al., 1994). This receptor is mostly limited to the central nervous system where it is expressed in the olfactory bulb, neocortex and habenula (Grailhe et al., 1999). While the presence of these receptors in limbic regions of the brain suggests their possible involvement in mood regulation, our knowledge of the pharmacology of the receptor is hindered by the absence of a selective ligand. As such, not much is known about their potential therapeutic contributions to the treatment of MDD.

5-HT$_6$ receptor

The 5-HT$_6$ receptor is expressed widely in the brain in regions including the striatum, nucleus accumbens, cortex, hippocampus, hypothalamus and amygdala (Gerard et al., 1997). Blockade of the 5-HT$_6$ receptor has been shown to result in enhanced cholinergic activity as well as increased glutamate levels in the cortex (Dawson, Nguyen, & Li, 2000; Reimer et al., 2003). Additionally, Dawson and colleagues demonstrated that drugs acting at the 5-HT$_6$ receptor site are also capable of modifying the activity of dopamine and GABA (Dawson et al., 2000). The mechanisms by which this is possible remain unclear however. Nevertheless, the evidence for antidepressant activity at the 5-HT$_6$ receptor is promising, as is demonstrated by a preclinical study in which a 5-HT$_6$ receptor agonist induced antidepressant-like effects similar to those observed by the administration of fluoxetine in mice (Svenningsson et al., 2007).

5-HT$_7$ receptor

Using in-situ hybridization studies, 5-HT$_7$ receptor mRNA has been shown to be present in the hypothalamus, thalamus, hippocampus and cortex (Bard et al., 1993). Once again, our knowledge of the pharmacology of the 5-HT$_7$ receptor is limited by the lack of availability of a specific agonist for the receptor. Most agonists for this receptor also show affinity for the 5-HT$_{1A}$ and a_{2A} -adrenergic receptor (Bonaventure et al., 2004). However, it has been demonstrated that

antagonism at the 5-HT_7 receptor results in dysregulation of body temperature as well as rapid eye movement (REM) sleep (Thomas & Hagan, 2004; Thomas et al., 2003). There is also significant evidence for the involvement of this receptor in depression. One example is that mice lacking the 5-HT_7 receptor show reduced immobility in the forced swimming test (FST) compared to controls, suggesting an antidepressant phenotype (Guscott et al., 2005). Additionally, antagonists for this receptor facilitate the anti-immobility effect of medications for the treatment of MDD (Wesolowska, Tatarczynska, Nikiforuk, & Chojnacka-Wojcik, 2007). For example, administration of the 5-HT_7 receptor antagonist SB-269970 for 7 days, was shown to produce behavioural, electrophysiological and neuro-anatomical changes similar to those observed after chronic SSRI administration (Mnie-Filali et al., 2011). It has also been suggested that the antidepressant activity of certain medications such as aripripazole is at least in part mediated by the 5-HT_7 receptor (Sarkisyan, Roberts, & Hedlund, 2010).

NE System:

Research on the mechanism of action of tricyclic medications revealed that in addition to their effects on the 5-HT system, they also significantly modulate the NE system. In fact, in a study of 25 medications for the treatment of depression, it was found that 72 % were more potent at blocking uptake of NE than 5-HT (Richelson & Pfenning, 1984). Additionally, the principal metabolite of NE in the brain is 3-methoxy-4-hydroxyphenylglycol (MHPG), and while studies have failed to find a consistent relationship between altered MHPG levels and MDD, patients with MDD have been shown to have a higher excretion of catecholamines compared to controls (Schatzberg et al., 2004). In addition to this, patient response to medications which target the NE system is evidence that there is a dysregulation of the system in MDD (Anand & Charney, 2000).

It is also important to note that the NE system is heavily involved in the hypothalamicpituitary-adrenal (HPA) axis stress response (Gold & Chrousos, 2002). As such, it can be conceived that dysregulations in this system have reciprocal interactions with symptoms

consistent with mood and anxiety disorders. For example, it has been shown that exposure of rats to stressors such as uncontrollable foot shocks results in significant decrease in brain NE levels as well as depression-like symptomatology (Weiss et al., 1994). In further support for the activity of NE in the antidepressant response, several electrophysiological studies in rats by Béïque and colleagues have shown that the antidepressant medication venlafaxine increases the time required for a 50% recovery (RT_{50}) of the firing activity of dorsal hippocampal CA3 pyramidal neurons after suppression by microiontophoretic application of both 5-HT and NE (Béïque, De Montigny, Blier, & Debonnel, 1998, 1999). The results of these experiments demonstrate that the combined activity of NE and 5-HT contribute to the efficacy of venlafaxine in the treatment of MDD.

Distribution of NE neurons

NE is synthesized from tyrosine through a series of reactions involving conversion to ldihydroxyphenylalanine (l-DOPA) and then conversion to DA before the addition of a hydroxy group to form NE (Galvin, 1985). NE neurons in the brain originate mainly from the locus coeruleus (LC), located in the brainstem reticular formation at the level of the isthmus (Moore & Bloom, 1979). They project to various brain regions including the amygdala, hippocampus, hypothalamus and frontal cortex (Moret & Briley, 2011). These are regions of the brain implicated in emotional and cognitive processing, functions that have been shown to be impaired in MDD. A radioautography study by Descarries and Droz showed that exogenous NE is mainly stored in nerve endings in presynaptic axons (Descarries & Droz, 1970). There are three major ascending pathways projecting from the LC. The first, and largest ascending projection is to the mesencephalic tegmentum, otherwise known as the dorsal catecholamine bundle which extends from the substantia nigra to the cerebral aqueduct (Moore & Bloom, 1979). It projects to regions of the brain which are known to be involved in emotional processing. A second projection enters the central gray and ascends a component of the dorsal longitudinal fasciculus, while the third

runs ventrally from the LC to the mesencephalic tegmentum in the central tegmental tract and ascends through the VTA into the medial forebrain bundle (Moore & Bloom, 1979). There are two broad classes of NE receptors with subtypes in each class, α-adrenergic and β-adrenergic receptors respectively.

α-adrenergic receptors

These receptors are differentiated from the β-adrenergic receptors by their role in modulating the release of catecholamines from nerve terminals (Langer, 1976). There are two subtypes of this receptor, the α_1 and α_2- adrenergic receptors (Gold & Chrousos, 2002). It is widely accepted that α_1- adrenergic receptors are located postsynaptically and are excitatory, while α_2 – adrenergic receptors are located presynaptically and are inhibitory (Bylund, 1992; Curet & de Montigny, 1988).

α_1- adrenergic receptors

Initial evidence for the α_1-adreno receptor came from studies on smooth muscle contraction (McGrath, Brown, & Wilson, 1989). Battaglia and colleagues reported that both phentolamine and WB4104 inhibited [^{3}H]prazoin binding in the rat frontal cortex in such a way that was consistent with the existence of more than one receptor, suggesting the existence of more than a single subtype of α_1 -adreno receptor (Battaglia, Shannon, Borgundvaag, & Titeler, 1983). This was confirmed by Morrow and Creese who coined the α_{1A} and α_{1B} subtypes based on the affinity of binding of these ligands at the various receptors (Morrow & Creese, 1986). Subsequently, radioligand studies were performed which confirmed this finding (Harrison, Pearson, & Lynch, 1991).

Northern blot analysis showed that α_{1A} is most abundant in the vas deferens, followed by the hippocampus, cerebral cortex, aorta, brain stem, heart and spleen (Bylund, 1992). While α_{1B}

is most abundant in the liver, heart, cerebral cortex, lateral geniculate nucleus (LGN) of the thalamus, brain stem, kidney, lungs and spleen (Bylund, 1992). It has also been demonstrated that a_{1B} receptors mediate a rapid increase in the formation of inositol 1,4,5-riphosphate and promote the release of calcium from intracellular stores (Han, Wilson, & Minneman, 1990; Wilson & Minneman, 1990). On the other hand, the α_{1A}- adreno receptors appear to mediate a signal transduction mechanism that is dependent on the influx of extracellular calcium (Bylund, 1992).

α_2- adrenergic receptors

As with α_1-adrenoreceptor subtypes, evidence of α_2 receptor subtypes derives from radioligand studies which showed that antagonists at this receptor site such as prazosin, oxymetazoline, and ARC239 were found to have different affinities in inhibiting [^{3}H]yohimbine binding to various rat tissues (α_{2B}) compared to human blood platelets (α_{2A}) (Bylund, 1992). Subsequently, a third subtype was identified from studies on opossum kidney (α_{2C}), and a fourth in bovine pineal gland (α_{2D}) (Blaxall, Murphy, Baker, Ray, & Bylund, 1991; Simonneauz, Ebadi, & Bylund, 1991).

Of the two main families of adrenergic receptors, the α_2 adrenoreceptors have shown the most relevance in the treatment of MDD. Animal studies by Blier and colleagues have demonstrated that the chronic administration of the α_2 antagonist mirtazapine, in rats, resulted in an increase in the firing activity of dorsal raphe nucleus (DRN) 5-HT and LC NE neurons (N Haddjeri, Blier, & De Montigny, 1996; Nasser Haddjeri & Blier, 1995). Additionally, sustained administration of the antidepressant medication bupropion had been shown to initially decrease the firing activity of NE neurons through the activation of α_2 adrenergic receptors, which then become desensitized, allowing an increase in firing rate and pattern. (Dong & Blier, 2001;

Ghanbari, El Mansari, & Blier, 2010). Furthermore, there are α_2- adrenergic autoreceptors on NE terminals which also desensitize following prolonged administration of drugs such as bupropion and reboxetine (Szabo & Blier, 2001). Finally, there are α_2 -adrenergic receptors on 5-HT terminals, and it has been shown that administration of mirtazapine in combination with paroxetine is more effective in the treatment of depression in patients than monotherapy alone (Blier, Gobbi, Turcotte, De Montigny, & Debonnel, 2009). These and other similar findings provide support for the involvement of α_2 adrenoreceptors in the pharmacological response to several types of medications in the treatment of MDD.

β-adrenergic receptors

Activation of the β-adrenergic receptors results in stimulation of adenylate cyclase which leads to increased intracellular levels of cAMP (Alexander, Davis, & Lefkowitz, 1975). These receptors belong to the family of G-coupled receptors, and radioligand studies have revealed the presence of at least three subtypes of β-adrenergic receptors ($\beta_{1, 2, 3}$) (Stiles, Caron, & Jefkowitz, 1984; Wallukat, 2002). Due to their involvement in myocardial metabolism regulation, they are mostly implicated in asthma and disorders of the cardiovascular system (Taylor, 2007). It has been demonstrated however, that chronic administration of medications for MDD can result in sustained activation of cAMP in specific brain regions, leading to upregulation of certain target genes such as brain derived neurotrophic factor (BDNF) in the hippocampus and cerebral cortex (Duman, Heninger, & Nestler, 1997). Additionally, studies have found evidence of increased $5HT_2$ and β-adrenergic receptor binding sites in the brains of individuals who died by suicide compared to healthy controls (Arango et al., 1990; Mann, Stanley, & McBride, 1986). Further support for the involvement of β-adrenergic receptors in the antidepressant response can be gleaned from the finding that the tricyclic antidepressant medication desipramine modulates these receptors and desensitizes them (Lacroix, Blier, Curet, & de Montigny, 1991; Lafaille,

Welner, & Suranyi-Cadotte, 1991). Nevertheless, one main objection to the β- adrenergic hypothesis for explaining the antidepressant response is the fact that the β-adrenergic antagonist propranolol, which penetrates the BBB does not produce an antidepressant effect.

DA system:

DA was not recognized as a neurotransmitter until the late 1950s (Carlsson, Lindqvist, & Magnusson, 1957; Carlsson, Lindqvist, Magnusson, & Waldeck, 1958; Montagu, 1957). As such, early studies of depression and the antidepressant response focused on 5-HT and NE. A compelling case was made for the involvement of the DA system in depression however, by Randrup and colleagues in 1975 and again in 1977 where they published findings showing that administration of several antidepressant medications resulted in significant DA reuptake inhibition (Randrup & Braestrup, 1977; Randrup et al., 1975). Since then, various studies in animals and humans have confirmed that there is a dysregulation of the DA system in MDD and other mood and anxiety disorders (Dunlop & Nemeroff, 2007; Nemeroff & Owens, 2002; Tye et al., 2013; Willner, 1983).

Using microdialysis in animals, it was shown that monoamine oxidase type A (MAO-A) inhibitors which are effective in the treatment of depression, produce a marked increase in DA output (Colzi, D'Agostini, Kettler, Borroni, & Da Prada, 1990). Additionally, one of the key symptoms of depression is loss of pleasure in activities that were formerly pleasurable or rewarding, otherwise known as anhedonia (DSM-5, 2015). Various studies have shown that activation of the brain DA system is essential in order to feel the rewarding effects of a drug or activity (Stein, 2008; Wise, 2008). Also, in addition to modulation of the DA system by MAO inhibitors, it has been shown that bupropion, a medication with significant antidepressant properties, induces a low occupancy of striatal DA transporter, observed 3 to 24 hours after repeated administration for 11 days in healthy volunteers (Learned-Coughlin et al., 2003). As

such, it is widely accepted that an increase in DA activity is pertinent to the antidepressant response of several medications.

It is also clear that there is significant cross-talk between the 5-HT, NE, and DA systems as was demonstrated by Guiard and colleagues (Guiard et al., 2008). Their study in rats showed that upon selective lesioning of DA neurons, there was a decrease in the firing activity of DRN 5-HT neurons while the activity of NE neurons was increased (Guiard et al., 2008). Subsequent experiments confirmed that this interaction was not unidirectional, as lesions or depletions in the other systems also had a significant effect on the activity of DA neurons (Guiard et al., 2008). Hence, the activity of DA neurons should also be taken into account when administering medications which modulate either of the other systems.

Distribution of DA neurons

Tyrosine Hydroxylase (TH) studies have confirmed that midbrain DA neurons are localized in two main regions; the substantia nigra (SN) with neurons projecting to the striatum as well as cortical and limbic areas of the brain, and the VTA which has neurons innervating the ventral striatum as well as the ventro-medial part of the head of the caudate-putamen (Bjorklund & Dunnett, 2007). In addition to modulation by projections from DRN 5-HT and LC NE neurons, VTA DA neurons also receive modulatory positive input from the laterodorsal tegmentum (LDTg) and negative input from lateral habenula (LH) respectively (Lammel et al., 2012). It has also been established that only about half of the population of DA neurons in the VTA discharge spontaneously due to inhibitory GABA input from the ventral pallidum (Grace & Bunney, 1984; Grace, Floresco, Goto, & Lodge, 2007). Another important electrophysiological parameter especially relevant in DA neurons is bursting. Bursting signals an increase in neurotransmitter release beyond the amount discharged by tonic firing (Cooper, 2002). Additionally, bursting of DA neurons predicts reward, failure of expected reward, as well as the

organism's motivation state (Cooper, 2002). There is evidence by Johnson and colleagues that depolarization of the medial prefrontal cortex (mPFC) neurons via NMDA receptors on distal dendritic branches are responsible for bursting activity of VTA DA neurons (Johnson, Seutin, & North, 1992).

Furthermore, Grace and Lodge showed that *in vivo*, bursting of these neurons requires the activity of the LDTg (Lodge & Grace, 2006). As such, activity of certain medications used in MDD to increase the number of spontaneously discharging DA neurons, or bursting activity of these neurons in the VTA, can be attributed to an alleviation of this inhibition as has been demonstrated by several electrophysiological studies from our lab and others (Olga Chernoloz et al., 2009; Grace et al., 2007).

DA receptors

The DA receptors are G-protein coupled receptors (GPCRs), and at least five DA receptor types ($D_{1,2,3,4,5}$) have been identified thus far (Missale, Nash, Robinson, Jaber, & Caron, 1998). They are further classified into D_1-like (D_1, D_5) and D_2-like (D_2, D_3, D_4) receptors based on their ability to activate (D_1-like) or inhibit (D_2-like) adenylate cyclase, as well as sequence homologies and pharmacological activity (Boyson, McGonigle, & Molnoff, 1986; Sibley & Monsama Jr., 1992; Van Tol et al., 1992). Studies have shown that D_1-and D_2-like receptors are localized in the striatum, substantia nigra, and olfactory bulb (Levey et al., 1993). D_1 localization however, is more dense in the substantia nigra pars reticulata and entoduncular nucleus than in the external segment of the globus pallidus where there is more D_2 localization (Levey et al., 1993). Both receptor families are also present in the basal ganglia, caudate, and putamen (Levey et al., 1993).

D_1-Like DA receptors

The D_1 and D_5 receptors have similar sensitivities to antagonists and as such are often simply referred to as D_1-like receptors (Seeman & Van Tol, 1994). It has been shown however,

that DA is roughly 10 times more potent at the D_5 than the D_1 receptor (Seeman & Van Tol, 1994). Additionally, the D_1 receptor is the most widespread DA receptor, expressed on both DA and GABA neurons (Gerfen et al., 1990; Le Moine, Normand, & Bloch, 1991). The D_5 receptor on the other hand, is more scantily expressed, restricted to the hippocampus, the lateral mamillary nucleus, and the parafacicular nucleus of the thalamus (Gerfen et al., 1990; Le Moine et al., 1991). Both receptors are co-expressed on pyramidal neurons of prefrontal, premotor, cingulate and entorhinal cortex, hippocampus and dentate gyrus (Huang et al., 1992; Smiley, Levey, Ciliax, & Goldman-Rakic, 1994). The D_1 receptors are required for long-term potentiation in the hippocampus, and it can be conceived that they are involved in the long-term effects of antidepressant administration which require activation of cAMP (Lemon & ManahanVaughan, 2006; Nibuya, Nestler, & Duman, 1996). In fact, it has been shown that there is an increase in the number of D_1 DA receptors in the nucleus accumbens in patients who died by suicide only in patients receiving medications specifically for MDD (Bowden et al., 1997).

D_2-Like DA receptors

The D_2 receptors are expressed mainly in the striatum, the olfactory tubercle, and in the core and shell of the nucleus accumbens (Jackson & Westlind-Danielsson, 1994). They are also expressed in limbic regions such as the amygdala, hypothalamus, substantia nigra pars compacta, and the VTA where they are expressed on DA neurons and function as autoreceptors to inhibit the activity of the neuron in the presence of DA (Carter & Muller, 1991; Jackson & WestlindDanielsson, 1994). The antidepressant medications fluoxetine, desipramine and tranylcypromine have been shown to cause a region-specific increase in D_2 receptor mRNA (Ainsworth et al., 1998). The D_3 receptors are also more prevalent in limbic areas of the brain such as the ventromedial shell of the nucleus accumbens, and to a lesser extent in the hippocampus and medial-temporal lobe (Diaz et al., 1994).

Moreover, it has been demonstrated that administration of the $D_{3/2}$ agonist, pramipexole, which has antidepressant properties, results in an increase in tonic activation of D_2 receptors in the mPFC, and 5-HT_{1A} receptors on CA3 pyramidal neurons of the hippocampus (O Chernoloz, El Mansari, & Blier, 2012). Consequently shedding light on a possible mechanism by which the medication produces an antidepressant response. D_4 receptors are highly expressed in the frontal cortex, amygdala, hippocampus, hypothalamus and mesencephalon, with limited expression in the basal ganglia (Van Tol et al., 1991). These receptors have also been shown to modulate GABAergic neurotransmission in the cerebral cortex, hippocampus, globus pallidus and sustantia nigra pars reticulata (Mrzljak et al., 1996). Additionally, D_4 DA receptors have been observed to be elevated in schizophrenia (Seeman, Guan, & Van Tol, 1993). There is also evidence of elevated D_4 receptor levels in the basal and central nuclei of the amygdala in postmortem brain tissue of patients with depression compared to controls (Xiang et al., 2008).

Currently, all first-line medications for MDD act primarily on one or more of the monoaminergic systems (Rush et al., 2006). Although they are efficacious in the treatment of MDD, there are two pervading problems. The first, as previously stated, is the time lag to clinically relevant effects of two weeks on average (Rush et al., 2006). Considering the significant burden of MDD, and risk of suicide in some patients, this lag between treatment and onset of therapeutic effects is not optimal (Angst, Angst, & Stassen, 1999; Ferrari et al., 2013). The second major concern is remission in patients undergoing treatment (Bakish, 2001). It is estimated that even after chronic antidepressant treatment for over eight weeks, a significant proportion (roughly 30 %) of patients do not achieve remission and are considered treatment resistant (Rush et al., 2006). This has caused clinicians and scientists to seek faster acting antidepressant medications. Over the last two decades, there has been substantial interest in

glutamate modulators as possible antidepressant medications with rapid onset of activity and efficacy in a treatment resistant population of depressed patients (Berman et al., 2000; Owen, 2012).

Glutamate system

Glutamate is the most abundant excitatory neurotransmitter in the brain (Altevogt, Davis, & Pankevich, 2011; Y. Zhou & Danbolt, 2014). In addition to being the major excitatory neurotransmitter in the mammalian brain, glutamate also serves as the precursor to the major inhibitory neurotransmitter, GABA (Niciu, Ionescu, Richards, & Zarate, 2014). Thus far, several studies have provided confirmation that there is a dysregulation of the glutamate system in numerous disorders as far ranging as mood and anxiety disorders, schizophrenia, autism spectrum disorders, epilepsy, Alzheimer's, Parkinson's and even stroke (Miladinovic, Nashed, & Singh, 2015). There are two main types of receptors for glutamate: metabotropic receptors (mGLURs) which require the activity of secondary messengers to produce their effects, and ionotropic receptors which as the name implies, are a single channel through the cellular membrane permitting the flow of ions in and out of the cell (Wisden & Seeburg, 1993). Results from clinical and pre-clinical studies have implicated both types of receptors in the therapeutics of MDD (Andrzej, Chaki, Nowak, & Witkin, 2008; Palucha & Pilc, 2005).

Metabotropic glutamate receptors

There are 8 types of mGLURs (mGLUR1 – mGLUR8) with variants for certain receptors as well (Conn & Pin, 1997). They can be further subclassified into group I (mGLUR1 and mGLUR5), group II (mGLUR2 and mGLUR3) and group III (mGLUR4, 6, 7 and 8) (Masu, Tanabe, Tsuchida, Shigemoto, & Nakanishi, 1991). Similar to 5-HT_2 receptors, group I mGLURs are $G_{q/11}$ coupled, and as such their activation results in increased levels of inositol phosphate and

DAG (Masu et al., 1991). They are also implicated in the activity of mTOR and other constituents of synaptic plasticity (Page et al., 2006). These receptors have complex pharmacology profiles and have been implicated in the treatment of MDD. For example, a positron emission tomography (PET) study by Deschwaden and colleagues found that in a sample of postmortem brain tissue from MDD patients, there is decreased mGLUR5 expression (Deschwanden et al., 2011). There is also evidence that the effects of ketamine involve some mGLURs (Krystal et al., 2005; Lorrain, Baccei, Bristow, Anderson, & Varney, 2003; Sou, Chan, & Chen, 2006). Our knowledge of the mGLURs and their implications in MDD is as yet limited however, and further studies are needed in order to further elucidate their involvement in the treatment of MDD.

