Electrophysiological Investigations of the Effects of a Subanesthetic Dose of Ketamine on Monoamine Systems.

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ABSTRACT

Ketamine is a non-competitive NMDA antagonist that has been shown to have antidepressant properties both clinically as well as in preclinical studies when administered at a subanesthetic dose. *In vivo* electrophysiological recordings were carried in male Sprague Dawley rats 30 minutes following ketamine administration (10 mg/kg) to first assess its effects on monoaminergic firing. Whilst no change in the firing activity of serotonin (5-HT) neurons was observed in the dorsal raphe nucleus (DRN), an increase in the firing activity was observed for dopamine (DA) and noradrenergic (NE) neurons in the ventral tegmental area (VTA) and locus coeruleus (LC), respectively. The effect of ketamine on these electrophysiological parameters was prevented by pre-administration of the AMPA receptor antagonist NBQX 10 minutes prior to ketamine administration. In a second series of experiments, an increase in AMPA-evoked response was observed within 30 minutes in the CA3 layer of the hippocampus (HPC) following acute ketamine administration. These findings suggest that acute ketamine administration produces a prompt enhancement of AMPA transmission in the forebrain and also results in increased catecholaminergic activity. These effects may play a crucial role in the rapid antidepressant effects of ketamine observed shortly following its infusion in the clinic.

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5-HT	5-hydroxytryptamine, serotonin
8-OH-DPAT	8-hydroxy-2-(di-n-propylamino)tetralin
ACC	anterior cingulate cortex
AD	antidepressant
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AMPT	α-methyl paratyrosine
ANOVA	analysis of variance
AP	anterior-posterior
APA	American Psychiatric Association
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CREB	cAMP response element-binding protein
DA	dopamine
DAT	dopamine transporter
DOS	duration of suppression
DRN	dorsal raphe nucleus
DLPFC	dorsolateral prefrontal cortex
DMPFC	dorsomedial prefrontal cortex
EAAT	excitatory amino acid transporter
EEF	eukaryotic elongation factor
FST	forced swim test
GABA	gamma-aminobutyric acid
HAM-D	Hamilton Depression Rating Scale
HPC	hippocampus
i.p.	intraperitoneal
i.v.	intravenous
LC	locus coeruleus

MAOI	monoamine oxidase inhibitor
MDD	major depressive disorder, unipolar depression, clinical depression
MDE	major depressive episode
mGluR	metabotropic glutamate receptor
mTOR	mammalian target of rapamycin
NAc	nucleus accumbens
NBQX	2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione
NE	norepinephrine
NET	norepinephrine transporter
NMDA	*N*-methyl-D-aspartate
nPGi	nucleus paragigantocellularis
NRI	norepinephrine reuptake inhibitor
OCC	occiptal cortex
PCP	phenylcyclidine
PFC	prefrontal cortex
SEM	standard error of the mean
SERT	serotonin transporter
SGZ	subgranular zone
SN	substantia nigra
SNRI	serotonin norepinephrine reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
SVZ	subventricular zone
TPH2	tryptophan hydroxylase 2
TST	tail suspension test
VGLUT	vesicular glutamate transporter
VMAT	vesicular monoamine transporter
VMPFC	ventromedial prefrontal cortex
VP	ventral pallidum
vSUB	ventral subiculum
VTA	ventral tegmental area
WHO	World Health Organization

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INTRODUCTION

1. Major depressive disorder

1.1 Background

Major depressive disorder (MDD) is an increasingly common and debilitating disorder affecting more than 121 million people worldwide. Nearly 1 in 5 people will experience a major depressive episode at some point in their lives [16]. According to the World Health Organization estimates, MDD is projected to be the world's leading cause of disability worldwide by 2030 [17]. Current estimates make MDD the leading burden of all diseases, measured in disability adjusted life years, in middle-high income countries, surpassing figures for ischemic heart disease [17].