Ionotropic glutamate receptors

There are three main ionotropic receptors, classified based on their affinity for α-amino3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), kainic acid, or N-methyl-D-aspartic acid (NMDA) respectively (Wisden & Seeburg, 1993). As such, they are called the AMPA, kainate and NMDA receptors. Ionotropic receptors are tetrameric, with specific subunit compositions which determine the biophysiological properties of the receptor (Dingledine & Dingledine, 1999)

AMPA receptors

AMPA and kainate receptors exhibit faster open and close kinetics than the NMDA receptor and as such are major mediators of fast glutamatergic neurotransmission in the brain (Kennedy, 1989; Nicholl, Kauer, & Malenka, 1988). AMPA receptors are composed of homo- or heterotetramers composed of GluA1 – 4 subunits, each subunit contributes different properties to the channel kinetics, ion selectivity, and receptor trafficking properties of the receptor (Greger, Watson, & Cull-Candy, 2017). For instance, the GluA2 subunit confers calcium impermeability or low calcium permeability while GluA2 lacking AMPA receptors are calcium permeable. This

has important implications for NMDA channels and subsequently synaptic signalling and plasticity (Cull-Candy, Kelly, & Farrant, 2006). It is thus not surprising that AMPA subunits are distributed differentially throughout the brain depending on synaptic plasticity requirements of various brain regions (Martin, Blackstone, Levey, Huganir, & Price, 1993).

There is also evidence that AMPA receptors are involved in the induction of BDNF for neurogenesis, an effect which is seen with chronic administration of antidepressant medications (Hayashi, Umemori, Mishina, & Yamamoto, 1999; Lindefors, Ballarin, Ernfors, Falkenberg, & Persson, 1992). Additionally, although ketamine binds primarily to the NMDA receptor, studies have shown that the activity of the AMPA receptor is required for the antidepressant-like effects of the drug (El Iskandrani, Oosterhof, El Mansari, & Blier, 2015; Maeng et al., 2008; W. Zhou et al., 2014). An example of this is the ability of AMPA antagonist 2, 3-dihidroxy-6-nitro-7sulfamoyl-benzo[f]quinoxaline (NBQX) to abolish the antidepressant-like effects of ketamine in animals (El Iskandrani et al., 2015; Koike, Lijima, & Chaki, 2011).

Kainate receptors

Unlike the AMPA and NMDA receptors, kainate receptors act more as modulators of synaptic transmission and neuronal excitability than in excitatory postsynaptic complexes (Contractor, Mulle, & Swanson, 2011). Kainate receptors are capable of inhibiting or facilitating glutamate release in various brain regions, as well as in modulating excitability of various cellular components such as in the CA1 region of the hippocampus and the axon of the dentate granule cells (Melyan, Wheal, & Lancaster, 2002; Schmitz, Frerking, & Nicoll, 2000).

Understanding the pharmacological contribution of kainate receptors has been challenging due to overlapping sensitivities between the kainate and AMPA receptors (Contractor et al., 2011). And while it is now clear that kainate receptors are involved in hippocampal mossy fiber activity as well as GABAergic transmission with CA1 pyramidal calls, much of the details of these interactions are still unclear (Contractor, Swanson, & Heinemann,

2001; Rodriguez-Moreno, Herreras, & Lerma, 1997). Studies have shown however, that kainate receptors are formed by GluR5 – 7, KA-1 and KA-2 subunits (Bahn, Volk, & Wisden, 1994; Feldmeye & Cull-Candy, 1994). They are distributed widely throughout the brain, and are involved in epileptogenesis and cell death (Feldmeye & Cull-Candy, 1994; Meldrum & Garthwaite, 1990).

NMDA receptors

The NMDA receptors are perhaps the most studied of the glutamate receptors. They are widely expressed throughout the nervous system, to differing degrees depending on the developmental stage of the animal (Bozic et al., 2017). Studies have shown that the NMDA receptors play a critical role in development, synaptic plasticity, as well as learning and memory (Bozic et al., 2017). Knockout studies in mice revealed that loss of any of the GluN subunits of the NMDA receptor result in serious defects. For instance, knockout of the GluN1/ NR1 subunit results in death immediately after birth due to respiratory failure (Forrest et al., 1994). Also, reductions in the functioning of GluN1 as well as a knockout of GluN2A/NR2A subunit results in a phenotype with schizophrenia-like characteristics (Mohn, Gainetdinov, Caron, & Koller, 1999).

In order for the NMDA channel to open, two conditions must be fulfilled. The first is the binding of agonists glutamate and glycine at their respective domains, which causes the channel to go from a closed to an open conformation (Sun et al., 2002). Secondly, the cell must be depolarized, which allows the Mg^{2+} block in the channel to be released (Sun et al., 2002). Agonists of the NMDA receptor such as glycine and D-serine have shown significant efficacy in the treatment of schizophrenia (Heresco-Levy & Javitt, 2004; Tsai & Lin, 2010).

It has also been shown that there are reduced levels of the NR2A and NR2B subunits in the PFC of MDD patients compared to controls in postmortem tissue (Feyissa, Chandran, Stockmeier, & Karolewicz, 2009). Additionally, several NMDA antagonists have been assessed

for their therapeutic efficacy in the treatment of MDD including ketamine, which received approval for the treatment of depression in 2019 by the food and drug administration (FDA) in the United States (Berman et al., 2000; Dang et al., 2014; Phillips et al., 2019; Zarate et al., 2006).

Ketamine

Ketamine was synthesized in 1962 as an anesthetic agent to replace phencyclidine (PCP) (Sinner & Graf, 2008). It is still routinely used in that capacity today for surgeries, mostly as an adjunct, and in emergency rooms especially in children (Sinner & Graf, 2008). It exists as a racemic mixture of (S) and (R) enantiomers with a half-life of roughly 3 hours, and it has been established that the (S) enantiomer is more potent at binding to the active site than the (R) enantiomer (Schuttler et al., 1987; Zeilhofer, Swandulla, Geisslinger, & Brune, 1992). Ketamine binds to the PCP site of the NMDA receptor (Sinner & Graf, 2008). In order to do this, it must go through the channel. As such, the pore must already be in the open conformation. By binding to the PCP site, ketamine is then able to block the channel thus preventing the flow of ions through it (Sun et al., 2002).

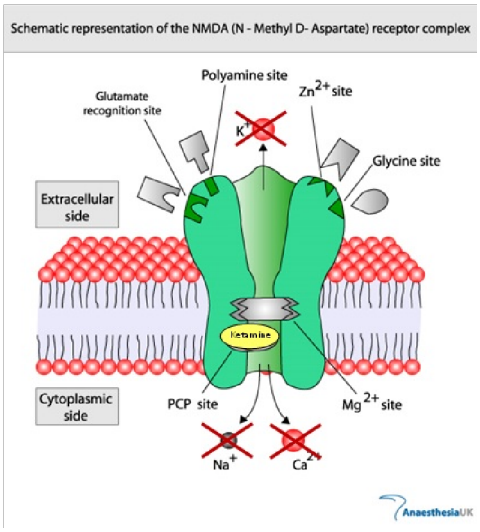


Figure 1. Schematic representation of the NMDA receptor with ketamine bound at PCP site.

In 2000, a pilot study led by John Krystal from Yale, showed that at a subanesthetic dose, ketamine is able to produce a rapid antidepressant effect in TR depressed patients (Berman et al., 2000). At a dose of 0.5 mg/kg delivered intravenously (i.v) over 40 mins, ketamine produces a reduction in depressive symptoms within 2 to 24 hours of administration in responders. These striking results were replicated in a placebo-controlled study by Zarate and colleagues. (Zarate et al., 2006), which triggered a series of studies using midazolam, a short acting benzodiazepine, as an active control (McGirr et al., 2015; Wilkinson et al., 2017). These studies established the efficacy of this strategy for the treatment of MDD. A clinical study by our lab showed that in addition to producing an antidepressant response in some patients after a single administration, the antidepressant effects of ketamine can be prolonged and enhanced by repeated administration such that more patients respond when the drug is given repeatedly (Phillips et al., 2019).

Additionally, in the basic science arm of the lab, using electrophysiology in rats, we had previously shown that when given in subanesthetic doses, ketamine produces an increase in the number of spontaneously discharging VTA DA neurons per tract, as well as an increase in the frequency of discharge and burst of LC NE neurons within 30 mins to 2 hours of administration, which may account for some of the rapid antidepressant effect of the drug (El Iskandrani et al., 2015)

Objectives of the study

Hence, the objective of the current study was to

1. Assess the presence of previously observed acute (30 mins to 2 hours) increase in VTA DA and LC NE neuron activity, 24 hours after a single sub-anesthetic dose of ketamine. The antidepressant effect of ketamine in the clinic has been shown to be transient, peaking after 24 hours and in most cases, dissipating within three to seven days (Zarate et

al., 2006). As such, we were curious as to whether or not the increase in activity of these neurons would show a similar pattern.

2. Measure DRN 5-HT neuron activity 24 hours after a single administration of ketamine. While the previous study by our lab found no changes in DRN-5HT activity 30 mins to 2 hours after a single administration of ketamine, we were interested in assessing if there would be any changes in neuron activity developing over 24 hours due to the established crosstalk between the monoaminergic systems, as well as the fact that majority of current first-line antidepressant medications act on the 5-HT system (Guiard et al., 2008; Rush et al., 2006).
3. As mentioned above, antidepressant response to ketamine can be maintained and enhanced by repeated administration (Murrough et al., 2013; Phillips et al., 2019). Thus, we investigated the effect of repeated administration of ketamine on DRN 5-HT, VTA DA and LC NE neurons in a longitudinal manner to observe the onset of possible increase in activity and the amount of time required for this increase in activity to dissipate.

Hypothesis

We hypothesized that:

1. The previously observed enhanced activity of VTA DA and NE LC neurons would still be present up to 24 hours after a single injection of ketamine but should dissipate within one week as is observed in the clinic.
2. Repeated administration of ketamine would sustain the enhanced catecholamine neuron activity.

Materials and Methods:

Animals

Male Sprague-Dawley rats, obtained from Charles River (St. Constant, Quebec) were used. They weighed between 250 – 340 g at the time of electrophysiological experiments. Rats were kept in a facility at a constant temperature of 22 ± 2 °C and housed in groups of 2 per cage under standard laboratory conditions (12:12h light-dark cycles with access to food and water ad libitum). They were not used for a week after arrival to allow for habituation. Body temperature was maintained at 37 °C during surgery and electrophysiological recordings. All animals were handled according to the guidelines of the Canadian Council on Animal Care (CCAC) and the local Animal Care Committee (Institute of Mental Health Research, Ottawa, Canada) approved all protocols.

Drug Administration

Ketamine hydrochloride was purchased from ERFA Canada Inc. (Ketalar ® Montreal, Quebec). It was dissolved in 0.9% aqueous saline solution and administered at a dose of 10 mg/kg intraperitoneally (i.p). Control rats received the vehicle (0.9% aqueous saline solution i.p.) In the single administration paradigm, rats received a single i.p. injection of ketamine (10 mg/kg while in the repeated administration paradigm, animals received the same dose of ketamine three times a week for two weeks as illustrated below.

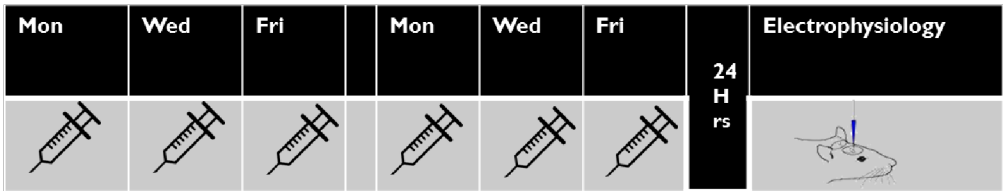


Figure 2. Repeated ketamine administration paradigm

Electrophysiological experiments were always conducted 24 hours after the last administration unless otherwise stated.

In vivo electrophysiological experiments

Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p) and placed on a stereotaxic frame (using the David Kopf Rat adaptor) with the skull positioned horizontally. Supplemental doses of anaesthetic (100 mg/kg, i.p) were given to maintain constant anesthesia and prevent any nociceptive response to palpebral reflex or pinching of the hind paw (pedal withdrawal reflex). Body temperature was maintained at 37 °C by a thermistor-controlled heating pad (Seabrook medical instruments, Saint-Hyacinthe, Quebec). Extracellular recordings of the monoaminergic neurons were performed using single-barrel glass micropipettes (Stoelting, USA) pulled on a pipette puller (Narishige, Japan) and filled with 2M NaCl solution at an impedance range of 2 – 4 MW. A burr hole was drilled at the stereotaxic coordinates corresponding to the monoaminergic structure of interest. The shape, duration of spikes, as well as the frequency of firing was used to identify neurons of interest and recorded in real-time using the Spike2 program (Cambridge Electronic Design, Cambridge, UK).

Recording of Serotonergic neurons

Electrodes were positioned 0.9 mm anterior to lambda (l) on the midline and lowered into the DRN, usually attained at a depth of 4.5 - 5.5 mm from the brain surface. The DRN 5-HT neurons were identified according to the following criteria: a slow (0.5 - 2.5 Hz), regular firing rate, long duration and a positive action potential (Sakai, Crochet 2001, Wang, Aghajanian 1982).

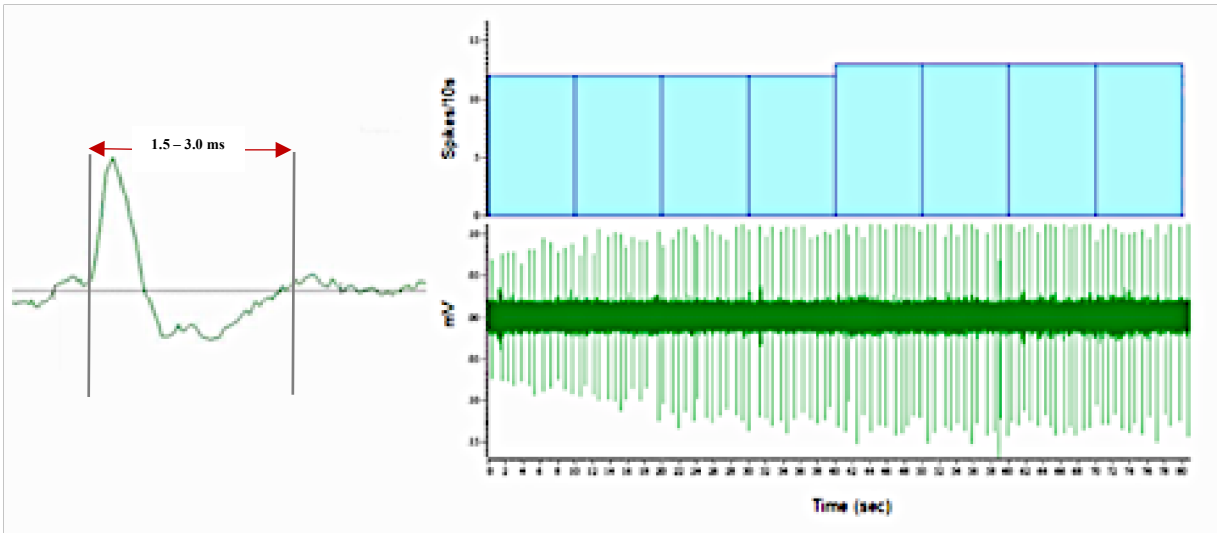


Figure 3. Electrophysiological recording of DRN 5-HT neuron. Single action potential (left) appears as uniform spikes (bottom right). Frequency of firing activity is slow and regular (upper right)

Recording of Dopaminergic neurons

Single-barrel glass micropipettes were positioned using the following coordinates from l: Anterior-Posterior (AP) +3.0 to +3.8 mm, laterally (L) 1-0.6 mm. The recording electrode was descended to a depth of 6.5 to 9 mm from the surface of the brain. Presumed DA neurons were identified according their well-established electrophysiological properties in vivo: a triphasic action potential with a marked negative deflection, a characteristic long duration (>2.5 ms) often with an inflection or 'notch' on the rising phase, a slow spontaneous firing rate (0.5 - 5 Hz) with an irregular single spiking pattern and slow bursting activity (Grace, Onn. 1989, Margolis et al. 2006).

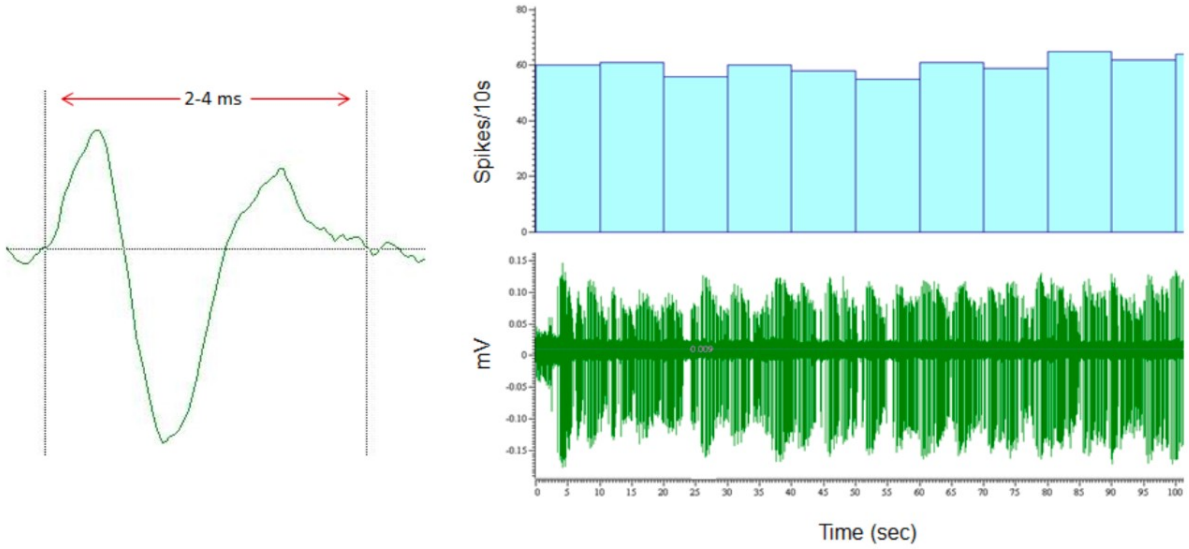


Figure 4. Electrophysiological recording of VTA DA neuron. Single action potential (left) appears as irregular spikes spikes (bottom right). Neurons often display rapid discharges (upper right), repeated bursting activity and amplitude of spikes decreasing with each discharge within a single burst.

Recording of Noradrenergic neurons

LC NE neurons were recorded with a single-barrel glass micropipette positioned at 1.1-1.2 mm posterior to λ and 0.9 - 1.3 mm from the midline suture and at a depth of 4.5 to 6.0 mm from the surface of the brain. The presumed NE neurons were identified by their regular firing rate (0.5 - 5 Hz), a biphasic action potential of long duration (~2 ms) and a characteristic burst discharge followed by a quiescent period in response to a nociceptive pinch of the contralateral hind paw (Cedarbaum, Aghajanian. 1977).

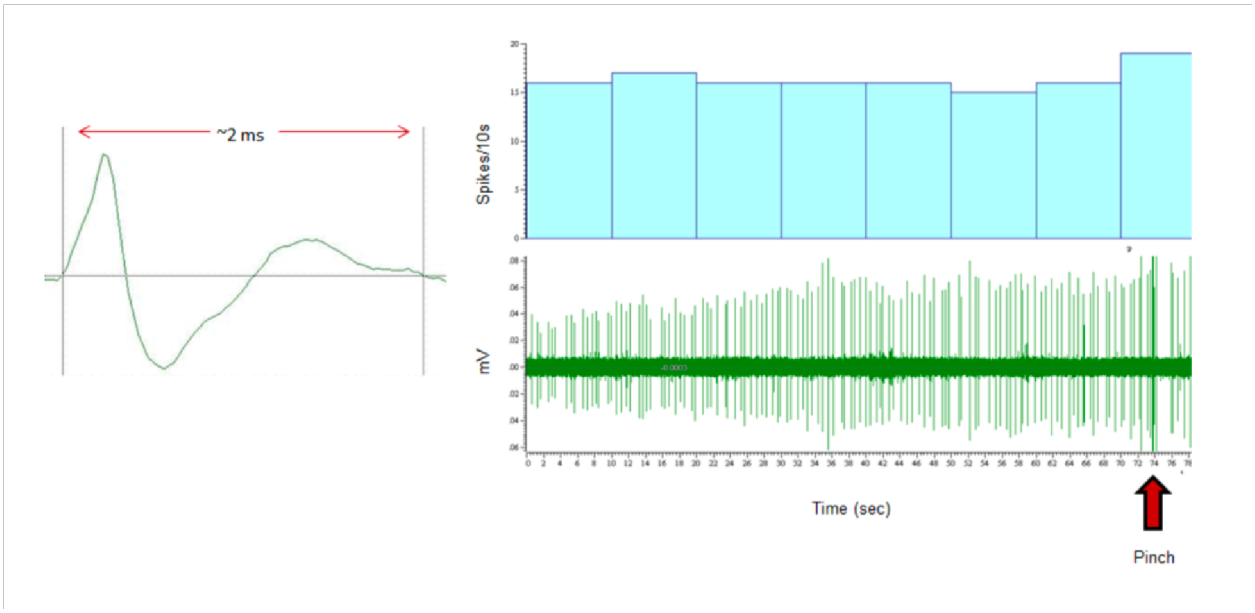


Figure 5. Electrophysiological recording of LC NE neuron. Single action potential (left) appears as uniform spikes (bottom right). Frequency of firing activity is regular, but higher than DRN 5-HT firing (upper right). NE neurons display rapid firing potentials followed by a period of pinch (arrow). silence in response to contralateral paw

Analysis

Data are expressed as means ± standard error of the mean (SEM). Firing activity of monoaminergic neurons were analyzed using a spike sorting software (www.github.com/nno/birstidator/releases). For bursting activity, a fixed threshold-based model was used, with the start of a burst signified by the occurrence of two spikes with interspike interval (ISI) < 0.08 s for NE and DA neurons, and <0.01 s for 5-HT neurons. The termination of a burst was defined as an ISI < 0.16 s for DA and NE (Dawe et al. 2001, Grace and Bunney 1983) and ISI > 0.01 s for 5-HT (Hajos and Sharp 1996).

Between group comparisons were carried out using one-way analysis of variance (ANOVA), followed by Tukey post hoc test. Statistical analysis and plots were done using Graphpad software (Prism Software Inc, La Jolla, CA) and Microsoft excel. Statistical significance was taken as $p < 0.05$.

Results:

Single administration paradigm

Dorsal raphe nucleus 5-HT neurons

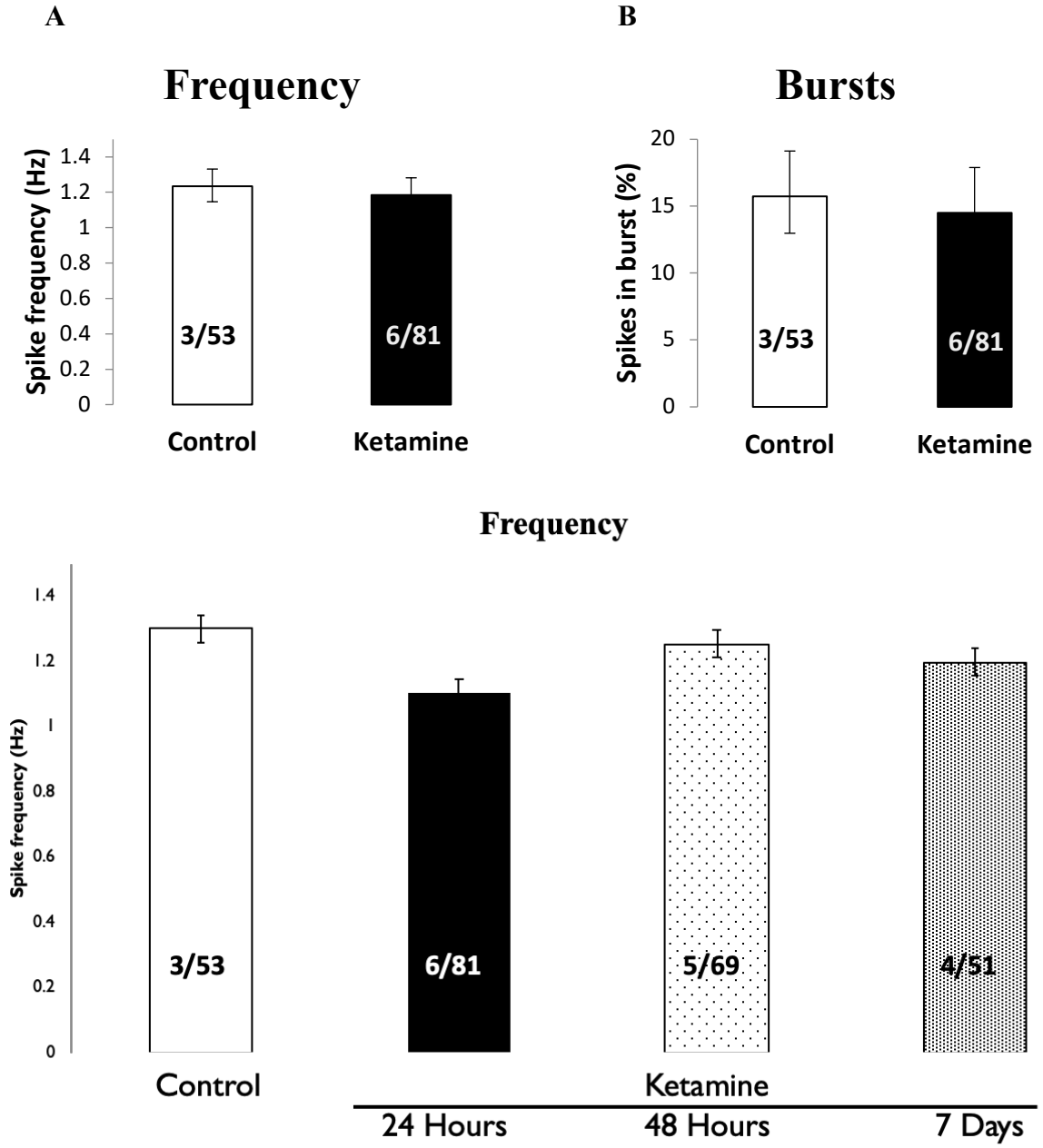


Figure 6: Effects of a single administration of ketamine on DRN 5-HT neurons. Mean (± SEM) firing rate and spikes occurring in bursts (A and B respectively). Numbers in bars refer to number of rats/number of neurons respectively. Figure 7: Effect of a single administration of ketamine on firing rate of DRN 5-HT neurons after 24 hours, 48 hours, and 7 days after a single administration respectively.

24 hours after a single administration of ketamine, there was no significant difference in the frequency of discharge Fig. 6A (controls: 1.30 ± 0.10, ketamine: 1.10 ± 0.08; t= 1.50, p=0.136), nor in the spikes occurring in bursts Fig 6B (controls: 15.7 ± 3.4, ketamine: 14.5 ± 2.8; t=0.638, p=0.525) of DRN 5-HT neurons in treated vs control animals. This is concordant with the previous study which also found no significant difference in the activity of DRN 5-HT neurons 30 mins – 2 hours after a single administration of ketamine.

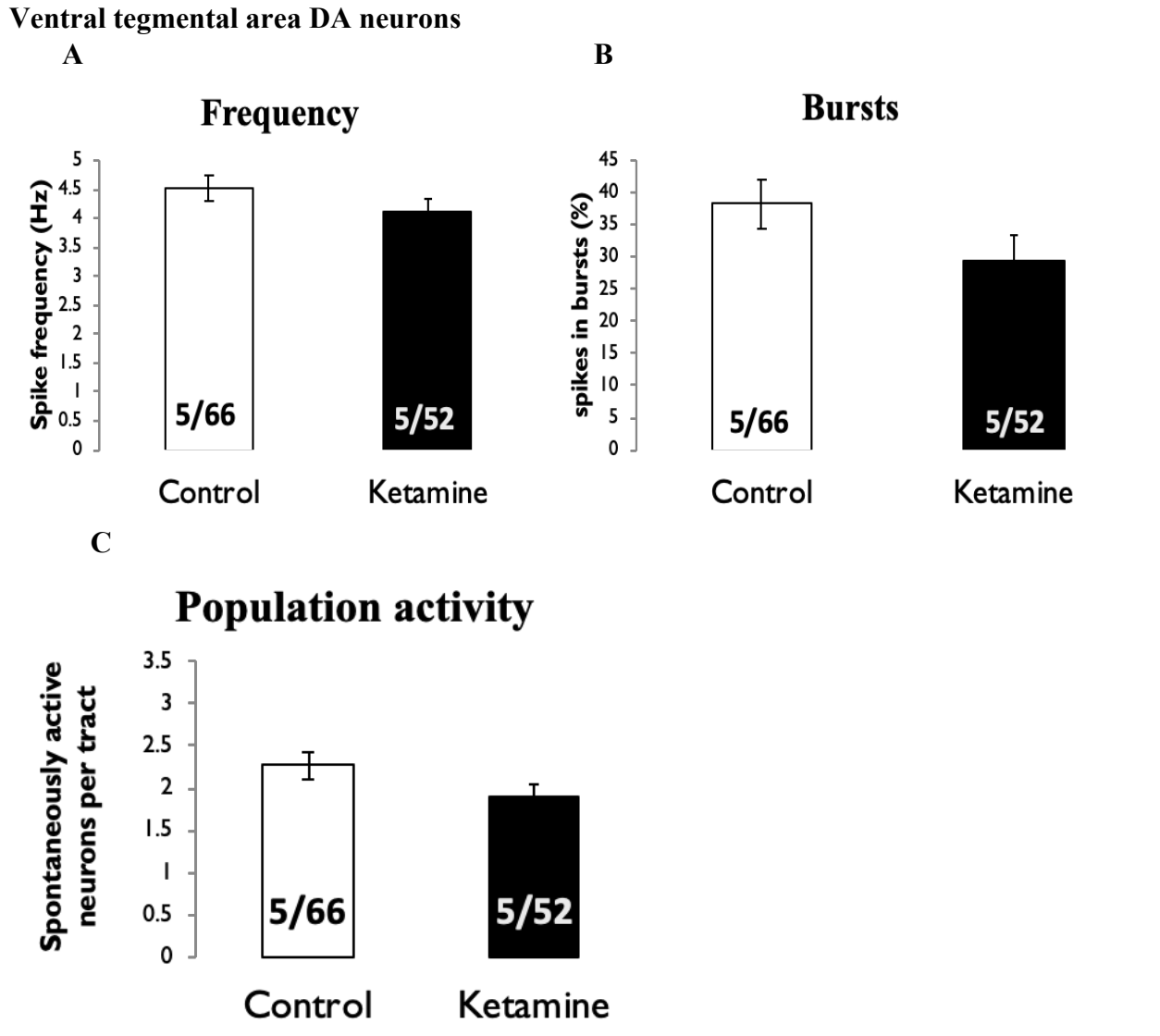


Figure 8: Effects of a single administration of ketamine on VTA DA neurons. Mean (± SEM) firing rate, spikes occurring in bursts and spontaneously active neurons per electrode descent (A, B and C respectively). Numbers in bars refer to number of rats/number of neurons respectively.

In VTA DA neurons, the previously observed (30 mins – 2 hrs) increase in spontaneously discharging neurons per tract was not present 24 hours after a single administration of ketamine Fig 7C (control: 2.3 ± 0.16, ketamine: 1.9 ± 0.19; t=2.20, p= 0.06). Additionally, neither the frequency of discharge nor spikes occurring in bursts were significantly different in treated compared to controls Fig 7A (controls: 4.52 ± 0.22, ketamine: 4.36 ± 0.25; t=1.317, p=0.190), Fig 7C (controls: 38.32 ± 3.8, ketamine: 29.40 ± 3.8; T= 2587, p=0.101), respectively.

Locus coeruleus NE neurons

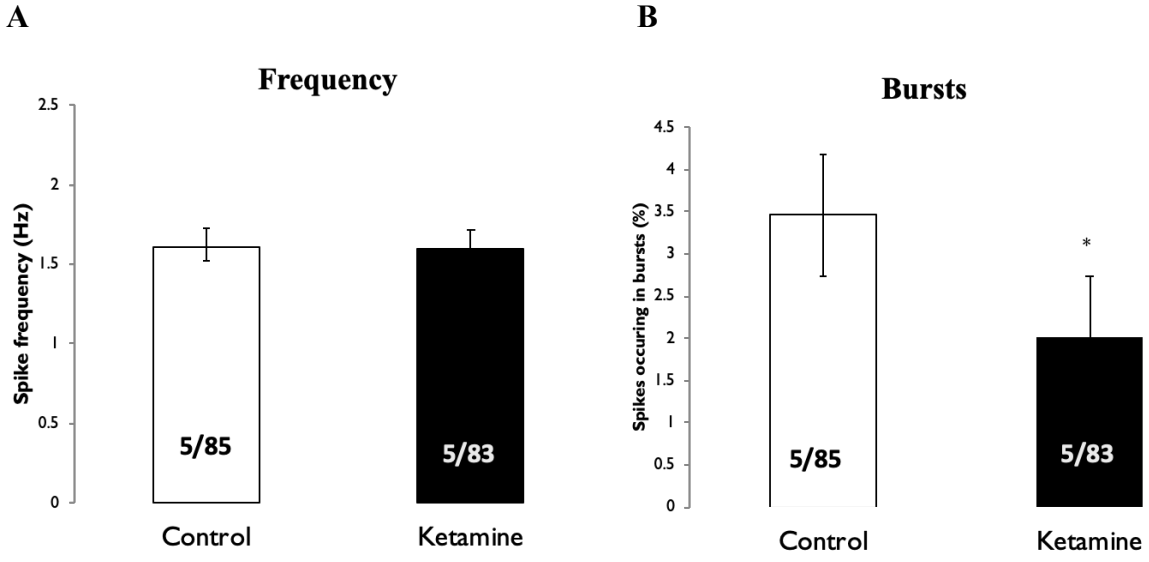


Figure 9: Effects of a single administration of ketamine on LC NE neurons. Mean (± SEM) firing rate and spikes occurring in bursrts (A and B respectively). Numbers in bars refer to number of rats/number of neurons respectively.
* p<0.05, ** p < 0.001

The previously reported increase (30 mins – 2 hrs) in frequency of discharge and bursts of LC NE neurons was not observed 24 hours after a single administration. On the contrary, spikes occurring in bursts was slightly but significantly decreased Fig 8A (control: 1.57 ± 0.09, ketamine: 1.60 ± 0.08; T= 7217, p=0.520), Fig 8B (control: 5.0 ± 1.70, ketamine: 2.0 ± 0.5; T= 791, p= 0.01) respectively.

Repeated administration paradigm

Repeated administration of ketamine has been shown to enhance and maintain the antidepressant effect in the clinic. Hence, the same paradigm was applied: rats received three administrations a week for two weeks while controls received saline. Subsequently, the activity of DRN 5-HT, VTA DA and LC NE neurons was measured 24 hours after the last of six injections.

Dorsal raphe nucleus 5-HT neurons

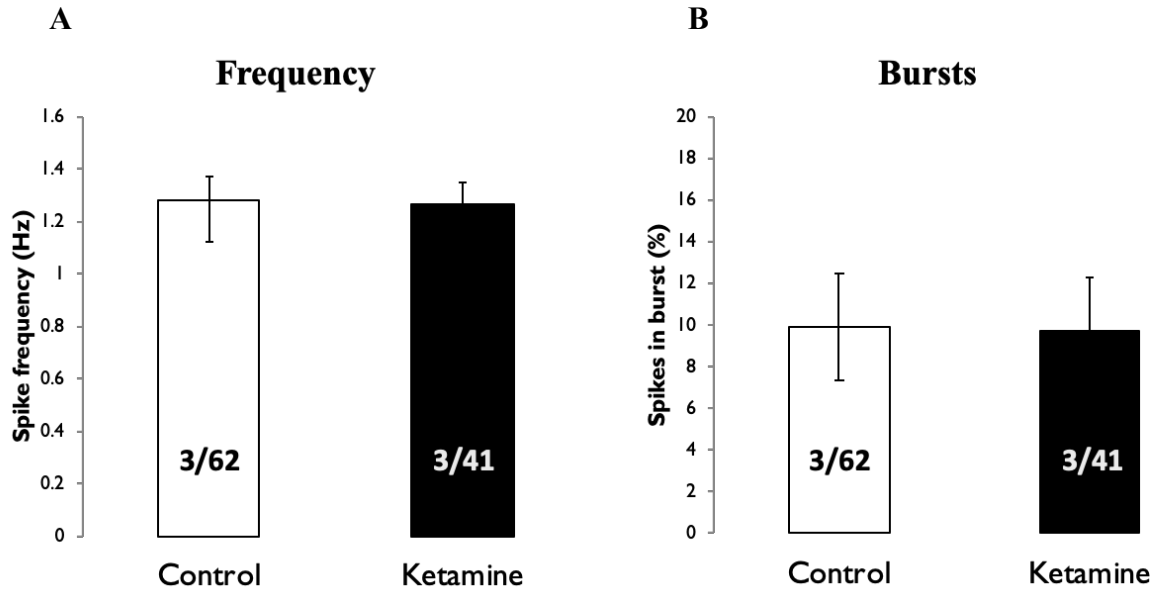


Figure 10: Effects of repeated administration of ketamine on DRN 5-HT neurons. Mean (± SEM) firing rate and spikes occurring in bursts (A and B respectively). Numbers in bars refer to number of rats/number of neurons respectively.

Twenty four hours after repeated administration of ketamine, there was no significant difference in the spike frequency nor burst of DRN 5-HT neurons compared to controls. Fig 9A (control: 1.28 ± 0.09, ketamine: 1.26 ± 0.16; $t = 0.107$, $p = 0.915$), Fig 9B (control: 10.0 ± 2.6, ketamine: 9.75 ± 2.6; $t = 0.139$, $p = 0.89$).

Ventral tegmental area DA neurons

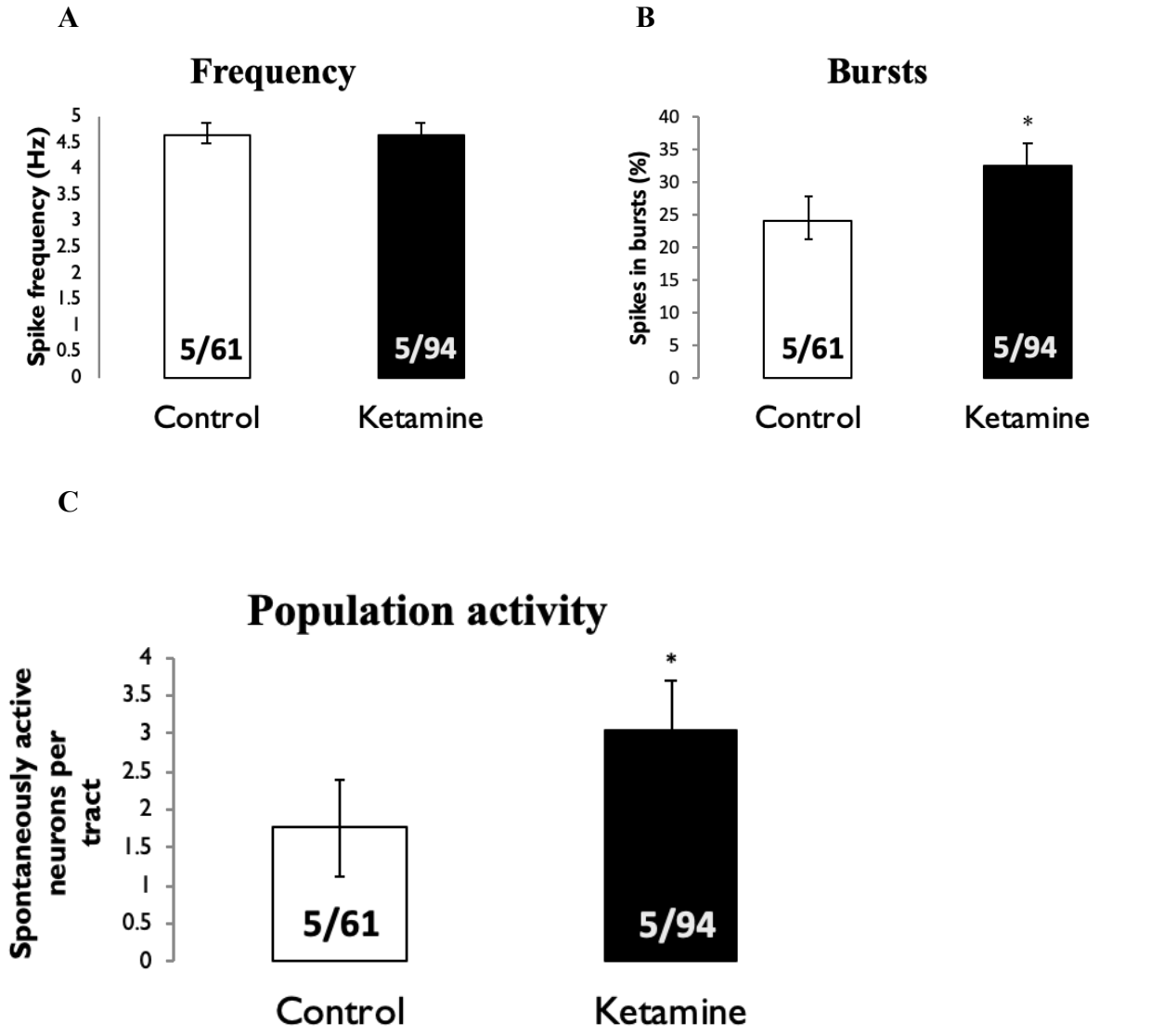
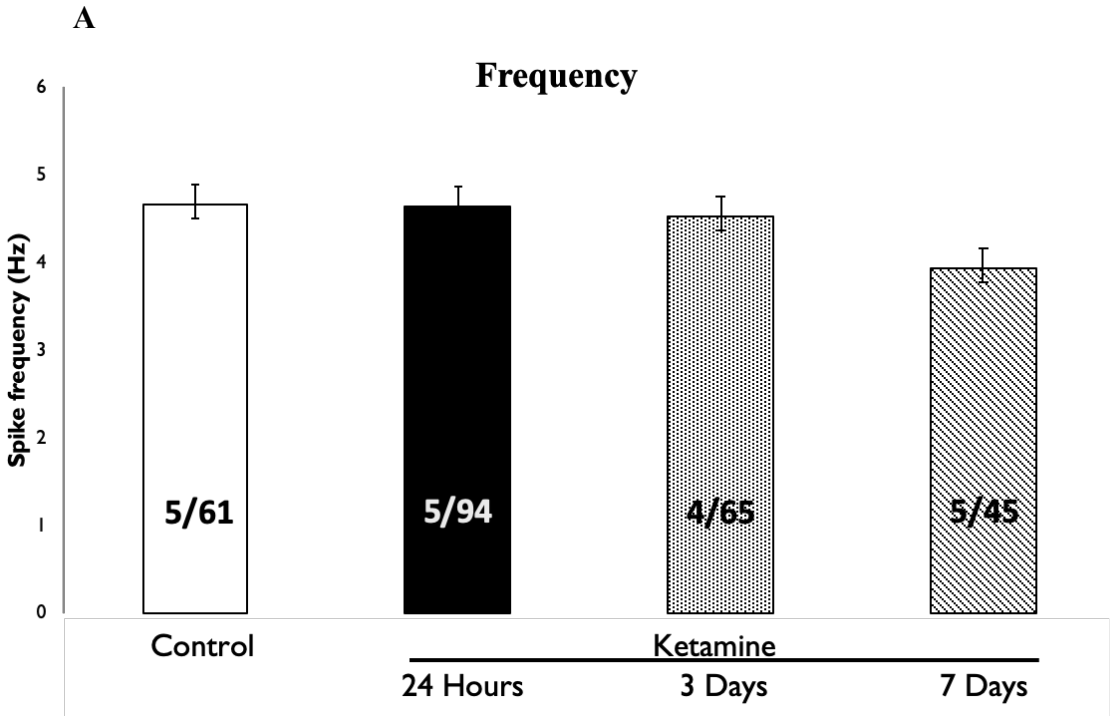
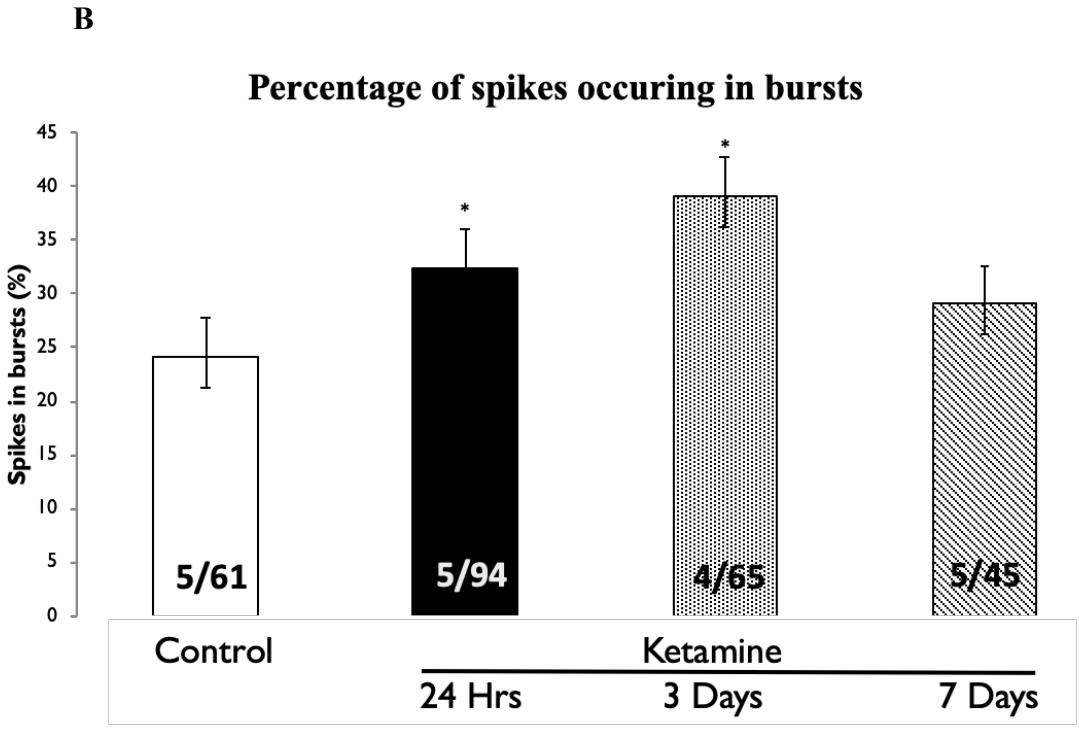


Figure 11: Effects of a repeated administration of ketamine on VTA DA neurons. Mean (± SEM) firing rate, spikes occurring in bursrts, and population activity (A, B and C respectively). Numbers in bars refer to number of rats/number of neurons respectively.
* $p<0.05$, ** $p < 0.001$

Twenty four hours following repeated administration however, there was a significant increase in bursting (Fig 10B: control: 24 $\pm$ 3.6, ketamine: 32 $\pm$ 2.8; T = 3983, p = 0.02) and spontaneously discharging neurons per tract of VTA DA neurons (Fig 10C: control: 1.6 $\pm$ 0.11, ketamine: 3.15 $\pm$ 0.61; T = 15.5, p = 0.02). This enhancement in spontaneously discharging neurons per tract was absent 3 days after repeated administration (Fig 11C, ketamine + 3 days:

2.23 ± 0.44; $F_{3, 15} = 4.35$, $p = 0.02$). Interestingly, the increase in bursting was still significantly different after 3 days, but absent after 7 days as revealed by post hoc analysis (Fig 11B, ketamine + 3 days: 38.0 ± 3.8, $F_{3, 266} = 2.9$, $p = 0.03$).