According to the American Psychiatric Association's (APA) Diagnostic and Statistical Manual of Mental Disorders 5th Edition Text Revision (DSM-5-TR; APA, 2013), MDD diagnosis requires the characterization of least one major depressive episode (MDE) of a minimum length of two weeks of depressed mood and/or anhedonia combined with at least five other depressive symptoms, including changes in weight, insomnia/hypersomnia, psychomotor agitation/retardation, diminished energy, and ability to concentration, feelings or worthlessness or inappropriate guilt, and recurrent suicidal ideation. Persistent symptoms of depression ultimately interfere with one's ability to work, study and deal with interpersonal relationships and enjoy activities that were once pleasurable.

In 1990, the economic burden of depression in the United States was estimated to be $77.4 billion, including direct treatment costs, lost earnings due to depression-related suicides, and indirect workplace costs [56]. In 10 years, this figure rose by 7% to $83.1

billion despite a documented increase in the rate of depression treatment [56]. In addition to the lost productivity and cost, suicidal ideation is a common symptom in patients with MDD. Strikingly, it is currently estimated that approximately 1 million people commit suicide each year globally [21], and suicide has become the third leading cause of death in individuals aged between 15 and 24 in the US [140]. The burden of MDD is both an economical concern as well as a human one. With the rising number of cases worldwide there now is an increased impetus for developing a better understanding of MDD, the antidepressant response, and the brain systems involved in depression. This can be accomplished in part by better understanding how antidepressants modulate the function of different brain regions to bring about a therapeutic effect.

1.2 Etiology

MDD is a multifactorial disorder and as a result, diagnosis as well as efforts to identify both genetic and environmental causes of the disorder is very challenging. Studies comparing the concordance rates for MDD between monozygotic and dizygotic twins suggest a heritability of about 37% [127]. Familial studies have suggested that there is no one "depression gene", but that MDD is a complex disorder with diverse genetic features. Such genetic variability could be the reason behind the heterogeneity of MDD, and explain why 2 patients can be similarly diagnosed with MDD despite not having a single symptom in common. Furthermore, while genetic susceptibility can predispose an individual to succumbing to MDD, experiencing external life stressors can also facilitate the developments of such symptoms [16]. Nevertheless, the role of genetic and environmental factors in the development of depressive symptoms has yet to be fully elucidated, thus hindering efforts to develop ideal animal models of depression. This has

led to a reliance on behavioral, biochemical and electrophysiological paradigms to determine the efficacy of different antidepressant treatments, as well as the development of enhanced treatment approaches including augmentation strategies.

1.3 Brain systems implicated in MDD

The heterogeneous nature of MDD, combined with the fact that there are no objective diagnostic tests available to assess depression in the clinic have confounded efforts to identify the brain regions involved in depression. Although the site of pathology is unknown, there is growing evidence implicating different brain regions in the variable depressive symptoms experienced by patients [18]. To date, the hippocampus (HPC) and the frontal regions of the cerebral cortex are the two forebrain regions that have received the most attention [18]. The cognitive processes implicated in these regions match with the cognitive abnormalities MDD patients experience. However other regions are also believed to be involved in MDD, including the amygdala, nucleus accumbens (NAc), and the hypothalamus [18].

Despite our limited knowledge on the etiology of MDD and the systems involved, diverse treatment strategies are available to treat patients experiencing depressive episodes. Tricyclics are the first generation of antidepressants, and their ability to alleviate depressive symptoms has been very well documented since their discovery in the 1950s [84]. Tricyclics mainly act by blocking monoamine transporters, thereby increasing extracellular levels of monoamine transmitters in the synapse. As the mechanism of action of tricyclics is predominantly to increase brain monoamine levels, their ability to alleviate depressive systems suggests that monoamines could play a very important role in the pathophysiology of MDD, and as a result much of the focus was

given to these systems because of their role in mood, emotion and cognitive function [123]. Additionally, these systems originate deep within the brain and innervate a wide network of structures, including the HPC and prefrontal cortex (PFC), which are known to play an important role in emotion regulation and cognitive function. Thus abnormalities in monoaminergic; serotonin (5-HT), norepinephrine (NE) and dopamine (DA) systems have been implicated in the pathophysiology of MDD and these systems will be discussed in detail.