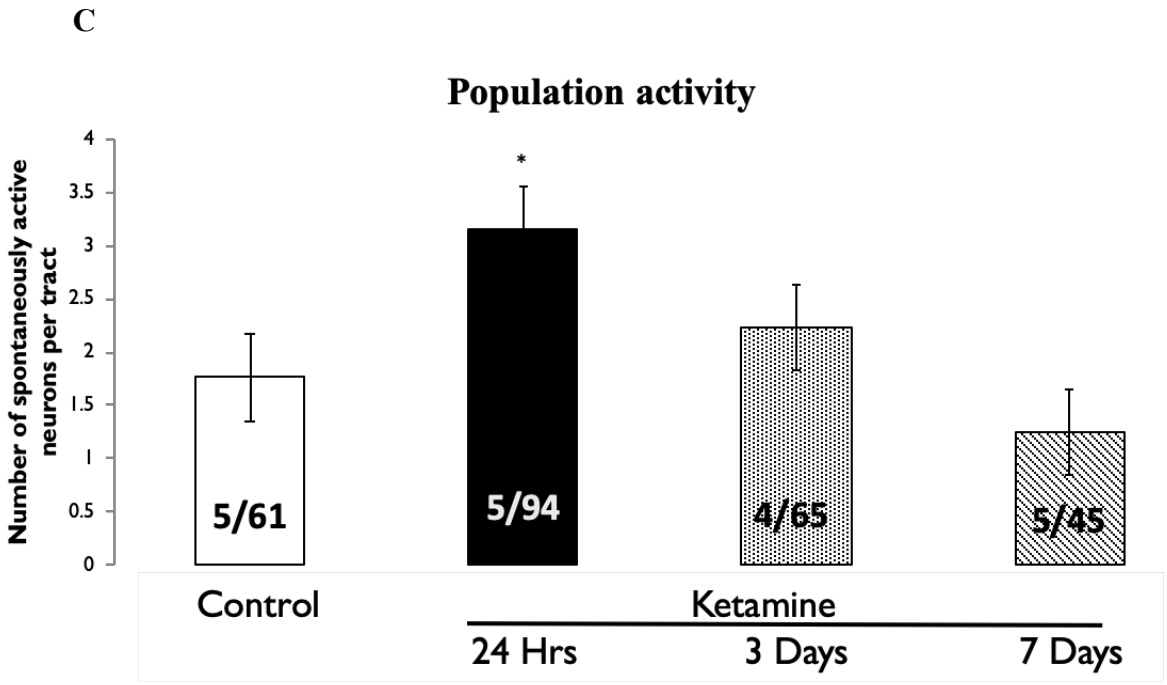


Figure 12: Effects of repeated administration of ketamine on VTA DA neurons after 3 and 7 days. Mean (± SEM) firing rate, spikes occurring in bursts, and population activity (A, B and C respectively). Numbers in bars refer to number of rats/number of neurons respectively.
* p<0.05, ** p < 0.001

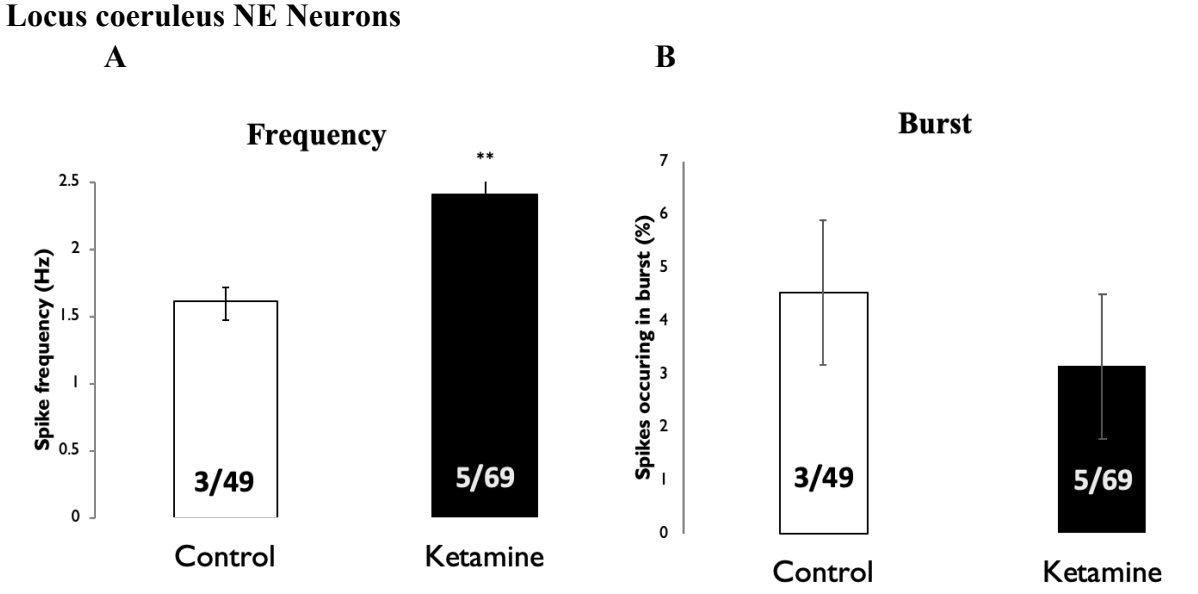
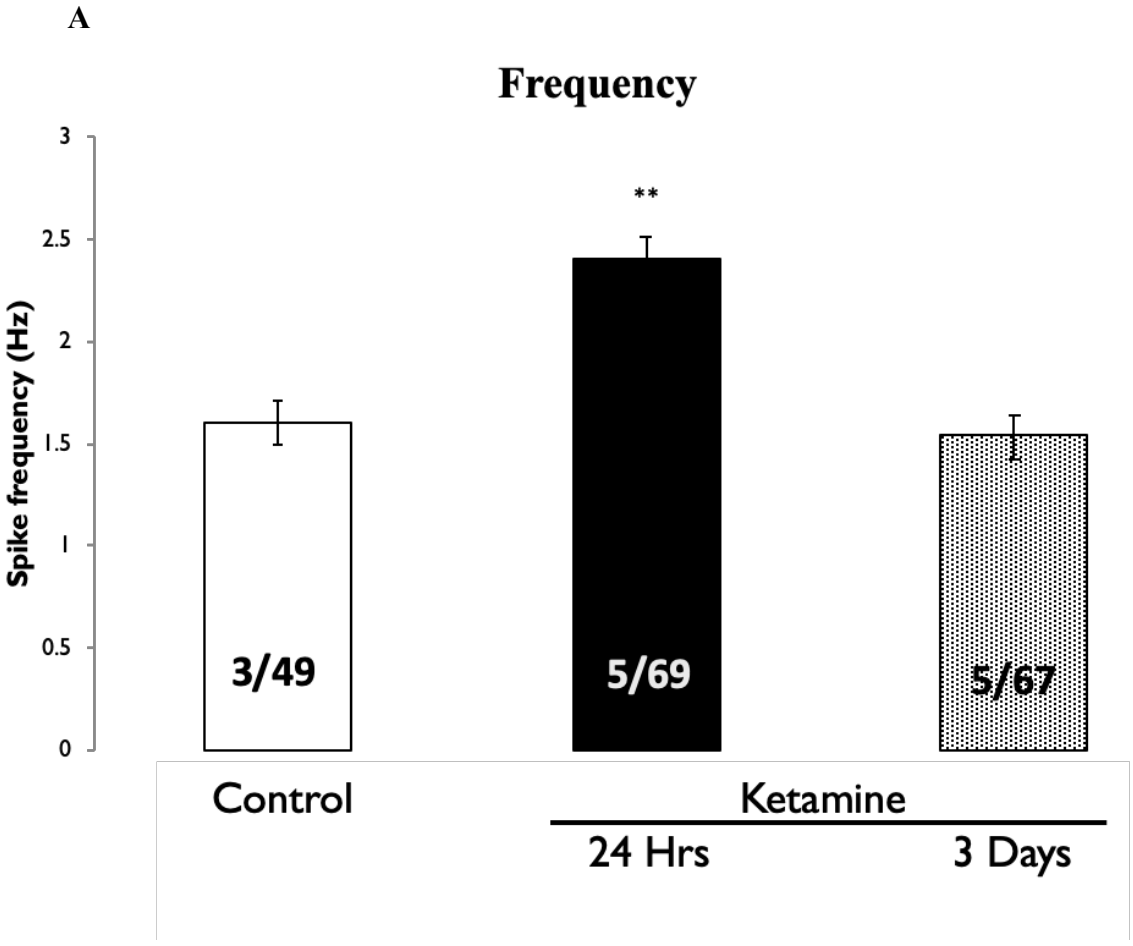


Figure 13: Effects of repeated administration of ketamine on LC NE neurons. Mean (± SEM) firing rate, spikes occurring in bursts, and spontaneously active neurons per tract (A and B respectively). Numbers in bars refer to number of rats/number of neurons respectively.
* p<0.05, ** p < 0.001

While the spikes occurring in bursts were unchanged in control compared to ketamine treated animals, Fig 12B (control: 2.0 ± 0.7, ketamine: 1.5 ± 0.4; F = 1.88, p = 0.17), the spike frequency was significantly increased in the ketamine treated group Fig 12A (control: 1.61 ±. 0.1, ketamine: 2.41± 0.12; F= 5.7, p= 0.02).

However, 3 days after repeated administration, this increase in the spike frequency was no longer significantly different Fig 13A (1.54 ± 0.08 $F_{2, 182} = 24$, $p < 0.001$). The spikes occurring in burst remained equivalent in control compared to ketamine treated animals Fig 13B ($F_{2, 182} = 0.49$, p= 0.6).



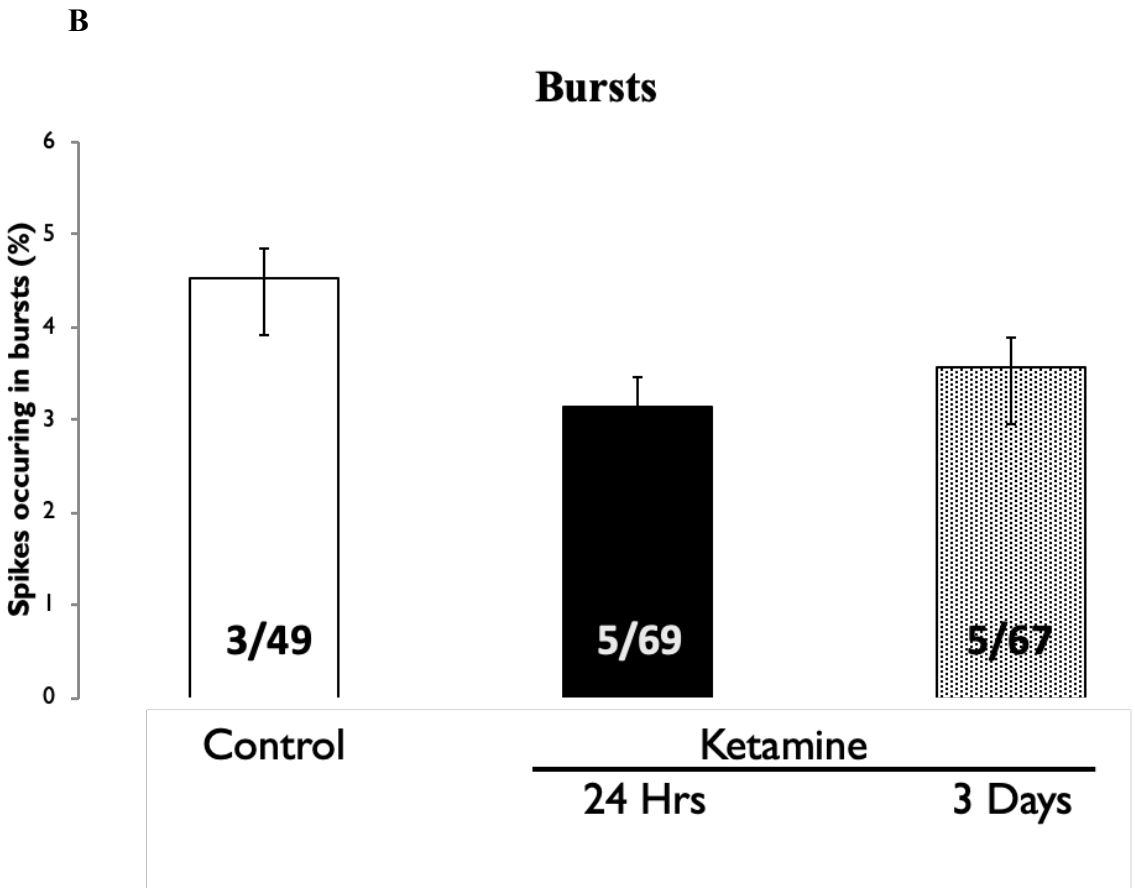


Figure 14: Effects of repeated administration of ketamine on LC NE neurons after 3 days. Mean (± SEM) firing rate, spikes occurring in bursts (A and B and respectively). Numbers in bars refer to number of rats/number of neurons respectively.

Discussion

Significant burden of disease, as well as increased risk of suicide make MDD a disorder which requires urgent attention (Angst et al., 1999; Ferrari et al., 2013). While response to firstline antidepressant medications is promising, there is a considerable lag to therapeutically relevant reduction in symptoms requiring two weeks or more (Rush et al., 2006). Additionally, only about one-third of patients achieve remission at first treatment trial and even with a change in medication or addition of an adjunct, there is still about 30 % of patients who do not achieve remission and are considered treatment resistant (Rush et al., 2006).

Within the last two decades, studies have shown that ketamine, in sub-anesthetic doses, is capable of producing a significant reduction in depressive symptoms in treatment resistant patients in 24 hours (Berman et al., 2000; Phillips et al., 2019; Zarate et al., 2006). This finding has been hailed as the biggest discovery in depression research in over 50 years, and the overwhelming evidence has led to the recognition of ketamine as an effective antidepressant strategy by the food and drug administration (Abdallah, Sanacora, Duman, & Krystal, 2015). However, the mechanisms by which ketamine produces antidepressant effects is as yet unclear.

Effect of ketamine on DRN 5-HT neurons

In a previous study, we reported on an increase in the number of spontaneously discharging VTA DA neurons per tract (population activity), as well as an increase in the frequency of discharge and bursts of LC NE neurons, while no significant change was found in the activity of DRN 5-HT neurons within 30 mins to 2 hours of ketamine administration (El Iskandrani et al., 2015). In the current study, we found that the activity of DRN 5-HT neurons was not significantly different in treated compared to control animals after 24 hours, under the parameters assessed. In addition to measuring the activity of these neurons 24 hours after a single administration, their activity was assessed after 48 hours and 7 days . This was done in order to evaluate the possibility that increased VTA DA and LC NE neuron activity might subsequently lead to an increase in the activity of 5-HT neurons via previously established crosstalk between the monoamine systems (Guiard et al., 2008). We found that even up to 7 days after a single administration, there was still no significant difference in the activity of DRN 5-HT neurons in treated compared to control animals. Next, we administered ketamine repeatedly, three times a week for two weeks and found still no significant change in the firing activity of DRN 5-HT neurons.

While the data from this study suggests no effect of ketamine on these neurons, a role for 5-HT in the antidepressant activity of ketamine cannot be ruled out yet. In fact, there is evidence

of 5-HT modulation by ketamine. A study by Gigliucci and colleagues in rats showed that administration of ketamine (25mg/kg) resulted in a decrease in the amount of time spent immobile in the FST (Gigliucci et al., 2013). This decrease was abolished by depletion of 5-HT using DL-4-chlorophenylalanine ethyl ester hydrochloride (*p*CPA), thus showing that 5-HT is required for the observed antidepressant-like response (Gigliucci et al., 2013). Also in rats, it has been shown that administration of 25 mg/kg of ketamine produces an increase in efflux of 5-HT in the mPFC (Amargós-Bosch, López-Gil, Artigas, & Adell, 2006). And a recently published study by the same group showed that following the systemic administration of ketamine in mice, there is an increase in extracellular 5-HT in the mPFC (López-Gil et al., 2019). In non-human primates, PET was used to show that subanesthetic doses of ketamine produce a transient decrease in 5-HT transporter activity, subsequently leading to increased 5-HT availability (Yamamoto et al., 2013). Interestingly, it had previously been demonstrated that ketamine inhibits the catecholamine transporters to varying degrees (Azzaro & Smith, 1977; Smith, Azzaro, Zaldivar, Plamer, & Lee, 1981).

Hence, it is clear that under the conditions utilized in the above-mentioned studies, there is an effect of ketamine on the 5-HT system. A few reasons can be suggested for why no changes were observed in the current study. The first is the possibility that the effects of ketamine on this system are more rapid and transient than effects on the DA and NE systems, appearing in under 30 mins and dissipating just as quickly. In the PET experiment by Yamamoto and colleagues in monkeys, they found that 5-HT levels in the extracellular fluid (ECF) were increased immediately following the start of ketamine infusion, rising to 2.4 times baseline levels and returning to control levels within 30 mins (Yamamoto et al., 2013). Also, using an analog NMDA antagonist MK-801 in rat brain slices, it was shown that electrical stimulation of the substantia nigra pars compacta resulted in an increase in 5-HT release only in the presence of

MK-801 (Iravani, Muscat, & Kruk, 1999). This increase was found to occur immediately upon electrical stimulation, and dissipate within 60 seconds (Iravani et al., 1999). While it is interesting to speculate on the rapid effects of ketamine on DRN 5-HT neurons, the logistics of in vivo electrophysiological recordings make it near impossible to adequately measure. One would have to ensure that a neuron would be found within minutes of drug administration, and if no increase is found, attempt to reduce the time between drug administration and recording in multiple follow-up experiments which, while possible, is inefficient. Additionally, there is some evidence that the changes in 5-HT activity due to ketamine might not be so rapid. For example, in a study of ketamine stereoisomers, both isomers caused an increased in stimulated 5-HT efflux which began after 20 mins and continued to be significantly increased for over 50 mins (Tso, Blatchford, Callado, McLaughlin, & Stamford, 2004). This study casts doubt on an argument for a rapid increase and dissipation of the firing of DRN 5-HT neurons.

The second possible explanation for the absence of a significant increase in 5-HT neuron activity in the current study is that increased 5-HT transmission is not always concordant with a change in DRN 5-HT neuron activity. An example of this is the drug Lamotrigine which upon chronic administration in rats, was been shown to decrease the firing of 5-HT cells while significantly increasing hippocampal 5-HT_{1A} tonic activation (Shim, El Mansari, & Blier, 2013). Additionally, it is known that activation of autoreceptors on the somatodendritic site causes a decrease in the firing activity of these cells, while activation of terminal autoreceptors for 5-HT modulates the release of 5-HT into the synaptic cleft (Aghajanian, 1978; Green, 1985). Hence, it can be extrapolated that in the absence of somatodendritic autoreceptor (5-HT_{1A}) activation resulting from ketamine or its metabolites, there will be no change in neuron activity. In support of this hypothesis, the study by López-Gil and colleagues found that in the DRN, no change in extracellular 5-HT concentrations was observed in treated compared to control animals (López-

Gil et al., 2019). They also found that systemic administration of ketamine did not alter the functionality of 5-HT_{1A} receptors in the DRN(López-Gil et al., 2019). As such, it can be argued that the rapid antidepressant effect of ketamine is in part because the effects of the drug are not constrained by this time-consuming step of autoreceptor desensitization, as is observed with the administration of most SSRIs, while still producing increased 5-HT transmission. However, further studies will have to be conducted to confirm or disprove this hypothesis.