2.0 The monoaminergic systems

2.1 The serotonergic system

Serotonin, also known as 5-hydoxytryptamine, is an indolamine and one of the three monoamine neurotransmitters located in the central nervous system (CNS). The neurons of the raphe nuclei are the cardinal source of 5-HT in the CNS. An estimated 50-60% of 5-HT neurons are located in the dorsal raphe nucleus (DRN) [10]. A good deal of interest and speculation has been directed to the possible role of 5-HT in depression as its role in emotion and mood is widely recognized and shown [36, 40]. Evidence of 5-HT involvement in MDD was further strengthened by observed findings in acute tryptophan depletion studies where a brief recurrence of depressive symptoms is observed in patients that had previously shown a response to serotonergic-related ADs such as selective serotonin reuptake inhibitors (SSRI) [31].

5-HT is synthesized inside the neuron terminal from its precursor tryptophan in 2 steps, with the first involving the neuron form of the rate limiting enzyme tryptophan hydroxylase (TPH2) [141]. Following its synthesis, 5-HT is packaged into vesicles via

the vesicular monoamine transporter (VMAAT). A depolarization of the presynaptic neuron facilitates 5-HT exocytosis into the synapse where it can act on 5-HT receptors. Subsequently, 5-HT reuptake occurs via the 5-HT transporters into the nerve terminal where it is either repackaged or metabolized by monoamine oxidase.

There are 14 5-HT receptors that are known to date, and these receptors can be located presynaptically on 5-HT neurons, or postsynaptically at downstream targets [143]. 5-HT receptors that are located presynaptically predominantly regulate the firing activity of 5-HT neurons [143]. 5-HT_{1A} receptors are one subtype of presynaptic autoreceptors that are located on the dendrites and cell body of 5-HT neurons. They play a crucial role in regulating the firing activity of 5-HT neurons via a feedback mechanism [1, 24]. As such, triggering these receptors results in an activation of the coupled inhibitory $G_{\alpha i3}$ proteins, which in turn leads to an influx of a hyperpolarizing K^+ current, and a reduction 5-HT release into the synapse [69, 143].

Postsynaptic 5-HT_{1A} receptors are $G_{\alpha o}$-protein coupled receptors and their activation by 5-HT leads to an influx of a hyperpolarizing K^+ current, and a subsequent inhibition of postsynaptic neuron firing [25, 69]. A hyperactivity of postsynaptic regions has been implicated in depression as both postmortem in situ hybridization and PET imaging studies have shown that there is a reduction in 5-HT_{1A} receptor levels in postsynaptic regions in unmedicated, depressives and depressed suicide victims [42, 80]. Thus, it is important to regard the postsynaptic effects when determining the consequence of antidepressant treatments on monoamine transmission. Assessment of the tonic activation of postsynaptic 5-HT_{1A} receptors is one way to achieve this. It has been demonstrated that an enhanced serotonergic neurotransmission incurred by AD drugs and

electroconvulsive shocks (ECS) is due to enhanced activation or sensitivity of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus CA3 pyramidal neurons, which can be measured in terms of the degree of disinhibition in the presence of the selective 5-HT_{1A} antagonist WAY 100635 [57]. Thus, it is important to determine the responsiveness of 5-HT_{1A} receptors in these neurons when assessing the efficacy of AD treatments.

Studies have suggested that depressive symptoms experienced by MDD patients might be due to abnormalities in the regulation of 5-HT activity. One such study revealed that binding of the 5-HT_{1A} receptor agonist [3H]8-hydroxy-2-(di-n-propyl)aminotetralin (8-OH-DPAT) was enhanced in the DRN of suicide victims with depression [125]. Such an increase in binding was attributed to an increase in autoreceptor density, which could lead to an increased activation of this negative feedback mechanism, a decreased firing, and ultimately neurotransmitter release. This finding would then support the hypothesis of diminished 5-HT activity in MDD patients.

The 5-HT_{1B} subfamily is another subset of presynaptic 5-HT autoreceptors that play a crucial role in regulating 5-HT release into the synapse [143]. These receptors are present at the nerve terminals of 5-HT neurons, and their stimulation results in an inhibition of exocytosis of packaged 5-HT due to inactivation of Ca^{2+} channels located on the terminal end [143]. Recent studies have suggested that 5-HT_{1B} receptors may be involved in the pathophysiology of MDD. Specifically, studies have demonstrated that p11, a binding protein closely associated with 5-HT_{1B} receptors, increases localization of 5-HT_{1B} at the cell surface and that both antidepressants and electroconvulsive therapy (ECT) increase p11 expression in the cingulate cortex in rodents [129]. Moreover, reductions in p11 expression were shown in both animal models of depression and in

postmortem brains from depressed patients [129]. Overexpression of p11 in transgenic mice resulted in antidepressant-like behavior in the tail suspension test (TST), whereas p11 knockout mice displayed depressive phenotypes and were not responsive to antidepressant administration. In a second series of experiments, a reduction of p11 expression in the NAc resulted in depressive-like behavior, and restoration of p11 expression reversed this effect [4]. Additionally, decreased p11 expression in the NAc in postmortem brains of depressed patients was reported, complementing earlier findings [4]. Whilst preliminary, such findings highlight the potential importance of 5-HT_{1B} receptor regulation in the pathophysiology of MDD.

2.2 The dopaminergic system

Most of the DA producing neurons are located in the brainstem nuclei [88]. These include the retro-rubro field (A8), the substantia nigra pars compacta (A9), and the ventral tegmental area (VTA; A10). There are three main projection pathways that axons arising from these nuclei follow [88]. One such pathway is the nigrostriatal pathway which projects from the substantia nigra to the dorsal striatum. This pathway plays a role in motor planning and execution of movement [88]. The second pathway is the mesocortical pathway which arises from the VTA and projects the frontal and temporal cortices, and plays an important role in concentration and working memory [88]. The third pathway is the mesolimbic pathway which also originates from the VTA, and projects to the ventral striatum [44]. This pathway is believed to play an important role in the experience of pleasure, motivation and reward [44]. A fourth major DA pathway is the tuberoinfundibular pathway which projects from the hypothalamus to the pituitary

gland, and plays an important role in regulating the secretion of hormones, including prolactin [101]. As DA is known to play a major role in regulating hedonic responses, and anhedonia is one of the cardinal symptoms of depression, a dysregulation in any of the aforementioned DA pathways may potentially put individuals at risk of developing depressive symptoms [44].

DA is synthesized at nerve terminals from tyrosine and phenylalanine, and its synthesis is catalyzed by the enzyme tyrosine hydroxylase. Like 5-HT, DA is packaged into vesicles, following its synthesis, via VMAAT. Following a depolarization of the presynaptic neuron, DA is exocytosed into the synapse [44]. Reuptake of DA from the synapse is arbitrated by the dopamine transporters (DAT) as well as norepinephrine transporters (NET), due to DA structural resemblance to NE [44]. Dopamine exerts its effects on postsynaptic neurons via its interaction with receptors belonging to one of two families. The D1 family (comprising D1 and D5 receptor subtypes) is coupled to stimulatory $G_{\alpha s}$ proteins, and their activation results in an activation of the adenylyl cyclase second messenger system [44]. The D2 family on the other hand (comprising D2, D3 and D4 receptor subtypes) is coupled to inhibitory $G_{\alpha i}$ proteins that reduce adenylyl cyclase activity [44]. The firing activity and synaptic release of DA neurons are regulated through D_2 somatodendritic and terminal autoreceptors, respectively [44].