Lastly, evidence suggests that glutamate modulation of DRN 5-HT neurons is both excitatory and inhibitory. In fact, the DRN which contains the highest population of 5-HT cells in the brain, is highly heterogeneous, containing glutamate and GABA neurons, as well as DA (Marinelli et al., 2004). It has been shown that in addition to a reduction in the overall firing rate of DRN 5-HT neurons shortly after the beginning of SSRI administration, there is also a reduction in the strength of AMPA receptor-mediated synaptic transmission (Geddes et al., 2015). This reduction in synaptic transmission is not only alleviated, but potentiated by sustained administration of an SSRI via minipump for 7 days (Geddes et al., 2015). The authors argue that the observed increase in AMPA receptor-mediated synaptic transmission after 7 days of SSRI administration in the rats is in response to the previous decrease in synaptic transmission after 2 days (Geddes et al., 2015). This is evidenced by a compensatory upregulation in the number of AMPA receptor-containing synapses and NMDA modulation (Geddes et al., 2015). Subsequently, it can be argued that since ketamine acts directly on NMDA receptors, and their activity has been shown to involve the AMPA receptors, homeostatic modulation of DRN 5-HT neurons is maintained and thus no change in firing activity is observed. It must be stated that evidence in support of this hypothesis is speculative and further experimentation is required in order to probe this relationship further.

Effect of ketamine on VTA DA neurons

Twenty-four hours after the administration of a single sub-anesthetic dose of ketamine, we observed that the previously reported increase in population activity of DA neurons was no longer present (El Iskandrani et al., 2015). Additionally, the bursting activity and frequency of discharge of the neurons was not significantly different in treated compared to control animals. This is in contrast with behavioural studies in animals which have shown that the antidepressant-like effects of ketamine are still present up to 7 days after a single administration (Koike et al., 2011; Maeng et al., 2008). However, there is also evidence that in animals, the rapid antidepressant-like effects of ketamine are not sustained (Popik, Kos, Kucma-Sowa, & Nowak, 2008).

Subsequently, we sought to investigate the ability of repeated administration of ketamine to enhance and extend the antidepressant response in the clinic, by measuring the change in VTA DA activity after repeated administration of ketamine in rats, utilizing the same paradigm of administration that is employed clinically (Murrough et al., 2013; Phillips et al., 2019). We found that repeated administration of ketamine was able to enhance the activity of VTA DA neurons as evidenced by an increase in the bursting of these neurons in addition to an increase in population activity 24 hours later. While the increase in population activity was not significantly different after 3 days, the bursting activity remained significant, returning to baseline by day 7.

The importance of the DA system in depression cannot be overstated. Beginning with MAO inhibitors, considered first generation antidepressant medications, we have known that medications which are effective in the treatment of depression act in part by increasing brain DA levels (Tekes, Tothfalusi, Gaal, & Magyar, 1988). And although some current first line SSRIs initially have a negative effect on the activity of DA neurons, their effect is enhanced in patients by the addition of adjuncts which increase VTA DA neuron activity (Chernoloz et al., 2009).

Additionally, it has been shown that anhedonia, one of the hallmark symptoms of MDD, is linked to dysfunctions in the DA system (Der-Avakian & Markou, 2012).

DA neurons of the mesolimbic system are necessary for predicting rewarding and motivating events (Cooper, 2002). As such, reduced neuron output, characterized as a reduction in the frequency of discharge or bursting, is associated with depressive-like symptoms. An example of this is the Flinders sensitive line rat model (Friedman, Friedman, Dremencov, & Yadid, 2008). These rats, which show increased immobility time in the forced swim test and reduced DA in the accumbens, also show decreased bursting of VTA DA neurons, which is rescued by chronic administration of desipramine (Friedman et al., 2008). In humans, it has been shown that pharmacological agents which block or decrease DA, induce depressive symptoms or worsen symptoms in already depressed patients (Kapur & Mann, 1992). Additionally, post-mortem studies revealed that in the brains of depressed subjects, there is reduced DA transporter density and elevated D2/D3 DA receptor binding in the central and basal nuclei of the amygdala (Pare, Yeung, Price, & Stacey, 1969). Also, in humans, there is evidence of antidepressant efficacy for the selective NE/DA reuptake inhibitor nomifensine (Kapur & Mann, 1992).

There is also substantial evidence in support of the activity of ketamine on the DA system. Early studies by Azzaro and Smith showed that ketamine inhibits catecholamine reuptake (Azzaro & Smith, 1977; Smith et al., 1981). And there is an indication that ketamine has affinity for the DA transporter (Lorrain et al., 2003). Over the last decade, other animal studies have investigated the effects of ketamine on the DA system. This is because of the established dysregulation of the DA system in the depressive-like state. It has been shown that the activity of DA neurons is dysregulated in animals exposed to highly stressful conditions (Grace et al., 2007; Lodge & Grace, 2011; Mizoguchi, Shoji, Ikeda, Tanaka, & Tabira, 2008). One of such conditions is learned helplessness (LH) where animals are exposed to uncontrollable and inescapable shock

for some time, leading to reduced escape attempts when escape is made possible (Maier & Seligman, 1976). There is evidence that the administration of ketamine abolishes inhibition of DA neurons imposed by exposure to LH stress (Belujon & Grace, 2014).

This activity of ketamine on DA neurons has been shown to be mediated in part by the LHb. Yang and colleagues convincingly demonstrated that in congenitally LH rats, there is increased bursting of LHb neurons which send inhibitory projections to the VTA (Yang et al., 2018).

They showed that LHb neurons are mostly glutamatergic, and require NMDA receptor activity (Y. Yang et al., 2018). These neurons are thought to encode negative stimuli, and systemic application of ketamine unto the LHb alleviated the inhibitory burst firing, thus relieving DA neurons of this inhibition (Yang et al., 2018). It is important to note that the inhibitory action of LHb neurons unto the VTA is both direct, and through the mostly GABAergic rostromedial tegmental nucleus (RMTg) (Yang et al., 2018).

As mentioned previously, other glutamatergic sources of input unto VTA DA neurons include the pedunculopontine tegmental nucleus (PPN) which has been shown to regulate firing of VTA DA neurons (Grace et al., 2007). As well as the ventral subiculum which influences the number of spontaneously discharging DA neurons (Valenti, Lodge, & Grace, 2011). While these regions have not been investigated directly in the antidepressant-like effects of ketamine, it would be interesting to see if their activity is changed due to ketamine since the corresponding DA effects are altered.

Effect of ketamine on LC NE neurons

Similar to the results obtained for VTA DA neurons, we observed that the previously recorded increase in activity of LC NE neurons was no longer present 24 hours later. Specifically, the enhanced firing and bursting of these neurons was no longer significant in treated compared to control animals. Following repeated administration of ketamine, we

observed a marked and significant enhancement (50 %) in the frequency of discharge of these neurons which was no longer significant after 3 days. The bursting activity however, remained unchanged even after repeated administration.

To our knowledge, this is the first study to assess the effects of repeated administration of sub-anesthetic doses of ketamine on LC NE neurons. The impetus for which stems from the relevance of NE in our current understanding of depression. In fact, there is convincing evidence linking the NE system to symptoms of depression including but not limited to disruption in sleep, concentration and energy. In rats, it has been demonstrated that LC NE neurons fire differentially during the sleep-wake cycle with the highest firing being during waking (Aston-Jones & Bloom, 1981). Similarly, evidence from human and animal studies have revealed ample evidence that the NE system is intrinsic to attention management (Gabay, Pertzov, & Henik, 2011; Viggiano, Ruocco, Arcieri, & Sadile, 2004). Also important is the role of the a_2 -adrenergic receptor as a regulatory feedback site (Siever & Uhde, 1984). There is evidence that the a_2 -adrenergic receptors are less responsive in some depressed patients compared to controls (Siever & Davis, 1985). This can be gleaned from studies assessing the effects of a_2 agonist clonidine on plasma MHPG levels in unmedicated depressed patients (Charney et al., 1982). The researchers reported that although there were no significant differences in MHPG levels in control compared to depressed patients, there was a blunted growth hormone response which suggests abnormality in the sensitivity of a_2 adrenergic receptors (Charney et al., 1982). Further, several medications which target the NE system have been shown to produce a reduction of depressive symptoms (Richelson & Pfenning, 1984). Interestingly, the serotonin/norepinephrine reuptake inhibitor (SNRI) levomilnacipran which has roughly two-fold greater affinity for the NE transporter has been shown to abolish depression-like behavior in rats, and potently decrease depressive symptoms in patients (Auclair et al., 2013; Saraceni, Venci, & Gandhi, 2014).

While it is clear from all these studies that the NE system is involved in the antidepressant response, not many studies have assessed its relevance in the antidepressant response to ketamine. However, there is some non-depression related evidence of the effects that ketamine produces on the NE system. Such as inhibition of the high affinity NE transporter by ketamine (Azzaro & Smith, 1977).

We can hypothesize on how ketamine is able to produce an effect on NE neurons in the LC by examining the main modulatory input projections to this nucleus. The first is a predominantly glutamatergic input from the paragigantocellularis (PGi) (Aston-Jones, Rajkowski, Kubiak, Valentino, & Shipley, 1996). The PGi is a highly intergrative nucleus in the ventral rostrolateral medulla which plays a pivotal role in controlling both LC and sympathetic activities (Aston-Jones et al., 1996). Evidence suggests that stimulation of the PGi potently activates most LC neurons, and is blocked by excitatory amino acid (EAA) antagonists (Ennis & Aston-Jones, 1988). The second major input is via the prepositus hypoglossi (PrH) (Shipley, Pieribone, & Aston-Jones, 1988). Unlike the former brain region, input from the PrH is mostly GABAergic and thus inhibitory (Ennis & Aston-Jones, 1989). However, relatively little else is known about the innervation of the LC by the PrH due to the difficulty of accessing the nucleus (Ennis & Aston-Jones, 1989). It would be interesting to see what effects, if any, ketamine has on these two nuclei which could shed more light on the mechanisms of action of the drug *in vivo*. In addition to offering a possible explanation for the rapid antidepressant effect of ketamine, the elevation in NE neuron activity also explains the transient increase in blood pressure observed during ketamine administration (Fond et al., 2014).

Mechanisms of action of ketamine not involving the monoamine systems

Inhibition of NMDA receptors on GABA interneurons

Since the discovery of rapid antidepressant activity by ketamine, several other studies have investigated the mechanisms by which this is possible. Most studies have focused on the glutamate system, exploring the modulatory action of the drug on NMDA and non-NMDA receptors, as well as on a number of molecular markers. One mechanism which has garnered the most interest is the inhibition of NMDA receptors on inhibitory GABA interneurons. In evidence for this hypothesis, Breier and colleagues showed that when ketamine is administered to healthy volunteers, there is an overall increase in prefrontal cortex activity, believed to be due to preferential inhibition of NMDA receptors expressed on GABAergic interneurons, and subsequent increase in glutamate release by pyramidal neurons (Breier, Malhotra, Pinals, Weisenfeld, & Pickar, 1997). This is often referred to as the disinhibition hypothesis and is supported by the finding that pyramidal neurons and GABA interneurons are differentially modulated by NMDA receptor inhibition (Homayoun & Moghaddam, 2007). Further, it was shown that the analog NMDA receptor antagonist MK-801 inhibits GABAergic interneurons first, due to their fast-spiking activity, which in turn leads to an increase in the activity of pyramidal neurons (Homayoun & Moghaddam, 2007). Also, there is evidence that ketamine has a greater affinity for GluN2D containing NMDA receptors which are more highly expressed in forebrain inhibitory interneurons (Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994). This increase in pyramidal neuron activity and subsequently excitatory glutamatergic neurotransmission in the mPFC and other relevant regions of the brain, could then contribute to the alleviation of depressive symptoms (Moghaddam, Adams, Verma, & Daly, 1997).

Additionally, administration of partial inverse agonists at the benzodiazepine binding site of a5-containing $GABA_A$ receptors has been shown to produce rapid antidepressant-like (Fischell, Van Dyke, Kvarta, LeGates, & Thompson, 2015). While there is substantial evidence to support the hypothesis that the primary activity of ketamine is via inhibition of GABAergic

interneurons, there are other studies which argue that this is not the case. For example, it has been shown that in mice with a global reduction in $GABA_A$ receptor function, administration of ketamine still reversed behavioral despair novelty-induced hyper-anxiety, and selectively potentiated GABAergic synaptic inhibition in the medial mPFC (Ren et al., 2016). Also, mice lacking the GluN1 subunit of the NMDA receptor in parvalbumin-expressing interneurons, which is expected to have the same effect as inhibition of inhibitory interneurons, still show antidepressant-like effects of ketamine (Pozzi, Dorocic, Wang, Carlen, & Meletis, 2014). As such, the contribution of GABA interneuron inhibition on the antidepressant effects of ketamine remains to be fully elucidated.

(R)-Ketamine Enantiomer metabolite

Another hypothesis that has generated significant interest recently, is that the antidepressant activity of ketamine is actually due to a metabolite of one of its enantiomers. Hashimoto and colleagues have published several studies demonstrating that the (R) enantiomer of ketamine produces better antidepressant-like effects in animals than the (S) enantiomer without producing any psychotomimetic effects (Fukumoto et al., 2017; C. Yang et al., 2017, 2015; Zhang, Li, & Hashimoto, 2014). Their studies compared both enantiomers of ketamine and showed that while the (S)-ketamine enantiomer also produced antidepressant-like effects in rodents, (R)-ketamine appeared to be more potent and produced longer lasting effects (Fukumoto et al., 2017). This finding has been confirmed by Gould and associates who demonstrated that the effects of the (R) enantiomer are due to its metabolite (2R,6R)-hydroxynorketamine (HNK) (Zanos et al., 2016). They showed that HNK exerts behavioral, electroencephalographic and cellular antidepressant-like effects (Zanos et al., 2016).

However, to date, no clinical studies have been published in patients to support this finding. On the contrary, in 2019, the FDA approved (S)-ketamine as an adjunctive medication for the treatment of difficult to treat depression in the United states, having passed several

clinical trials (Andrade, 2017). This casts doubt on the hypothesis that the antidepressant effects of ketamine are independent of the NMDA receptor, as (2R, 6R)-HNK does not require the activity of this receptor to produce its effects. Nevertheless, (2R, 6R)-HNK has been shown to increase AMPA receptor-mediated excitatory post-synaptic potential in the CA1 region of the hippocampus (Zanos et al., 2016). This was confirmed by administration of NBQX prior to (2R, 6R)-HNK. Interestingly, this abolished the antidepressant-like effects of the drug similar to what is observed with the administration of the racemic mixture in animals (El Iskandrani et al., 2015; Koike et al., 2011; Maeng et al., 2008).

AMPA receptor involvement in effects of ketamine

Although the involvements of the AMPA receptor in the activity of ketamine are considered downstream effects, several studies have consistently confirmed that this receptor is necessary for the antidepressant actions of ketamine (Chaki & Koike, 2014; El Iskandrani et al., 2015; Koike et al., 2011; Maeng et al., 2008). According to the disinhibition hypothesis, increased glutamatergic neurotransmission results in acute activation of postsynaptic AMPA receptors (Zanos et al., 2016). In humans, Sanacora and colleagues employed electroencephalography to demonstrate that ketamine induces increases in gamma-band power which is an indicator of fast ionotropic receptors such as AMPA channels (Jaworska, de la Salle, Ibrahim, Blier, & Knott, 2019; Sanacora et al., 2014). More direct evidence for the involvement of the AMPA receptor in ketamine activity can be found in numerous studies using rats and mice where blocking of the AMPA receptor abolished the behavioural and electrophysiological antidepressant-like effects of ketamine (Chaki & Koike, 2014; El Iskandrani et al., 2015; Gigliucci et al., 2013; Koike et al., 2011; W. Zhou et al., 2014). Additionally, pre-treatment with the AMPA agonist CX546 appears to enhance the effects of ketamine assessed by FST in rats (W. Zhou et al., 2014).

One possibility for the involvement of the AMPA receptor could be that due to the fast open-close mechanics of the receptor, the cell is depolarized, leading to release of the Mg^{2+} block and thus permitting the binding of ketamine to the NMDA receptor (Wisden & Seeburg, 1993). Hence, blocking of the AMPA receptor could be resulting in an indirect blocking of ketamine activity on the NMDA receptor. Intriguingly however, ketamine administration also results in an increase in AMPA receptor subunits GluA1 and GluA2 in the hippocampus three hours after administration, and in the mPFC after 24 hours (Li et al., 2010; Nosyreva et al., 2013; Zanos et al., 2016). As such, further studies are necessary to fully elucidate the involvement of the AMPA receptor in the activity of ketamine.

BDNF and mechanistic target of rapamycin (mTOR)

BDNF is a growth factor which regulates the growth of neurites, functional connections, synapse formation, and synaptic plasticity in the central nervous system (Autry & Monteggia, 2012). Interestingly, it has been shown that subsequent to the chronic administration of first line antidepressant medications, there is an increase in BDNF-related activity which is evident within 30 mins of ketamine administration (Castren & Kojima, 2017; Castrén, Võikar, & Rantamäki, 2007; Garcia et al., 2008). Also, systemic administration of BDNF as well as intra-hippocampal administration of BDNF has been shown to confer resilience to chronic stress (Taliaz et al., 2011).

Importantly, Autry and colleagues showed that in an animal model with a knockdown of forebrain BDNF, ketamine produced no antidepressant-like effects (Autry et al., 2011). Additionally, Duman et al demonstrated that infusion of a BDNF-neutralizing antibody into the mPFC was sufficient to prevent the antidepressant-like effect of ketamine, thus indicating that BDNF activity is essential for the effects of ketamine (Lepack, Fuchikami, Dwyer, Banasr, & Duman, 2014). In further support of this, it has been shown that a single nucleotide

polymorphism of the BDNF gene ($BDNF^{Val66met}$) in mice and humans, which confers deficits in BDNF processing and activity-dependent secretion, do not respond to ketamine (Chen et al., 2006; Liu et al., 2012).

Activity of BDNF drives activation of the mTOR complex via one of two pathways. The first is binding to tropomyosin receptor kinase B (TrkB), which activates phosphatidyl-inositol 3kinase and subsequently protein kinase B (Akt) (Reichardt, 2006). Alternatively, binding to TrkB can result in activation of MEK-MAPK/Erk signalling pathway (Yoshii & Constantine-Paton, 2010). Regardless of which of the pathways is mobilized, mTOR activation ultimately regulates neurogenesis, dendritic spine growth, protein translation initiation and protein synthesis in the hippocampus and mPFC (Li et al., 2010). Mobilization of mTOR has been shown to be transient, returning to baseline levels within two hours (Li et al., 2010). However, the synaptogenesis triggered by this activation requires significantly longer time to take effect (Nibuya et al., 1996).

Additionally, activation of mTOR signalling is linked to the deactivation of glycogen synthase kinase-3 (GSK-3), and it has been shown that mice with a knock-in at both *GSK-3α* and *GSK-3β* genes do not show antidepressant-like effects of ketamine (Beurel, Song, & Jope, 2011; Zanos & Gould, 2018). Taken together, the evidence suggests that BDNF and mTOR are essential components of downstream ketamine activity and as such, are relevant for the rapid antidepressant effects of the drug.

Conclusion

Ketamine is an anesthetic agent which acts by blocking the NMDA receptor channel (Lorrain et al., 2003). Recently, clinical studies have demonstrated convincingly that in responders, sub-anesthetic doses of ketamine produce rapid antidepressant effects beginning 2 – 24 hours after administration (Berman et al., 2000; Zarate et al., 2006). Which, when compared to current first line antidepressant medications that can take up to two weeks to produce clinically

relevant effects, has been hailed as the most significant finding in depression research over the last 50 years (Abdallah et al., 2015). While the antidepressant effect of ketamine is rapid, it is also transient, dissipating over seven days in patients (Zarate et al., 2006). As such, clinicians administer the drug repeatedly, twice or three times a week, which has been shown to prolong the antidepressant effects (Murrough et al., 2013; Phillips et al., 2019).

Various hypotheses have been offered to explain the rapid antidepressant activity of ketamine. In our lab, an electrophysiological study in rats showed that a single administration of ketamine results in an increase in the frequency of discharge and bursts of NE neurons, as well as an increase in the number of spontaneously discharging DA neurons within 30 mins – 2 hours (El Iskandrani et al., 2015). Subsequently, the current project investigated these changes after 24 hours. We were interested in assessing how perseverant the ketamine-induced changes to these neurons are, as well as whether or not any changes in the activity of 5-HT neurons would become apparent over this time. Additionally, we assessed the effects of repeated administration of ketamine on VTA DA, LC NE and DRN 5-HT neurons after 24 hours utilizing a similar procedure to that employed in the clinic.

We found that the increase in activity of LC NE and VTA DA neurons which was observed 30 mins to 2 hours after a single administration had dissipated after 24 hours. However, 24 hours after repeated administration, there was a robust and significant increase in the population activity and bursting of VTA DA neurons as well as in the frequency of discharge of LC NE neurons While the increase in frequency of discharge of NE neurons and population activity of DA neurons was no longer significantly different after three days, the enhanced bursting of DA neurons remained significant, but dissipated over seven days.

Disparately, the activity of DRN 5-HT neurons did not differ significantly in treated compared to control animals in any of the parameters assessed. Hence, the results of this study

suggest that the antidepressant effects of ketamine are at least in part due to the rapid increase in VTA DA and LC NE neuron activity. As such, while further studies are required to effectively elucidate how ketamine produces these increases in catecholamine activity, it is clear that monoamine systems are relevant for the activity of ketamine, and should be taken into account in the clinical administration of ketamine for depression.

Future directions

The results of the current investigation can be furthered through studies in animals and humans. In rats, it would be interesting to confirm that similar to findings in humans, there are no significant differences in response to ketamine between males and females. Subsequently, it would be necessary to investigate 5-HT neurotransmission in the hippocampus in order to further probe the effects of ketamine on the 5-HT system.

In humans, our lab in the mood disorders research unit of the Royal's Institute of Mental Health Research is one of the few labs investigating the involvement of the monoamine systems in the antidepressant effects of ketamine. Having shown that DA and NE are increased in rats as a result of ketamine administration, an obvious next step would be to investigate this increase in humans. To do this, one possibility would be to administer the radioactive DA D_2 antagonist [C11] raclopride and image it using PET while co-administering ketamine. The hypothesis is that if ketamine does indeed increase DA in humans as it has been shown to do in rats, we would expect an increased fluorescence with [C-11] raclopride. Consequently, if this increase corresponds to treatment response, it would serve as a confirmation that the increase in DA activity is necessary for the antidepressant effect of ketamine. I believe these studies would be helpful in furthering our understanding of the rapid antidepressant effects of ketamine.

References

Abdallah, C. ., Sanacora, G., Duman, R. S., & Krystal, J. H. (2015). Ketamine and rapid-acting antidepressants: a window into a new neurobiology for mood disorder therapeutics. *Annual Review of Medicine*, *66*, 509–523.