2.3 The noradrenergic system

Noradrenergic neurons arise from the locus coeruleus, a distinct nucleus located near the wall of the fourth ventricle in the posterior area of the rostral pons [50]. Despite its localized origins, projections out of the locus coeruleus are widespread throughout the CNS reaching targets including the amygdala PFC and the NAc [50]. The noradrenergic

system plays an important role in emotional pain and mediating many of the sympathetic effects that occur during stress. This is achieved by initiating the fight-or-flight response via the hypothalamic-pituitary-adrenal axis [2]. Thus, an imbalanced system would result in the inability to adequately respond to external stressors which is one of the key characteristics of MDD [130].

NE and DA are structurally alike; the difference between DA and NE is one hydroxyl group, and as a result they share the same synthetic pathway. NE is directly synthesized from DA via the enzyme dopamine-β-hydroxylase before it is packaged into vesicles by VMAAT. Following a depolarization event, exocytosis of NE containing vesicles occurs, resulting in the release of NE into the synaptic cleft [118]. Excess NE is then taken up by NET for subsequent recycling or degradation by monoamine oxidase (MAO). Firing of NE neurons is regulated by α2-arenergic autoreceptors that located on the cell body. Activation of these somatodendritic autoreceptors results in an influx of a hyperpolarizing K^+ current, ultimately leading to decreased NE neuronal firing [5, 31].

Reduced NE function has been linked to MDD. Post mortem studies on depressed patients who have committed suicide reveal an elevated binding of the agonist iodoclonidine to the α2-arenergic autoreceptor; indicating an upregulation of these receptors [100]. Moreover, clinical studies have revealed that not unlike tryptophan depletion, catecholamine depletion in remitted patients using a NET synthesis inhibitor also resulted in a relapse of depressive symptom, therefore directly implicating NE in the antidepressant response [92].

3.0 Monoamine deficiency hypothesis for MDD

The central and current view on the pathophysiology of depressive disorders has been the "monoamine hypothesis" which considers shortages of monoamines in the brain as an underlying factor in the pathophysiology of the disorder [18, 34]. This hypothesis has been supported by knowledge of the acute mechanism of action of first generation and subsequent generations of antidepressants. Although the mechanism of action of such antidepressants, including SSRIs, norepinephrine reuptake inhibitors (NRIs), tricyclic antidepressants (TCAs) and MAO inhibitors (MAOIs) may be different, they all ultimately produce an increase in brain monoamine levels [16]. However this early theory has suffered several setbacks, the most major of which was the fact that changes in monoamine levels at some synapses can be detected within minutes after antidepressant administration; however a therapeutic response requires continuous administration of these drugs for weeks [36]. Following extensive research focusing on the long term effects of such treatments, a modified theory emerged suggesting that the transient increase in 5-HT levels that occur around the cell body and dendrites following acute antidepressant administration are not observed in the postsynaptic areas due to a negative feedback mechanism enforced by activation of the somatodendritic 5-HT_{1A} autoreceptors, and as a result the firing activity is reduced [14]. Following chronic treatment however, firing activity return to normal and postsynaptic 5-HT levels are elevated due to desensitization of the 5-HT_{1A} autoreceptors [15]

There are several lines of evidence supporting the monoamine theory for MDD. 5-HT and NE can be depleted experimentally in humans by oral treatments [113]. A drink containing all the amino acids with the exception of tryptophan stimulates the liver

to produce protein and depletes the plasma and the brain of tryptophan. As tryptophan is the rate limiting enzyme for 5-HT synthesis, its depletion produces a reduction in available 5-HT. NE and DA depletion can be induced with treatments of α-methyl paratyrosine (AMPT), which blocks the tyrosine hydroxylase enzyme and prevents the production of NE and DA from its precursor, L-tyrosine. MDD patients who remit following treatment with selective SSRIs show a reoccurrence of signs and symptoms of depression following such tryptophan depletion, whilst patients who remit following NRI treatments show a reoccurrence of signs and symptoms of depression following an AMPT depletion paradigm [11, 72]. It is important to note that almost every compound that has been synthesized for the purpose of increasing monoamine levels has demonstrated antidepressant-like properties in the clinic, further validating this theory [16].