Adham, N., Romanienko, P., Hartig, P., Weinshank, R. L., & Branchek, T. (1992). The Rat 5 . HydroxytryptaminelB Receptor Is the Species logue of the Human 5-HydroxytryptaminelD beta Receptor. *Molecular Pharmacology*, *41*(1), 1–7.

Aghajanian, G. . (1978). Feedback regulation of central monoaminergic neurons: evidence from single cell recording studies. In *Essays in neurochemistry and neuropharmacology* (3rd ed., pp. 1–32).

Ainsworth, K., Smith, S. ., Zetterstrom, T. ., Pei, Q., Franklin, M., & Sharp, T. (1998). Effect of antidepressant drugs on dopamine D1 and D2 receptor expression and dopamine release in the nucleus accumbens of the rat. *Pharmacology*, *140*(4), 470–477.

Alexander, R. ., Davis, J. ., & Lefkowitz, R. . (1975). Direct identification and characterization of Beta-adrenergic receptors in rat brain. *Nature*, *5534*, 437.

Altevogt, B. M., Davis, M., & Pankevich, D. E. (2011). *Glutamate-related biomarkers in drug development for disorders of the nervous system: Workshop summary* (1st ed.). National Academies Press.

Amargós-Bosch, M., López-Gil, X., Artigas, F., & Adell, A. (2006). Clozapine and olanzapine, but not haloperidol, suppress serotonin efflux in the medial prefrontal cortex elicited by phencyclidine and ketamine. *International Journal of Neuropsychopharmacology*, *9*(5), 565–573. https://doi.org/10.1017/S1461145705005900

Anand, A., & Charney, D. S. (2000). Norepinephrine dysfunction in depression. *The Journal of Clinical Psychiatry*, *61*(Suppl 10), 16–24.

Andrade, C. (2017). Ketamine for depression, 3: does chirality matter? *The Journal of Clinical Psychiatry*, *78*(6), e674–e677.

Andrzej, P., Chaki, S., Nowak, G., & Witkin, J. M. (2008). Mood disorders: regulation by metabotropic glutamate receptors. *Biochemical Pharmacology*, *75*(5), 997–1006.

Angel, I., Schoemaker, H., Prouteau, M., Garreau, M., & Langer, S. . (1993). Litoxetine: a selective 5-HT uptake inhibitor with concomitant 5-HT3 receptor antagonist and antiemetic properties. *European Journal of Pharmacology*, *232*(2–3), 139–145.

Angst, J., Angst, F., & Stassen, H. . (1999). Suicide risk in pateints with major depressive disorder. *The Journal of Clinical Psychiatry*, *60*(Supplement 2), 57–62.

Arango, V., Ernsberger, P., Marzuk, P. M., Chen, J. S., Tierney, H., Stanley, M., … Mann, J. J. (1990). Serotonin 5-HT2 and beta-adrenergic receptor binding sites in the brain of suicide victims. *Archives of General Psychiatry*, *47*, 1038–1047.

Aston-Jones, G., & Bloom, F. E. (1981). Activity of norepinephrine-containing locus coreeulus neurons in behavng rats anticipates fluctuations in the sleep-waking cycle. *Journal of Neuroscience*, *1*(8), 876–886.

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R. ., & Shipley, M. . (1996). Role of the locus coeruleus in emotional activation. *In Progress in Brain Research*, *107*, 379–402.

Auclair, A. ., Martel, J. ., Assie, M. ., Bardin, L., Heusler, P., Cussac, D., Marien, M., NewmanTancredi, A., O'Connor, J.A., Deportere, R. (2013). Levomilnacipran (F2695), a norepinephrine-preferring SNRI: Profile in vitro and in models of depression and anxiety. *Neuropharmacology*, *70*, 338–347.

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

Autry, A. ., & Monteggia, L. . (2012). Brain-derived neurotrophic factor and neuropsychiatric disorders. *Pharmacological Reviews*, *64*(2), 238–258.

Azzaro, A. ., & Smith, D. . (1977). The inhibitory action of ketamine HCL on [3H] 5hydroxytryptamine accumulation by rat brain synaptosomal-rich fractions: comparison with [3H] catecholamine and [3H] γ-aminobutyric acid uptake. *Neuropharmacology*, *16*(5), 349– 356.

Bahn, S., Volk, B., & Wisden, W. (1994). Kainate receptor gene expression in the developing rat brain. *Journal of Neuroscience*, *14*, 5525–5547.

Bai, F., Yin, T., Johnstone, E. M., Chen, S., Gaboor, V., Little, S. P., & Nelson, D. (2004). Molecular cloning and pharmacological characterization of the guinea pig 5-HT1E receptor. *European Journal of Pharmacology*, *484*(2–3), 127–139.

Bailey, M. R., Williamson, C., Mezias, C., Winiger, V., Silver, R., Balsam, P. D., & Simpson, E. H. (2016). The effects of pharmacological modulation of the serotonin 2C receptor on goaldirected behavior in mice. *Psychopharmacology*, *233*, 615–624.

Bakish, D. (2001). New standard of depression treatment: remission and full recovery. *The Journal of Clinical Psychiatry*, *62*(Supplement 26), 5–9.

Bard, J. ., Zgombick, J., Adham, N., Vaysse, P., Branchek, T. ., & Weinshank, R. L. (1993). Cloning of a novel human serotonin receptor (5-HT7) positively linked to adenylate cyclase. *Journal of Biological Chemistry*, *268*(31), 23422–23426.

Battaglia, G., Shannon, M., Borgundvaag, B., & Titeler, M. (1983). Properties of 3H-prazosin labeled alpha1-adrenergic receptors in rat brain and porcine neurointermediate lobe tissue. *Journal of Neurochemistry*, *41*, 538–542.

Béïque, J. C., De Montigny, C., Blier, P., & Debonnel, G. (1998). Blockade of 5hydroxytryptamine and noradrenaline uptake by venlafaxine: A comparative study with paroxetine and desipramine. *British Journal of Pharmacology*, *125*(3), 526–532. https://doi.org/10.1038/sj.bjp.0702074

Béïque, J. C., De Montigny, C., Blier, P., & Debonnel, G. (1999). Venlafaxine: Discrepancy between in vivo 5-HT and NE reuptake blockade and affinity for reuptake sites. *Synapse*, *32*(3), 198–211. https://doi.org/10.1002/(SICI)1098-2396(19990601)32:3<198::AIDSYN6>3.0.CO;2-2

Belujon, P., & Grace, A. A. (2014). Restoring Mood Balance in Depression: Ketamine Reverses Deficit in Dopamine-Dependent Synaptic Plasticity. *Biological Psychiatry*, *76*(12), 927–936.

Berendsen, H. ., Broekkamp, C. ., & Pinder, R. . (1998). Mirtazapine enhances the effect of haloperidol on apomorphine-induced climbing behaviour in mice and attenuates haloperidol-induced catalepsy in rats. *Psychopharmacology*, *135*(3), 284–289.

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. https://doi.org/10.1016/S0006-3223(99)00230-9

Beurel, E., Song, L., & Jope, R. . (2011). Inhibition of glycogen synthase kinase-3 is necessary for the rapid antidepressant effect of ketamine in mice. *Molecular Psychiatry*, *16*(11), 1068.

Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., … Belmer, A. (2010). A population-specific HTR2B stop codon predisposes to severe impulsivity. *Nature*, *468*(7327), 1061.

Bezzina, G., Body, S., Cheung, T. H. ., Hampson, C. ., Bradshaw, C. ., Glennon, J. ., & Szabadi, E. (2015). Evidence for a role of 5-HT 2C receptors in the motor aspects of performance, but not the efficacy of food reinforcers, in a progressive ratio schedule. *Psychopharmacology*, *232*(4), 699–711.

Bjorklund, A., & Dunnett, S. . (2007). Dopamine neuron systems in the brain: an update. *TRENDS in Neurosciences*, *30*(5), 194–202.

Blaxall, H. ., Murphy, T. ., Baker, J. ., Ray, C., & Bylund, D. . (1991). CHaracterization of the alpha-2C adrenergic receptor subtype in the opposum kidney and in the OK cell line. *Journal of Pharmacology*, *259*, 323–329.

Blier, P., & Bouchard, C. (1994). Modulation of 5-HT release in the guinea-pig brain following long-term administration of antidepressant drugs. *British Journal of Pharmacology*, *113*(2), 485–495. https://doi.org/10.1111/j.1476-5381.1994.tb17015.x

Blier, P., & De Montigny, C. (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT1A agonist gepirone: electrophysiological studies in the rat brain. *Synapse*, *1*(5), 470–480.

Blier, P., Gobbi, G., Turcotte, J., De Montigny, C., & Debonnel, G. (2009). Mirtazapine and paroxetine in major depression: a comparison of monotherapy versus their combination from treatment initiation. *European Neuropsychopharmacology*, *19*(7), 457–465.

Blier, P., & Ward, N. M. (2003). Is there a role for 5-HT1A agonists in the treatment of depression? *Bilogical Psychiatry*, *53*(3), 193–203.

Bockaert, J., Claeysen, S., Compan, V., & Dumuis, A. (2004). Current drug targets. *CNS & Neurological Disorders*, *3*(1), 39–51.

Bonaventure, P., Nepomuceno, D., Hein, L., Sutcliffe, J. ., Lovenberg, T., & Hedlund, P. . (2004). Radioligand binding analysis of knockout mice reveals 5-hydroxytryptamine7 receptor distribution and uncovers 8-hydroxy-2-(di-n-propylamino) tetralin interaction with alpha2 adrenergic receptors. *Neuroscience*, *124*(4), 901–911.

Bowden, C., Theodorou, A., Cheetham, S. ., Lowther, S., Katona, C. ., Crompton, R., & Horton, R. . (1997). Dopamine D1 and D2 receptor binding sites in brain samples from depressed suicides and controls. *Brain Research*, *752*(1–2), 227–233.

Boyson, S. ., McGonigle, P., & Molnoff, P. . (1986). Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *Journal of Neuroscience*, *6*(11), 3177–3188.

Bozic, M., Chen, S.-R., Errico, F., Hashimoto, K., Lammert, E., Laumet, G., … Yoneda, Y. (2017). *The NMDA Receptors*. (K. Hashimoto, Ed.). Cham: Springer International Publishing.

Bradshaw, C. M., Roberts, M. H., & Szabadi, E. (1973). Comparison of the effects of imipramine and desipramine on single cortical neurones. *British Journal of Pharmacology*, *48*(2), 358P-359P.

Breier, A., Malhotra, A. ., Pinals, D. ., Weisenfeld, N. ., & Pickar, D. (1997). Association of ketamine-induced psychosis with focal activation of the prefrontal cortex in healthy volunteers. *American Journal of Psychiatry*, *154*, 805–811.

Bruinvels, A. T., Landwehrmeyer, B., Gustafson, E. L., Durkin, M. M., Mengod, G., Branchek, T. ., … Palacios, J. M. (1994). Localization of 5-HT1B, 5-HT1Då, 5-HT1E and 5-HT1F receptor messenger RNA in rodent and primate brain. *Neuropsychopharmacology*, *33*(3–4), 367–386.

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

Bylund, D. B. (1992). Subtypes of alpha1- and alpha2-adrenergic receptors. *The FASEB Journal*, *6*(3), 832–839.
Carlsson, A., Lindqvist, M., & Magnusson, T. O. . (1957). 3,4-Dihydroxyphenylalanine and 5hydroxytryptophan as reserpine antagonists. *Nature*, *180*(4596), 1200.
Carlsson, A., Lindqvist, M., Magnusson, T., & Waldeck, B. (1958). On the presence of 3hydroxytyramine in brain. *Science*, *127*(3296), 471.
Carter, A. ., & Muller, R. . (1991). Pramipexole, a dopamine D2 autoreceptor agonist, decreases the extracellular concentration of dopamine in vivo. *European Journal of Pharmacology*, *200*(1), 65–72.
Castren, E., & Kojima, M. (2017). Brain-derived neurotrophic factor in mood disorders and antidepressant treatments. *Neurobiology of Disease*, *97*, 119–126.
Castrén, E., Võikar, V., & Rantamäki, T. (2007). Role of neurotrophic factors in depression. *Current Opinion in Pharmacology*, *7*(1), 18–21.
Chaki, S., & Koike, H. (2014). Requirement of AMPA receptor stimulation for the sustained antidepressant activity of ketamine and LY341495 during the forced swim test in rats. *Behavioural Brain Research*, *271*(1), 111–115.
Chaput, Y., De Montigny, C., & Blier, P. (1991). Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments: An in vivo electrophysiological study in the rat. *Neuropsychopharmacology*, *5*(4), 219–229.
Charney, D. S., Heninger, G. R., Sternberg, D. E., Hafstad, K. ., Giddings, S., & Landis, H. . (1982). Adrenergic receptor sensitivity in depression: effects of clonidine in depressed patients and healthy subjects. *Archives of General Psychiatry*, *39*(3), 290–294.
Chen, J., van Praag, H., & Gardner, E. (1991). Activation of 5-HT3 receptor by 1phenylbiguanide increases dopamine release in the rat nucleus accumbens. *Brain Research*, *543*(2), 354–357.
Chen, Z., Jng, D., Bath, K. ., Leraci, A., Khan, T., Siao, C. ., … Hempstead, B. . (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, *314*(5796), 140–143.
Chenu, F., El Mansari, M., & Blier, P. (2013). Electrophysiological effects of repeated administration of agomelatine on the dopamine, norepinephrine, and serotonin systems in the rat brain. *Neuropsychopharmacology*, *38*(2), 275–284. https://doi.org/10.1038/npp.2012.140
Chernoloz, O, El Mansari, M., & Blier, P. (2012). Long-term administration of the dopamine D3/2 receptor agonist pramipexole increases dopamine and serotonin neurotransmission in the male rat forebrain. *Journal of Neuroscience*, *37*(2), 113–121.
Chernoloz, Olga, El Mansari, M., & Blier, P. (2009). Electrophysiological studies in the rat brain on the basis for aripripazole augmentation of antidepressants in major depressive disorder. *Psychopharmacology*, *206*(2), 335–344.
Colzi, A., D'Agostini, F., Kettler, R., Borroni, E., & Da Prada, M. (1990). Effective selective and reversible MAO inhibitors on dopamine outflow in rat striatum: A microdialysis study. In P. Riederer & M. B. . Youdim (Eds.), *Amine Oxidases and Their Impact on Neurobiology* (Volume 32, pp. 79–84). Vienna: Springer.
Conn, P. J., & Pin, J.-P. (1997). Pharmacology and Functions of Metabotropic Glutamate Receptors. *Annual Review of Pharmacology and Toxicology*, *37*(1), 205–237. https://doi.org/10.1146/annurev.pharmtox.37.1.205
Contractor, A., Mulle, C., & Swanson, G. (2011). Kainate receptors coming of age: milestones of two decades of research. *TRENDS in Neurosciences*, *34*(3), 154–163.

Contractor, A., Swanson, G., & Heinemann, S. (2001). Kainate receptors are involved in short- and long-term plasticity at mossy fiber synapses in the hippocampus. *Neuron*, *29*(1), 209–216.

Cooper, D. . (2002). The significance of action potential bursting in the brain reward circuit. *Neurochemistry International*, *41*(5), 333–340.

Cuijpers, P., Sijbrandij, M., Koole, S. ., Andersson, G., Beekman, A. ., & Reynolds III, C. . (2013). The efficacy of psychotherapy and pharmacotherapy in treating depressive and anxiety disorders: A meta-analysis of direct comparisons. *World Psychiatry*, *12*(2), 137–148.

Cull-Candy, S. G., Kelly, L., & Farrant, M. (2006). Regulation of ca2+ permeable AMPA receptors: synaptic plasticity and beyond. *Current Opinion in Neurobiology*, *16*, 288–297.

Curet, O., & de Montigny, C. (1988). Electrophysiological characterization of adrenoreceptors in the rat dorsal hippocampus. II. Receptors mediating the effect of synaptically released norepinephrine. *Brain Research*, *475*(1), 47–57.

Cutler, N. ., & Heiser, J. . (1978). The tricyclic antidepressants. *JAMA*, *240*(21), 2264–2266.

Dang, Y. ., Ma, X. ., Zhang, J. ., Ren, Q., Wu, J., Gao, C. ., & Hashimoto, K. (2014). Targeting of NMDA receptors in the treatment of major depression. *Current Pharmaceutical Design*, *20*(32), 5151–5159.

Dawson, L. ., Nguyen, H. ., & Li, P. (2000). In vivo effects of the 5-HT6 antagonist SB-271046 on striatal and frontal cortex extracellular concentrations of noradrenaline, dopamine, 5-HT, glutamate and aspartate. *British Journal of Pharmacology*, *130*(1), 23–26.

de Montigny, C, & Aghajanian, G. (1978). Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. *Science*, *202*(4378), 1303–1306.
https://doi.org/10.1126/science.619465

de Montigny, Claude, & Blier, P. (1983). Electrophysiological repeated zimelidine neurotransmission investigations administration in the rat. *The Journal of Neuroscience*, *3*(6), 1270–1278.

DeLeon, A., Patel, N. ., & Crismon, M. L. (2004). Aripriprazole: a comprehensive review of its pharmacology, clinical efficacy and tolerability. *Clinical Therapeutics*, *26*(5), 649–666.

Delgado, P. L. (2000). Depression: the case for a monoamine deficiency. *The Journal of Clinical Psychiatry*.

Der-Avakian, A., & Markou, A. (2012). The neurobology of anhedonia and other reward-related deficits. *Trends in Neurosciences*, *35*(1), 68–77.

Descarries, L., & Droz, B. (1970). Intraneural Distribution of Exogenous Norepinephrine in the Central Nervous System of the Rat. *The Journal of Cell Biology*, *44*(2), 385–399. https://doi.org/10.1083/jcb.44.2.385

Deschwanden, A., Karolewicz, B., Feyissa, A. ., Treyer, V., Ametamey, S. ., Johayem, A., … Hasler, G. (2011). Reduced metabotropic glutamate receptor 5 density in major depression determined by [(11)C]ABP688 PET and postmortem study. *American Journal of Psychiatry*, *168*(7), 727–734.

Diaz, J., Levesque, D., Griffon, N., Lammers, C. ., Martres, M. ., Sokoloff, P., & Schwartz, J. . (1994). Opposing roles for dopamine D2 and D3 receptors on neurotensin mRNA expression in nucleus accumbens. *European Journal of Pharmacology*, *6*(8), 1384–1387.

Diaz, S. L., & Maroteaux, L. (2011). Implication of 5-HT2B receptors in the serotonin syndrome. *Neuropsychopharmacology*, *61*(3), 495–502.

Dingledine, & Dingledine, R. . B. (1999). The glutamate receptor ion channels. *Pharmacological Reviews*, *51*(1), 7–61.

Dong, J., & Blier, P. (2001). Modification of norepinephrine and serotonin, but not dopamine neuron firing by sustained bupropion treatment. *Psychopharmacology*, *155*(1), 52–57. DSM-5. (2015). *Depressive Disorders: DSM-5 Selections*. American Psychiatric Pub.

Duman, R. S., Heninger, G. R., & Nestler, E. J. (1997). A molecular and cellular theory of depression. *Archives of General Psychiatry*, *54*(7), 597–606.

Dunlop, W. B., & Nemeroff, C. B. (2007). The Role of Dopamine in the Pathophysiology of Depression. *Archives of General Psychiatry*, *64*(3), 327–337. https://doi.org/10.1001/archpsyc.64.3.327

Eglen, R. M., Wong, E. H. ., Dumuis, A., & Bockaert, J. (1995). Central 5-HT4 receptors. *Trends in Pharmacological Sciences*, *16*(11), 391–398.

El Iskandrani, K. ., Oosterhof, C., El Mansari, M., & Blier, P. (2015). Impact of subanesthetic doses of ketamine on AMPA-mediated responses in rats: an in vivo electrophysiological study on monoaminergic and glutamatergic neurons. *Journal of Psychopharmacology*, *29*(7), 792–801.

Ennis, M., & Aston-Jones, G. (1988). Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. *Journal of Neuroscience*, *8*(10), 3644–3657.

Ennis, M., & Aston-Jones, G. (1989). GABA-mediated inhibition of locus coeruleus from the dorsomedial rostral medulla. *Journal of Neuroscience*, *9*(8), 2973–2981.

Feldmeye, D., & Cull-Candy, S. G. (1994). Neurotransmitters; Elusive glutamate receptors. *Current Biology*, *4*(1), 82–84.

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J. L., … Whiteford, H. A. (2013). Burden of Depressive Disorders by Country, Sex, Age, and Year: Findings from the Global Burden of Disease Study 2010. *PLoS Medicine*, *10*(11), e1001547. https://doi.org/10.1371/journal.pmed.1001547

Feyissa, A. ., Chandran, A., Stockmeier, C. ., & Karolewicz, B. (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *33*(1), 70–75.

Fischell, J., Van Dyke, A. ., Kvarta, M. ., LeGates, T. ., & Thompson, S. . (2015). Rapid antidepressant action and restoration of excitatory synaptic strength after chronic stress by negative modulators of alpha5-containing GABAA receptors. *Neuropsychopharmacology*, *40*, 2499–2509.

Fletcher, P., Sinyard, J., & Higgins, G. A. (2010). Genetic and pharmacological evidence that 5HT2C receptor activation, but not inhibition, affects motivation to feed under a progressive ratio schedule of reinforcement. *Pharmacology Biochemistry and Behavior*, *97*(1), 170– 178.

Fond, G., Loundou, A., Rabu, C., Macgregor, A., Lançon, C., Brittner, M., … Roger, M. (2014). Ketamine administration in depressive disorders: a systematic review and meta-analysis. *Psychopharmacology*, *231*(18), 3663–3676.

Forrest, D., Yuzaki, M., Soares, H. ., Ng, L., Luk, D. ., Sheng, M., … Curran, T. (1994). Targeted dysruption of NMDA receptor 1 gene abolished NMD response and results in neonatal death. *Neuron*, *13*, 325–338.

Friedman, A., Friedman, Y., Dremencov, E., & Yadid, G. (2008). VTA Dopamine Neuron Bursting is Altered in an Animal Model of Depression and Corrected by Desipramine. *Journal of Molecular Neuroscience*, *34*, 201–209.

Fukumoto, K., Toki, H., Lijima, M., Hashihayata, T., Yamaguchi, J. ., Hashimoto, K., & Chaki, S. (2017). Antidepressant potential of (R)-ketamine in rodent models: comparison with (S)ketamine. *Journal of Pharmacology and Experimental Therapeutics*, *361*(1), 9–16.

Gabay, S., Pertzov, Y., & Henik, A. (2011). Orienting of attention, pupl size, and the norepinephrine system. *Attention, Perception, & Psychophysics*, *73*(1), 123–129.

Galvin, G. . (1985). Stress and Brain Noradrenaline: A review. *Neuroscience & Biobehavioral Reviews*, *9*, 233–243.