4.0 Monoaminergic antidepressants

Almost all of the antidepressants that are available and used today are based on the serendipitous discovery of antidepressant-like properties of TCAs over 50 years ago. TCAs and ECT quickly became the initial standard treatment for depression following the discovery of their antidepressant efficacies. However the use of TCAs was limited due to their adverse side effects, as well as concerns over the dangers of overdose. As a result, subsequent drug discoveries focused on finding more selective agents with similar efficacies, but fewer adverse side effects and improved tolerability. Such efforts yielded what are called the next generation antidepressants; SSRIs, NRIs and serotonin and norepinephrine reuptake inhibitors (SNRIs) that selectively target and block 5-HT and

NE reuptake transporters, leading to a subsequent increase in extracellular 5-HT and NE levels.

Although there are a wide variety of treatments available for those afflicted with MDD, clinical trials suggest that these medications are only partially effective for a large proportion of these patients, and completely ineffective for some. One such study is the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial that was conducted on a large adult outpatient sample with MDD (N= 2,876), which demonstrated that only 26.8% of patients achieved remission following an optimized trial of the SSRI citalopram for up to 12 weeks [63, 134]. Therefore, more than two thirds of MDD patients will remain ill despite an optimized first trial for an antidepressant [87]. Another major drawback of these first generation antidepressants is their delayed onset of clinical action. There is always urgency to treat MDD because although patients may have been ill for several weeks, months, and sometimes years, they are often in a crisis situation when they consult.

Rapid therapeutic action and higher remission rates represent the two major unmet needs for MDD. Since the initial discovery of the antidepressant-like properties of TCAs, there have not been any major strides in the development of antidepressants that induce a rapid and more potent antidepressant response. This is not very surprising as efforts have focused on producing antidepressants with similar modes of action as the first generation antidepressants, but with fewer side effects. The fact that so many people still struggle to find effective treatments for depression underlines the urgent need to develop innovative, mechanistically distinct agents to further reduce the burden of illness. In recent years, a growing body of evidence has implicated the glutamate system in mood

disorders. Initial evidence came from preclinical studies displaying the antidepressant-like properties of glutamatergic agents in rodents. Such studies sparked further clinical and preclinical investigations to identify whether glutamate modulating agents can produce an antidepressant effect in patients.

5.0 The glutamate system

5.1 Background

Although glutamate neurotransmitter was first discovered in the 1950s, it was not until the 1970s that it became widely recognized as the main and most abundant excitatory neurotransmitter in the CNS and spinal cord [39]. Glutamate acts on three cellular compartments: presynaptic neurons, postsynaptic neurons, and glia [86]. This is often characterized as the tripartite glutamatergic synapse. This neuron-glia complex serves as the basis of synthesis, release, reuptake and inactivation by receptors and amino acid transporters.

Glutamate neurotransmitter is utilized in more than 80% of neurons [68]. The glutamate system is notoriously involved in several physiological functions including cognition, neurogenesis, learning and memory. Furthermore, glutamate neurons project within the cortex and to subcortical regions including the dorsal raphe nucleus (DRN), locus coeruleus (LC) and the VTA, where monoaminergic systems are modulated, further solidifying its possible role in the pathophysiology of MDD [30]. Glutamate is synthesized from the precursor glutamine via the enzyme glutaminase [86]. Following synthesis, it is packaged into vesicles through the vesicular glutamate transporter (VGLUT) and its release is then regulated by voltage-dependent sodium channels [86]. Upon release, glutamate can bind to two different types of receptors; ionotropic

glutamate receptors and metabotropic glutamate receptors (mGluR). Glutamate clearance from the extracellular space takes place via high affinity excitatory amino acid transporters (EAATs) [86].

Ionotropic receptors include the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, N-Methyl-D-aspartic acid (NMDA) receptors, and kainate receptors [86]. These receptors are ligand-gated non-selective cation channels that allow the passage of Na^+, K^+ and Ca^{2+} ions. Activation of these receptors results in a subsequent intracellular signaling cascade involving downstream effectors including cyclic adenosine monophosphate response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) [34].

NMDA receptor channels are composed of several subunits, including NR1, NR2 (NR2A-NR2D) and NR3 (NR3A and NR3B) subunits [86]. AMPA receptors on the other hand are comprised of four functionally diverse AMPA receptor subunits (GLUR1-GLUR4) and are co-expressed with NMDA receptors at mature synapses [86].

Metabotropic glutamate receptors (mGluR) are G-protein coupled receptors which share a common characteristic seven transmembrane domains with an extracellular N-terminal and an intracellular –COOH terminal [90]. Whilst ionotropic receptors are primarily expressed at the postsynaptic density, mGluRs can be found both presynaptically and postsynaptically. Upon glutamate binding they can indirectly activate ion channels on the plasma membrane, ultimately resulting in either increased or decreased excitability of synaptic terminals [90].

5.2 Glutamate's link to depression

The involvement of the glutamate system in the pathophysiology of MDD was first hypothesized in the 1980s after studies demonstrated that depressed patients exhibit increased serum glutamate levels [66]. Studies conducted later replicated the observed increases of glutamate levels in plasma [94] and cerebrospinal fluid (CSF) [72]. Although these finding provided insight into the possible role of glutamate in depression and mood disorders, these results are difficult to interpret as it is difficult to account for the effect of medications and post mortem metabolism.

To address these limitations, studies are now assessing cerebral glutamate levels in depressed patients using proton magnetic resonance spectroscopy (^{1}H-MRS). This imaging technique allows for a more direct, non-invasive, *in vivo* assessment of cerebral glutamate levels, and is currently the most direct way of assessing brain glutamate content. ^{1}H-MRS studies carried out on depressed patients focused on assessing glutamine (Glx) levels in various brain regions [81]. Measuring Glx levels provides insight into the total glutamatergic pool that is available for synaptic and metabolic activity [81]. Such studies revealed elevated levels of glutamine in the occipital cortex (OCC), and decreased levels of glutamine in the anterior cingulate cortex (ACC), dorsolateral PFC (DLPFC), dorsomedial PFC (DMPFC), ventromedial prefrontal cortex (VMPFC), amgydala and the HPC of MDD patients [146]. These findings suggest that glutamate dysfunction is much more complicated than the simplistic view that either increased or decreased glutamate activity is associated with depressive symptoms. Additionally, a large number of clinical neuroimaging studies of mood and anxiety disorders show regional volumetric reductions in the HPC, PFC and amygdala, three

areas where glutamate synapses are predominant [75]. These findings almost certainly implicate glutamatergic systems in the pathophysiology of MDD, providing a rationale for the investigation of the antidepressant potential of glutamatergic agents.