Garcia, L. S. ., Comim, C. ., Valvassori, S. ., Reus, G. ., Barbosa, L. ., Andreazza, A. ., … Quevedo, J. (2008). Acute administrsation of ketamine induces antidepressant-lke effects in the forced swimming test and increases BDNF levels in the rat hippocampus. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *32*(1), 140–144.

Geddes, S. D., Assadzada, S., Sokolovski, A., Bergeron, R., Haj-Dahmane, S., & Béïque, J. C. (2015). Time-dependent modulation of glutamate synapses onto 5-HT neurons by antidepressant treatment. *Neuropharmacology*, *95*, 130–143. https://doi.org/10.1016/j.neuropharm.2015.02.027

Gerard, C., Martres, M. ., Lefevre, K., Miguel, M. ., Verge, D., Lanfumey, L., … El Mestikawy, S. (1997). Immuno-localization of serotonin 5-HT6 receptor-like material in the rat central nervous system. *Brain Research*, *746*(1–2), 207–219.

Gerfen, C. ., Engber, T. ., Mahan, L. ., Susel, Z. V. ., Chase, T. ., Monsama Jr., F. ., & Sibley, D. . (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429–1432.

Ghanbari, R., El Mansari, M., & Blier, P. (2010). Electrophysiological effects of the coadministration of escitalopram and bupropion on rat serotonin and norepinephrine neurons. *Journal of Psychopharmacology*, *24*(1), 39–50. https://doi.org/10.1177/0269881108095714

Gigliucci, V., O'Dowd, G., Casey, S., Egan, D., Gibney, S., & Harkin, A. (2013). Ketamine elicits sustained antidepressant-like activity via a serotonin-dependent mechanism. *Psychopharmacology*, *228*(1), 157–166. https://doi.org/10.1007/s00213-013-3024-x

Gold, P. W., & Chrousos, G. P. (2002). Organization of the stress sysatem and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Nature*, *7*, 254–275. https://doi.org/10.1038/sj/mp/4001032

Grace, A. A., & Bunney, B. . (1984). The control of fring pattern in nigral dopamine neurons: sngle spike firing. *Journal of Neuroscience*, *4*(11), 2866–2876.

Grace, A. A., Floresco, S. ., Goto, Y., & Lodge, D. . (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviours. *TRENDS in Neurosciences*, *30*(5), 220–227.

Grailhe, R., Waeber, C., Dulawa, S., Hornung, J., Zhuang, X., Brunner, D., … Hen, R. (1999). Increased exploratory activity and altered response to LSD in mice lacking the 5-HT5A receptor. *Neuron*, *22*(3), 581–591.

Gravel, P., & De Montigny, C. (1987). Noradrenergic denervation prevents sensitization of rat forebrain neurons to serotonin by tricyclic antidepressant treatment. *Synapse*, *1*(3), 233–239. https://doi.org/10.1002/syn.890010303

Green, A. . (1985). *Neuropharmacology of serotonin*. Oxford University Press, USA.

Greger, I. H., Watson, J. F., & Cull-Candy, S. G. (2017). Structural and Functional Architecture of AMPA-Type Glutamate Receptors and their Auxiliary Proteins. *Neuron*, *94*, 713–730.

Groenink, L., Van Bogaert, M. J. V, Van Der Gugten, J., Oosting, R. S., & Olivier, B. (2003). 5HT1A receptor and 5-HT1B receptor knockout mice in stress and anxiety paradigms. *Behavioural Pharmacology*, *14*(5), 369–383.

Guiard, B. P., El Mansari, M., Merali, Z., & Blier, P. (2008). Functional Interactions between dopamine, serotonin and norepinephrine neurons: an in-vivo electrophysiological study in rats with monoaminergic lesions. *International Journal of Neuropsychopharmacology*, *11*, 625–639.

Guscott, M., Bristow, L. J., Hadingham, K., Rosahl, T. ., Beer, M. ., Stamton, J. ., … Rupniak, N. . (2005). Genetic knockout and pharmacological blockade studies of the 5-HT7 receptor suggest therapeutic potential in depression. *Neuropharmacology*, *48*(4), 492–502.

Haddjeri, N, Blier, P., & De Montigny, C. (1996). Effects of long-term treatment with the α2adrenoreceptor antagonist mirtazapine on 5-HT neurotransmission. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *355*(1), 20–29.

Haddjeri, Nasser, & Blier, P. (1995). Noradrenergic modulation of central serotonergic neurotransmission: acute and long-term actions of mirtazapine. *International Clinical Psychopharmacology*, *10*, 11–17.

Hale, A., Corral, R.-M., Mencacci, C., Ruiz, S. J., Severo, C. A., & Gentil, V. (2010). Superior antidepressant efficacy results of agomelatine versus fluoxetine in severe MDD patients: a randomized, double-blind study. *International Clinical Psychopharmacology*, *25*(6), 305–314.

Hamati, R., El Mansari, M., & Blier, P. (2019). Synergistic action of aripripazole and escitalopram potentiates medial prefrontal cortical activity through blockade of 5-HT2B receptors. *Biological Psychiatry*, *85*(10), S346–S347.

Hamblin, M. W., Metcalf, M. A., McGuffin, R. W., & Karpells, S. (1992). Molecular cloning and functional characterization of a human 5-HT 1B serotonin receptor: a homologue of the rat 5-HT-1B receptor with 5-HT1D-like pharmacological specificity. *Biochemical and Biophysical Research Communications*, *184*(2), 752–759.

Han, C., Wilson, K. ., & Minneman, K. . (1990). Adrenergic receptor subtypes and formation of inositol phosphates in dispersed hepatocytes and renal cells. *Molecular Pharmacology*, *37*, 903–910.

Harrison, J. ., Pearson, W. ., & Lynch, K. R. (1991). Molecular characterization of alpha1- and alpha2-adrenoreceptors. *TIPS*, *12*, 62–67.

Hayakawa, H., Shimizu, M., Nishida, A., Motohashi, N., & Yamawaki, S. (1994). Increase in serotonin 1A receptors in the dentate gyrus as revealed by autoradiographic analysis following repeated electroconvulsive shock but not imipramine treatment. *Neuropsychobiology*, *30*(2), 53–56.

Hayashi, T., Umemori, H., Mishina, M., & Yamamoto, T. (1999). The AMPA receptor interacts with and signals through the protein tyrosine kinase Lyn. *Nature*, *397*(6714), 72.

Heisler, L. ., Zhou, L., Bajwa, P., Hsu, J., & Tecott, L. . (2007). Serotonin 5-HT2C receptors regulate anxiety-like behavior. *Genes, Brain and Behavior*, *6*, 491–496.

Heresco-Levy, U., & Javitt, D. . (2004). Comparative effects of glycine and D-cycloserine on persisten negative symptoms in shcizophrenia: a retrospective analysis. *Schizpphrenia Research*, *66*, 89–96.

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

Homayoun, H., & Moghaddam, B. (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *Journal of Neuroscience*, *27*, 11496–11500.

Huang, Q., Zhou, D., Chase, K., Gusella, J. F., Aronin, N., & DiFiglia, M. (1992). Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre-and postsynaptic localization, and prevalence in the basal ganglia,

limbic system, and thalamic reticular nucleus. *Proceedings of the National Academy of Sciences*, *89*(24), 11988–11992.
Iravani, M., Muscat, R., & Kruk, Z. (1999). MK-801 INteraction with the 5-HT Transporter: A real-time Study in Brain Slices Using Fasr Cyclic Voltammetry. *Synapse*, *32*, 212–224.
Ishihara, K., Taku, A., Hiroshi, H., Shigeto, Y., & Masashi, S. (1999). Enhancement of serotonin 1A receptor function following repeated electroconvulsive shock in young rat hippocampus neurons in vitro. *International Journal of Neuropsychopharmacology*, *2*(2), 101–104.
Jackson, D. ., & Westlind-Danielsson, A. (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacology & Therapeutics*, *64*(2), 291–370.
Jaworska, N., de la Salle, S., Ibrahim, M. ., Blier, P., & Knott, V. (2019). Leveraging machine learning approaches for predicting antidepressant treatment response using electroencephalography (EEG) and clinical data. *Frontiers in Psychiatry*, *9*, 768.
Johnson, S. ., Seutin, V., & North, R. . (1992). Burst firing in dopamine neurons induced by Nmethyl-D-aspartate: role of electrogenic sodium pump. *Science*, *258*(5082), 665–667.
Kapur, S., & Mann, J. . (1992). Role of the dopaminergic system in depression. *Biological Psychiatry*, *32*(1), 1–17.
Kemp, A., & Manahan-Vaughan, D. (2004). The 5-hydroxytryptamine4 receptor exhibits frequency-dependent properties in a synaptic plasticity and behavioural metaplasticity in the hippocampal CA1 region in vivo. *Cerebral Cortex*, *15*(7), 1037–1043.
Kennedy, M. B. (1989). Regulation of synaptic transmission in the central nervous system: longterm potentiation. *Cell*, *59*(5), 777–787.
Kennett, G. ., Bright, F., Blackburn, T., & Sanger, G. (1997). Anxiolytic-like actions of the selective 5-HT4 receptor antagonists SB 204070A and SB 207266A in rats. *Neuropharmacology*, *36*(4–5), 707–712.
Kilpatrick, G. ., Jones, B. ., & Tyers, M. . (1987). Identification and distribution of 5-HT3 receptors in rat brain using radioligand binding. *Nature*, *330*, 24–31.
Koike, H., Lijima, 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.
Krystal, J. ., Abi-Saab, W., Perry, E., D'Souza, D. ., Liu, N., Gueorguieva, R., … Breier, A. (2005). Preliminary evidence of attenuation of the disruptive effects of the NMDA glutamate receptor antagonist ketamine, on working memory by treatment with the group II metabotropic glutamate receptor agonist LY354740, in healthy human subjects. *Psychopharmacology*, *179*(1), 303–309.
Lacroix, D., Blier, P., Curet, O., & de Montigny, C. (1991). Effects of long-term desipramine administration on noradrenergic neurotransmission: electrophysiological studies in the rat brain. *Journal of Pharmacology and Experimental Therapeutics*, *257*(3), 1081–1090.
Lafaille, F., Welner, S. ., & Suranyi-Cadotte, B. . (1991). Regulation of serotonin type 2 (5-HT2) and beta-adrenergic receptors in rat cerebral cortex following novel and classical antidepressant treatment. *Journal of Neuroscience*, *16*(4), 209–214.
Lammel, S., Lim, B. ., Ran, C., Huang, W. ., Betley, M. ., Tye, K. ., … Malenka, R. . (2012). Input-specifc control of reward and aversion in the ventral tegmental area. *Nature*, *491*, 212–217.
Langer, S. . (1976). The role of alpha- and beta- presynaptic receptors in the regulation of noradrenaline release elicited by nerve stimulation. *Clinical Science and Molecular Medicine*, *51*, 423–426.

Le Moine, C., Normand, E., & Bloch, B. (1991). Phenotypical characterization of the rat striatal neurons expressing the D1 dopamine receptor gene. *Proceedings of the National Academy of Sciences*, *88*(10), 4205–4209.

Learned-Coughlin, S. ., Bergstrom, M., Savitcheva, I., Ascher, J., Schmith, V. ., & Langstrom, B. (2003). In vivo activity of bupropion at the human dopamine transporter as measured by positron emission tomography. *Biological Psychiatry*, *54*(8), 800–805.

Leichsenring, F., Steinert, C., & Hoyer, J. (2016). Psychotherapy versus pharmacotherapy of depression: What's the evidence? *Medizin Und Psychotherapie*, *62*(2), 190–195.

Lemon, N., & Manahan-Vaughan, D. (2006). Dopamine D1/D5 receptors gate the acquisition of novel information through hippocampal long-term potentiation and long-term depression. *Journal of Neuroscience*, *26*(29), 7723–7729.

Lepack, A. ., Fuchikami, M., Dwyer, J. ., Banasr, M., & Duman, R. S. (2014). BDNF release is required for the behavioral actions of ketamine. *International Journal of Neuropsychopharmacology*, *18*(1), pyu003.

Levey, A. ., Hersch, S. ., Rye, D. ., Sunahara, R. ., Niznik, H. ., Kitt, C. ., … Ciliax, B. . (1993). Localzation of D1 and D2 dopamine receptors in brain with subtype-specific antibodies. *Neurobiology*, *90*, 8861–8865.

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

Lindefors, N., Ballarin, M., Ernfors, P., Falkenberg, T., & Persson, H. (1992). Stimulation of Glutamate Receptors Increases Expression of Brain-Derived Neurotrophic Factor mRNA in Rat Hippocampus. *Annals of the New York Academy of Sciences*, *648*(1), 296–299.

Liu, R. ., Lee, F. ., Li, X. ., Bambico, F., Duman, R. ., & Aghajanian, G. . (2012). Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biological Psychiatry*, *71*(11), 996–1005.

Lodge, D. ., & Grace, A. A. (2006). The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *Proceedings of the National Academy of Sciences*, *103*(13), 5167–5172.

Lodge, D. ., & Grace, A. A. (2011). Developmental pathology, dopamine, stress and schizophrenia. *Journal of Developmental Neuroscience*, *29*(3), 207–213.

López-Gil, X., Jiménez-Sánchez, L., Campa, L., Castro, E., Frago, C., & Adell, A. (2019). Role of Serotonin and Noradrenaline in the Rapid Antidepressant Action of Ketamine. *ACS Chemical Neuroscience*, *10*(7), 3318–3326. https://doi.org/10.1021/acschemneuro.9b00288

Lorrain, D., Baccei, C. S., Bristow, L. J., Anderson, J. J., & Varney, M. A. (2003). Effects of Ketamine N-methyl-D-aspartate on glutamate and dopamine release in the rat prefrontal cortex: modulation by a group II selective metabotropic glutamate receptor agonist LY379268. *Neuroscience*, *117*(3), 697–706.

Lucas, G., Rymar, V., Du, J., Mnie-Filali, O., Bisgaard, C., Manta, S., … Sadikot, A. . (2007). Serotonin4 (5-HT4) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron*, *55*(5), 712–725.

Luscher, C., Jan, L. Y., Markus, S., Malenka, R. C., & Nicoll, R. A. (1997). G Protein-Coupled Inwardly Rectifying K Channels (GIRKs) Mediate Postsynaptic but Not Presynaptic Transmitter Actions in Hippocampal Neurons. *Neuron*, *19*, 3687–3695.
https://doi.org/10.1109/IECON.2006.347563

Maeng, S., Zarate, C. A., Schloesser, R. ., McCammon, J., Chen, G., & Manji, H. K. (2008). Cellular Mechanism Underlying the Antidepressant Effects of Ketamine: Role of α-

Amino3-Hydroxy-5-Methylisoxazole-4-Propionic Acid Receptors. *Biological Psychiatry*, *63*(4), 349–352.
Maier, S. ., & Seligman, M. . (1976). Learned helplessness: theory and evidence. *Journal of Experimental Psychology*, *105*(1), 3.
Mann, J. J., Stanley, M., & McBride, A. (1986). Increased serotonin2 and beta-adrenergic receptor binding in the frontal cortices of suicide victims. *Archives of General Psychiatry*, *43*(10), 954–959.
Marcinkiewicz, M., Verge, D., Gozlan, H., Pichat, L., & Hamon, M. (1984). Autoradiographic evidence for the heterogeneity of 5-HT1 sites in the rat brain. *Brain Research*, *291*(1), 159–163.
Marek, G. J., Carpenter, L. L., McDougle, C. J., & Price, L. H. (2003). Synergistic action of 5HT2A antagonists and selective serotonin reuptake inhibitors in neuropsychiatric disorders. *Neuropsychopharmacology*, *28*(2), 402–412.
Marinelli, S., Schnell, S. ., Hack, S. ., Christie, M. ., Wessendorf, M. ., & Vaughan, C. . (2004). Serotonergic and nonserotonergic dorsal raphe neurons are pharmacologically heterogeneous. *Journal of Neurophysiology*, *92*(6), 3532–3537.
Martin, K., Hannon, S., Phillips, I., & Heal, D. (1992). Opposing roles for 5-HT1B and 5-HT3 receptors in the control of 5-HT release in rat hippocampus in vivo. *British Journal of Pharmacology*, *106*(1), 139–142.
Martin, L. ., Blackstone, C. ., Levey, A. ., Huganir, R. ., & Price, D. . (1993). AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience*, *53*(2), 327–358.
Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R., & Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. *Nature*, *349*(6312), 760.
McGirr, A., Berlim, M. T., Bond, D. J., Fleck, M. P., Yatham, L. N., & Lam, R. W. (2015). A systematic review and meta-analysis of randomized, double-blind, placebo-controlled trials of ketamine in the rapid treatment of major depressive episodes. *Psychological Medicine*, *45*(4), 693–704. https://doi.org/10.1017/S0033291714001603
McGrath, J. C., Brown, C. ., & Wilson, V. G. (1989). Alpha-adrenoreceptors: a critical review. *Medical Research Reviews*, *9*, 407–533.
Meldrum, B., & Garthwaite, J. (1990). Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends in Pharmacological Sciences*, *11*, 379–387.
Melyan, Z., Wheal, H. ., & Lancaster, B. (2002). Metabotropic-mediated kainate receptor regulation of IsAHP and excitability in pyramidal cells. *Neuron*, *34*(1), 107–114.
Miladinovic, T., Nashed, M. G., & Singh, G. (2015). Overview of Glutamatergic Dysregulation in Central Pathologies. *Biomolecules*, *5*(4), 3112–3141.
Millan, M. J. (2005). Serotonin 5-HT2C receptors as a target for the treatment of depressive and anxious states: focus on novel therapeutic strategies. *Therapies*, *60*(5), 441–460.
Missale, C. ., Nash, R., Robinson, S. ., Jaber, M., & Caron, M. . (1998). Dopamine Receptors: From Structure to Function. *Physiological Reviews*, *78*(1), 189–225.
Mizoguchi, K., Shoji, H., Ikeda, R., Tanaka, Y., & Tabira, T. (2008). Persisten depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacology Biochemistry and Behavior*, *91*(1), 170–175.
Mnie-Filali, O., Faure, C., Lambás-Señas, L., Mansari, M. El, Belblidia, H., Gondard, E., … Haddjeri, N. (2011). Pharmacological Blockade of 5-HT7 Receptors as a Putative Fast Acting Antidepressant Strategy. *Neuropsychopharmacology*, *36*, 1275.
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*, 2921–2927.
Mohn, A. ., Gainetdinov, R. ., Caron, M. ., & Koller, B. . (1999). Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. *Cell*, *98*, 427–436.
Montagu, K. . (1957). Catechol compounds in rat tissues and in brains of different animals. *Nature*, *180*(4579), 244.
Monyer, H., Burnashev, N., Laurie, D. ., Sakmann, B., & Seeburg, P. H. (1994). Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. *Neuron*, *12*, 529–540.
Moore, R. Y., & Bloom, F. . (1979). Central Catecholamine Neuron Systems: Anatomy and Physiology of the Norepinephrine and Epinephrine Systems. *Annual Review of Neuroscience*, *2*(1), 113–168. https://doi.org/10.1146/annurev.ne.02.030179.000553
Moore, R. Y., Halaris, A. E., & Jones, B. E. (1978). Serotonin neurons of the midbrain raphe: Ascending projections. *Journal of Comparative Neurology*, *180*(3), 417–438. https://doi.org/10.1002/cne.901800302
Moret, C., & Briley, M. (2011). The importance of norepinephrine in depression. *Neuropsyhciatric Disease and Treatment*, *7*(Suppl 1), 9–13.
Morilak, D. A., Garlow, S. J., & Ciaranello, R. D. (1993). Immunocytochemical localization and description of neurons expressing serotonin2 receptors in the rat brain. *Neuroscience*, *54*(3), 701–717.
Morilak, D. A., Somogyi, P., Lujan-Miras, R., & Ciaranello, R. D. (1994). Neurons expressing 5HT 2 receptors in the rat brain: neurochemical identification of cell types by immunocytochemistry. *Neuropsychopharmacology*, *11*(3), 157.
Morrow, A. ., & Creese, I. (1986). Characterization of alpha1-adrenergic receptor subtypes in rat brain: a reevaluation of [3H]WB101 and [3H]prazosin binding. *Molecular Pharmacology*, *29*, 321–330.
Mrzljak, L., Bergson, C., Pappy, M., Huff, R., Levenson, R., & Goldman-Rakic, P. . (1996). Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. *Nature*, *381*(6579), 245.
Muller, J. ., Pryor, W. ., Gibbons, J. ., & Orgain, E. . (1955). Depression and anxiety occuring during Rauwolfia therapy. *Journal of the American Medical Association*, *159*(9), 836–839.
Murrough, J. ., Perez, A. ., Pillemer, S., Stern, J., Parides, M. ., aan het Rot, M., … Iosifescu, D. . (2013). Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biological Psychiatry*, *74*(4), 250–256.
Nemeroff, C. B., & Owens, M. . (2002). Treatment of mood disorders. *Nature Neuroscience*, *5*(11s), 1068.
Nibuya, M., Nestler, E. J., & Duman, R. S. (1996). Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. *Journal of Neuroscience*, *16*(7), 2365–2372.
Nicholl, R. ., Kauer, J. ., & Malenka, R. C. (1988). The current excitement in long term potentiation. *Neuron*, *1*(2), 97–103.
Nichols, D. E., & Nichols, C. D. (2008). Serotonin Receptors. *Chemical Reviewd*, *108*(5), 1614–1641. https://doi.org/10.1021/cr078224o
Niciu, M. ., Ionescu, D. ., Richards, E. ., & Zarate, C. A. (2014). Glutamate and its receptors in the pathophysiology and treatment of major depression disorder. *Journal of Neural Transmission*, *121*(8), 907–924.

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

Owen, R. . (2012). Glutamatergic approaches in major depressive disorder: focus on ketamine, memantine and riluzole. *Drugs of Today*, *48*(7), 469–478.

Page, G., Khidir, F., Pain, S., Barrier, L., Fauconneau, B., Olivier, G., … Hugon, J. (2006). Group I metabotropic glutamate receptors activate the p70S6 kinase via both mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase (ERK 1/2) signaling pathways in rat striatal and hippocampal synaptoneurosomes. *Neurochemistry International*, *49*(4), 413–421.

Palacios, J. M. (2016). Serotonin receptors in brain revisited. *Brain Research*, *1645*, 46–49. https://doi.org/10.1016/j.brainres.2015.12.042

Palucha, A., & Pilc, A. (2005). The involvement of glutamate in the pathophysiology of depression. *Drug News & Perspectives*, *18*(4), 262–268.

Pare, C. M. ., Yeung, D. P. H., Price, K., & Stacey, R. . (1969). 5-Hydroxytryptamine, noradrenaline, and dopamine in brainstem, hypothalamus, and caudate nucleus of controls and of patients committing suicide by coal-gas poisoning. *Lancet*, *294*(7612), 133–135.

Pazos, A., & Palacios, J. M. (1985). Quantitative Autoradiographic Mapping of Serotonin Receptors in the Rat Brain ., *346*, 205–230.

Peroutka, S. J. (1986). Pharmacological differentiation and characterization of 5-HT1A, 5-HT1B, and 5-HT1C binding sites in rat frontal cortex. *Journal of Neurochemistry*, *47*(2), 529–540.

Peroutka, S. J., Lebovitz, R. M., & Snyder, S. H. (1981). Two distinct central serotonin receptors with different physiological functions. *Science*, *212*(4496), 827–829. https://doi.org/10.1126/science.7221567

Phillips, J. ., Norris, S., Talbot, J., Birmingham, M., Hatchard, T., Ortiz, A., … Blier, P. (2019). Single, Repeated, and Maintenance Ketamine Infusions for Treatment-Resistant Depression: A Randomized Controlled Trial. *American Journal of Psychiatry*, *176*(5), 401–409.

Pitychoutis, P. M., Belmer, A., Moutkine, I., Adrien, J., & Maroteaux, L. (2015). Mice lacking the serotonin Htr 2B receptor gene present an antipsychotic-sensitive schizophrenic-like phenotype. *Neuropsychopharmacology*, *40*(12). https://doi.org/10.1038/npp.2015.126

Pompeiano, M., Palacios, J. M., & Mengod, G. (1994). Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Molecular Brain Research*, *23*(1–2), 163–178. https://doi.org/10.1016/0169-328X(94)90223-2

Popik, P., Kos, T., Kucma-Sowa, M., & Nowak, G. (2008). Lack of persistent effects of ketamine in rodent models of depression. *Psychopharmacology*, *198*, 421–430.

Pozzi, L., Dorocic, I. ., Wang, X., Carlen, M., & Meletis, K. (2014). Mice lacking NMDA receptors in parvalbumin neurons display normal depression-related behavior and response to antidepressant action of NMDAR antagonists. *PloS One*, *9*(1), e83879.

Randrup, A., & Braestrup, C. (1977). Uptake Inhibition of Biogenic Amines by Newer Antidepressant Drugs: Relevance to the Dopamine Hypothesis of Depression. *Psychopharmacology*, *53*, 309–314.

Randrup, A., Munkvad, I., Fog, R., Gerlach, J., Molander, L., Kjellberg, B., & Scheel-Kruger, J. (1975). Mania, depression and brain dopamine. In W. . Essman & L. Valzelli (Eds.), *Current developments in psychopharmacology* (2nd ed., pp. 206–248). New York: Spectrum Publications.

Rees, S., den Daas, I., Foord, S., Goodson, S., Bull, D., Kilpatrick, G., & Lee, M. (1994). Clonin and characterisation of the human 5-HT5A serotonin receptor. *FEBS Letters*, *355*(3), 242–246.

Reichardt, L. . (2006). Neurotrophn-regulated signalling pathways. *Philosophical Translations of the Royal Society B: Biological Sciences*, *361*(1473), 1545–1564.

Reimer, C., Borroni, E., Levet-Trafit, B., Martin, J. ., Poli, S., Porter, R. ., & Bos, M. (2003). INfluence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl) phenylamine, a potent and selective 5-HT6 receptor antagonist. *Journal of Medicinal Chemistry*, *46*(7), 1273–1276.

Ren, Z., Pribiag, H., Jefferson, S. ., Shorey, M., FUchs, T., Stellwagen, D., & Luscher, B. (2016). Bidirectional homeostatic regulation of a depression-related brain state by gammaaminobutyric acidergic deficits and ketamine treatment. *Biological Psychiatry*, *80*(6), 457– 468.

Richelson, E., & Pfenning, M. (1984). Blockade by antidepressants and related compounds of biogenic amine uptake into rat brain synaptosomes: Most antidepressants selectively block norepinephrine uptake. *European Journal of Pharmacology*, *104*(3–4), 277–286.

Rodriguez-Moreno, A., Herreras, O., & Lerma, J. (1997). Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. *Neuron*, *19*(4), 893–901. Rush, A. J., Trivedi, M. ., Wisniewski, S. ., Nierenberg, A. ., Stewart, J. ., Warden, D., … MacGrath, P. . (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.

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

Saraceni, M. ., Venci, J. ., & Gandhi, M. . (2014). Levomilnacipran (Fetzima) a new serotoninnorepinephrine reuptake inhibitor for the treatment of major depressive disorder. *Journal of Pharmacy Practice*, *27*(4), 389–395.

Sari, Y. (2004). Serotonin 1B receptors: from protein to physiological function and behavior. *Neuroscience & Biobehavioral Reviews*, *28*(6), 565–582.

Sarkisyan, G., Roberts, A. J., & Hedlund, P. . (2010). The 5-HT7 receptor as a mediator and modulator of antidepressant-like behavior. *Behavioural Brain Research*, *209*(1), 99–108.

Savitz, J., Lucki, I., & Drevets, W. C. (2009). 5-HT 1A receptor function in major depressive disorder. *Progress in Neurobiology*, *88*, 17–31. https://doi.org/10.1016/j.pneurobio.2009.01.009

Schatzberg, A. F., Rosenbaum, A. H., Orsulak, P. J., Rohde, W. A., Maruta, T., Kruger, E. R., … Schildkraut, J. J. (2004). Toward a biochemical classification of depressive disorders. *Psychopharmacology*, *75*(1), 34–38. https://doi.org/10.1007/bf00433498

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

Schmitz, D., Frerking, M., & Nicoll, R. A. (2000). Synaptic activation of presynaptic kainate receptors on hippocampal mossy fiber synapses. *Neuron*, *27*(2), 327–338.

Schuttler, J., Stanski, D. ., White, P. ., Trevor, A. ., Horai, Y., Verotta, D., & Sheiner, L. . (1987). Pharmacodynamic modeling of the EEG effects of ketamine and its enantiomers in man. *Journal of Pharmacokinetics and Biopharmaceutics*, *15*(3), 241–253.

Seeman, P., Guan, H. ., & Van Tol, H. . (1993). Dopamine D4 receptors elevated in schizophrenia. *Nature*, *365*(6445), 441.

Seeman, P., & Van Tol, H. . (1994). Dopamine receptor pharmacology. *Trends in Pharmacological Sciences*, *15*(7), 264–270.
Shaw D. M., Camps F. E., E. E. G. (1967). 5-Hydroxytryptamine in the Hind-Brain of Depressive Suicides. *The British Journal of Psychiatry*, *113*(505), 1407–1411.
Shim, S., El Mansari, M., & Blier, P. (2013). Modulation of the antidepressant-like effects of sustained administration of carisbamate and lamotrigine on monoaminergic systems: electrophysiological studies in the rat brain. *Journal of Pharmacology and Experimental Therapeutics*, *347*(2), 487–496.
Shipley, M. ., Pieribone, V. ., & Aston-Jones, G. (1988). GABA-ergic innervation of the rat locus coeruleus. *Society for Neuroscience Abstracts*, *14*, 406.
Sibley, D. ., & Monsama Jr., F. . (1992). Molecular Biology of dopamine receptors. *Trends in Pharmacological Sciences*, *13*, 61–69.
Siever, L. ., & Davis, K. . (1985). Overview: Toward a Dysregulation Hypothesis of Depression. *American Journal of Psychiatry*, *142*(9), 1017–1031.
Siever, L. ., & Uhde, T. . (1984). New studies and perspectives on the noradrenergic receptor system in depression: effects of the alpha 2-adrenergic agonist clonidine. *Biological Psychiatry*, *19*(2), 131–156.
Simonneauz, V., Ebadi, M., & Bylund, D. . (1991). Identification and characterization of alpha2D adrenergic receptors in bovine pineal gland. *Molecular Pharmacology*, *40*, 235–241.
Sinner, B., & Graf, B. M. (2008). *Modern Anesthetics. Handbook of Experimental Pharmacology*.
Smiley, J. ., Levey, A. ., Ciliax, B. ., & Goldman-Rakic, P. . (1994). D1 dopamine receptor immunoreactivity in human and monkey cerebral cortex: predominant and extrasynaptic localization in dendritic spines. *Proceedings of the National Academy of Sciences*, *91*(2), 5720–5724.
Smith, D. ., Azzaro, A. ., Zaldivar, S. ., Plamer, S., & Lee, H. . (1981). Properties of the optical isomers and metabolites of ketamine on the high affinity transport and catabolism of monoamines. *Neuropharmacology*, *20*, 391–396.
Sou, J. H., Chan, M. H., & Chen, H. H. (2006). Ketamine, but not propofol, anesthesia is regulated by metabotropic glutamate 5 receptors. *British Journal of Anesthesia*, *96*(5), 597– 601.
Stein, D. J. (2008). Depression, Anhedonia, and Psychomotor Symptoms: The Role of Dopaminergic Neurocircuitry. *CNS Spectrums*, *13*(7), 561–565.
Stiles, G. ., Caron, M. ., & Jefkowitz, R. (1984). Beta-adrenergic Receptors: Biochemical Mechanisms of Physiological Regulation. *Physiological Reviews*, *64*(2), 661–734.
Sun, Y., Olson, R., Horning, M., Armstrong, N., Mayer, M., & Gouaux, E. (2002). Mechanism of glutamate receptor desensitization. *Nature*, *417*, 245–253.
Svenningsson, P., Tzavara, E. T., Qi, H., Carruthers, R., Witkin, J. M., Nomikos, G. G., & Greengard, P. (2007). Biochemical and Behavioral Evidence for Antidepressant-Like Effects of 5-HT6 Receptor Stimulation. *Journal of Neuroscience*, *27*(15), 4201–4209. https://doi.org/10.1523/jneurosci.3110-06.2007
Szabo, S. T., & Blier, P. (2001). Effect of the selective noradrenergic reuotake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons. *European Journal of Neuroscience*, *13*(11), 2077–2087.
Taliaz, D., Loya, A., Gersner, R., Haramati, S., Chen, A., & Zangen, A. (2011). Resilience to chronic stress is mediated by hippocampal bran-derived neurotrophic factor. *Journal of Neuroscience*, *31*(12), 4475–4483.
Taylor, M. R. G. (2007). Pharmacogenetics of the human beta-adrenergic receptors. *Pharmacogenomics Journal*, *7*(1), 29–37. https://doi.org/10.1038/sj.tpj.6500393

Tekes, K., Tothfalusi, L., Gaal, J., & Magyar, K. (1988). Effect of MAO inhbitors on the uptake and metabolism of dopamine in rat and human brain. *Polish Journal of Pharmacology and Pharmacy*, *40*(6), 653–658.

Thomas, D. ., & Hagan, J. . (2004). 5-HT7 receptors. *Current Drug Targets-CNS & Neurological Disorders*, *3*(1), 81–90.

Thomas, D. ., Melotto, S., Massagrande, M., Gribble, A. ., Jeffrey, P., Stevens, A. ., … Stean, T. (2003). SB-656104-A, a novel selective 5-HT7 receptor antagonist, modulates REM sleep in rats. *British Journal of Pharmacology*, *139*(4), 705–714.

Tsai, G. ., & Lin, P. . (2010). Strategies to enhance N-methyl-D-aspartate receptor-mediated neurotransmission in schizophrenia, a critical review and meta-analysis. *Current Pharmaceutical Design*, *16*, 522–537.

Tso, M. ., Blatchford, K. ., Callado, L. ., McLaughlin, D. ., & Stamford, J. . (2004). Stereoselctive effects of ketamine on dopamine, serotonin and noradrenaline release and uptake in rat brain slices. *Neurochemistry International*, *44*, 1–7.

Tye, K. ., Mirzabekov, J. ., Warden, M. R., Ferenczi, E. ., Tsai, H.-C., Finkelstein, J., … Deisseroth, K. (2013). Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*, *493*, 537–541.

Valenti, O., Lodge, D. ., & Grace, A. A. (2011). Aversive Stimuli Alter Ventral Tegmental Area Dopamine Neuron Activity via a Common Action in the Ventral Hippocampus. *The Journal of Neuroscience*, *31*(11), 4280–4289. van Hooft, J. ., & Yakel, J. . (2003). 5-HT3 receptors in the CNS: 3B or not 3B? *Trends in Pharmacological Sciences*, *24*(4), 157–160.

Van Tol, H. ., Bunzow, J. ., Guan, H. ., Sunahara, R. ., Seeman, P., Niznik, H. ., & Civelli, O. (1991). Clonin of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature*, *350*(6319), 610.

Van Tol, H. ., Wu, C. ., Guan, H. ., Ohara, K., Bunzow, J. ., Civelli, O., … Jovanovic, V. (1992). Multiple dopamine D4 receptor variants in the human population. *Nature*, *358*(6382), 149.

Viggiano, D., Ruocco, L. ., Arcieri, S., & Sadile, A. . (2004). Involvement of norepinephrine in the control of activity and attentive processes in animal models of attention deficit hyperactivity disorder. *Neural Plasticity*, *11*(1–2), 133–149.

Wallukat, G. (2002). The beta-adrenergic receptors. *Herz*, *27*(7), 683–690.

Weiss, J. ., Stout, J. ., Aaron, M. ., Quan, N., Owens, M. ., Butler, P. ., & Nemeroff, C. B. (1994).
Depression and Anxiety: Role of the Locus Coeruleus and Corticotropin-releasing Factor. *Brain Research Bulletin*, *35*(5/6), 561–572.

Wesolowska, A., Tatarczynska, E., Nikiforuk, A., & Chojnacka-Wojcik, E. (2007). Enhancement of the anti-immobility action of antidepressants by a selective 5-HT7 receptor antagonist in the forced swimming test in mice. *European Journal of Pharmacology*, *555*(1), 43–47.

Wilkinson, S. ., Ballard, E. ., Bloch, M. ., Matthew, S. ., Murrough, J. ., Feder, A., … Sanacora, G. (2017). The effect of a single dose of intravenous ketamine on suicidal ideation: a systematic review and individual participant data meta-analysis. *American Journal of Psychiatry*, *175*(2), 150–158.

Willins, D. L., Deutch, Ariel, Y., & Roth, B. L. (1997). Serotonin 5-HT2A receptors are expressed on pyramidal cells and interneurons in the rat cortex. *Synapse*, *27*, 79–82.

Willner, P. (1983). Dopamine and Depression: A review of recent Evidence. *Brain Research Reviews*, *6*, 211–224.

Wilson, K. ., & Minneman, K. . (1990). Different pathways of [3H]inositol phosphate formation medicated by alpha1a- and alpha1b-adrenergic receptors. *Journal of Biological Chemistry*, *265*, 17601–17606.

Wisden, W., & Seeburg, P. H. (1993). Mammalian ionotropic glutamate receptors. *Current Opinion in Neurobiology*, *3*(3), 291–298.

Wise, R. A. (2008). Dopamine and Reward: The Anhedonia Hypothesis 30 years on. *Neurotoxicity Research*, *14*(2, 3), 169–183.

Xiang, L., Szebeni, K., Szebeni, A., Klimek, V., Stockmeier, C. A., Karolewicz, B., … Ordway, G. A. (2008). Dopoamine receptor gene expression in human amygdaloid nuclei: Elevated D4 receptor mRNA in major depression. *Brain Research*, *1207*, 214–224.

Yamamoto, S., Ohba, H., Nishiyama, S., Harada, N., Kakiuchi, T., Tsukada, H., & Domino, E. F.

(2013). Subanesthetic doses of ketamine transiently decrease serotonin transporter activity: A PET study in conscious monkeys. *Neuropsychopharmacology*, *38*(13), 2666–2674. https://doi.org/10.1038/npp.2013.176

Yang, C., Qu, Y., Abe, M., Nozawa, D., Chaki, S., & Hashimoto, K. (2017). (R)-ketamine shows greater potency and longer lasting antidepressant effects than its metabolite (2R, 6R)hydroxynorketamine. *Biological Psychiatry*, *82*(5), e43–e44.

Yang, C., Shirayama, Y., Zhang, J. ., Ren, Q., Yao, W., Ma, M., … Hashimoto, K. (2015). Rketamine: a rapid-onset and sustained antidepressant without psychotomimetic side effects. *Translational Psychiatry*, *5*(9), e632.

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., & Hu, H. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature*, *554*(7692), 317–322. https://doi.org/10.1038/nature25509

Yoshii, A., & Constantine-Paton, M. (2010). Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. *Developmental Neurobiology*, *70*(5), 304–322.

Zanos, P., & Gould, T. D. (2018). Mechanisms of ketamine action as an antidepressant. *Molecular Psychiatry*, *23*(4), 801–811. https://doi.org/10.1038/mp.2017.255

Zanos, P., Moaddel, R., Morris, P. ., Georgiou, P., Fischell, J., Elmer, G., … Dossou, K. . (2016). NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, *533*(7604), 481.

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), 856–864.

Zeilhofer, H. ., Swandulla, D., Geisslinger, G., & Brune, K. (1992). Differential effeccts of ketamine enantiomers on NMDA receptor currents in cultured neurons. *European Journal of Pharmacology*, *213*(1), 155–158.

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

Zhou, W., Wang, N., Yang, C., Li, X. ., Zhou, Z. ., & Yang, J. . (2014). Ketamine-induced antidepressant effects are associated with AMPA receptors-mediated upregulation of mTOR and BDNF in rat hippocampus nad prefrontal cortex. *European Psychiatry*, *29*(7), 419–423.

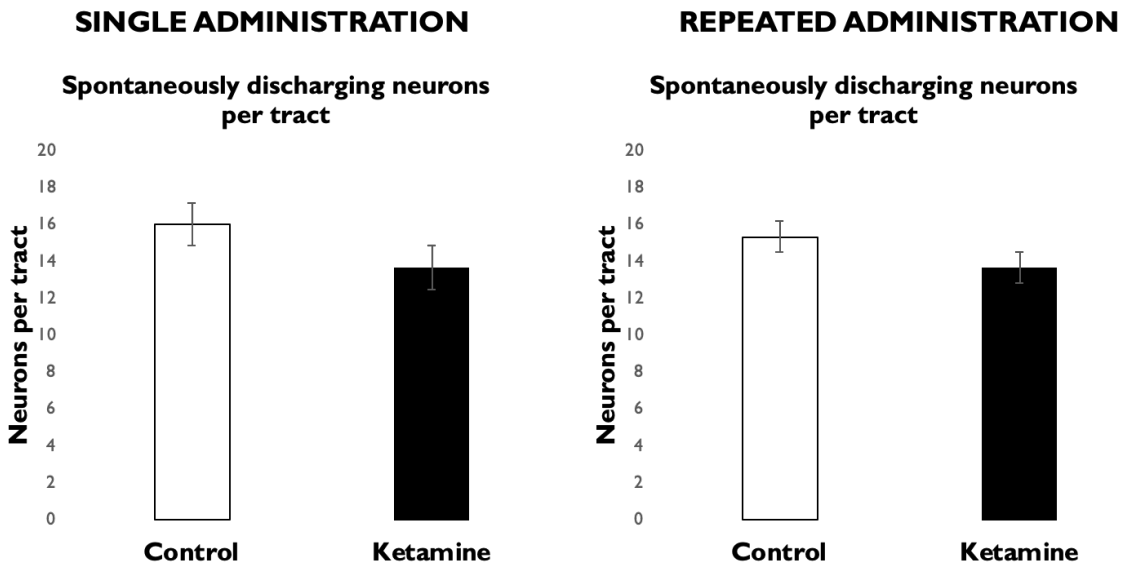
Zhou, Y., & Danbolt, N. C. (2014). Glutamate as a neurotransmitter in the healthy brain. *Journal of Neural Transmission*, *121*(8), 799–817.

Appendix

Single Administration						Repeated Administration		
		Firing	Burst	Neurons /tract		Firing	Burst	Neurons/ tract
DRN 5-HT	30 [2] mins - 2 hrs	—	—	N/A		N/A	N/A	N/A
	24 hrs	—	—	N/A		—	—	N/A
VTA DA	30[2] mins - 2 hrs	—	—	↗		N/A	N/A	N/A
	24 hrs	—	—	—		—	↗	↗
	3 days	N/A	N/A	N/A		—	↗	—
	7 days	N/A	N/A	N/A		—	—	—
LC NE	30[2] mins – 2 hrs	↗	↗	N/A		N/A	N/A	N/A
	24 hrs	—	—	N/A		↗	—	N/A
	3 days	N/A	N/A	N/A		—	—	N/A

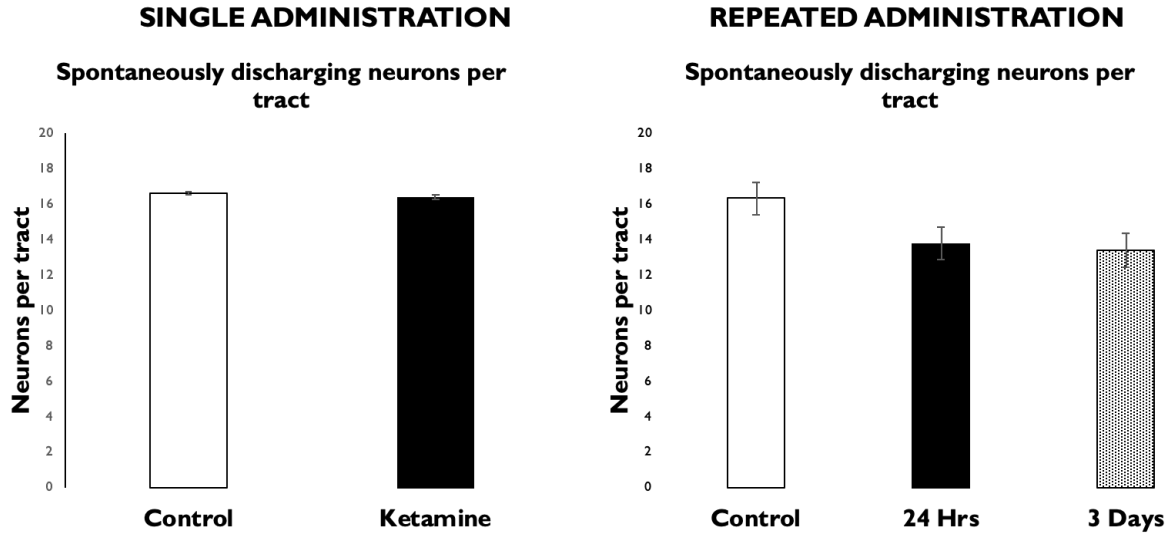
Figure A1. Summary of findings from electrophysiological experiments examining effects of single and repeated ketamine administration on monoamine neurons.

DRN 5-HT neurons



Effects of single and repeated administration of ketamine on number of spontaneously discharging 5-HT neurons per tract recorded in the DRN

LC NE neurons



Effects of single and repeated administration of ketamine on number of spontaneously discharging NE neurons per tract recorded in the LC