5.3 Neurotrophic hypothesis of MDD

Neurogenesis is defined as the process by which neurons are generated from neural stem and progenitor cells. Whilst this process is most active during development, it remains ongoing throughout life in several regions of the brain including the subventricular zone (SVZ), lining the lateral ventricles, and in the subgranular zone (SGZ) of the dentate gyrus [93]. The extent to which neurogenesis occurs in adults is a topic of debate. In an extensive investigation of neurogenesis in macaque monkeys, Rakic et al. (2002) conclude that apart from granule cells, all other classes of neurons examined were generated during specific and well-defined developmental periods and showed no credible evidence of natural turnover in the adult brain [108]. Moreover, Arellano et al. (2011) demonstrated a decrease in the number of neuroblasts in the SVZ of humans that begins during the first postnatal year and then declines more moderately through childhood and adult life [8]. The authors conclude that this decline in neuroblast number and their migratory pathway suggests that there is little to no olfactory bulb neurogenesis in humans [8]. However in a more recent investigation, Spalding et al. (2013) assessed the generation of hippocampal cells in humans by measuring the concentration of nuclear-bomb-test-derived ^{14}C in genomic DNA [122]. They concluded that in adult humans, 700 new neurons are added in each hippocampus every day, convincingly suggesting that neurogenesis remains ongoing throughout adulthood [122].

The neurotrophic hypothesis of depression was postulated in the early 1990s, and states that the generation of neurons in the postnatal brain is important for understanding and treating depression [106]. The neurotrophic hypothesis has two main postulates: 1) decreased neurogenesis results in depression, and 2) treatment for MDD requires the existence of intact hippocampal neurogenesis [106]. Although the neurotrophic hypothesis for MDD is relatively recent, evidence in favor of this theory has been accumulating. Indeed, in the early 1990s Gould et al (1991) demonstrated that stress robustly decreases generation of hippocampal neurons and results in neuronal cell death [54]. Moreover, Brezun et al. (1999) show that serotonin depletion led to an inhibition of adult neurogenesis [27]. Soon after, studies have shown that chronic but not acute antidepressant treatments increased SGZ cell proliferation and neurogenesis [84], and mice no longer responded behaviorally to antidepressants once neurogenesis was ablated by means of radiation [115], highlighting the potential importance of neurogenesis in the antidepressant response .

It is important to note that the neurotrophic hypothesis is not without its weaknesses. Whilst some studies show that intact neurogenesis is required for antidepressants to induce their behavioral responses in rodents, other studies fail to replicate these findings [19, 119]. Moreover, studies have mostly failed to produce depressive or anxious phenotypes following ablation of neurogenesis in naïve and stressed animals. [60, 150]. Whilst the role and importance of neurogenesis in the antidepressant response is yet to be fully elucidated, the neurotrophic hypothesis is a refreshing alternative to the predominant monoaminergic theory of MDD, and is in agreement with the existing view that depression is a limbic disorder involving hippocampal abnormalities [106].

5.4 Antidepressants regulate glutamate receptors

Current monoaminergic-based antidepressants have been shown to modulate glutamate transmission and receptor function, further indicating glutamate's role in mood disorders. In particular, chronic administration of the antidepressants agents imipramine, citalopram, and tianeptine cause a dampening of glutamate release presynaptically in the frontal cortex [26, 136]. Studies have also shown that chronic antidepressant administration modulates ionotropic glutamate receptor; producing a reduction of NMDA function. In particular, chronic administration of the TCA imipramine has been shown to result in a reduction of radioligand binding to rat cortical NMDA receptors [99]. Additionally, studies have revealed that chronic administration of the NRI reboxetine, and the SSRI fluoxetine, results in a marked increase in AMPA receptor subunit expression and a subsequent potentiation of AMPAR mediated transmission [98], [12]. These findings suggest that antidepressants can exhibit their therapeutic effects via a cascade of AMPA and NMDA-mediated events

Antidepressants have also been shown to induce changes in mGluR expression and function. Repeated ECT combined with chronic imipramine administration has been shown to result in increased expression of group I mGluR (mGluR 1/5) in the HPC [120]. Moreover, chronic imipramine administration increased the expression of group II mGluR (mGluR2/3) in the hippocampus, cortex, caudate nucleus and nucleus accumbens [120]. With the ever increasing number of studies revealing the involvement of the glutamate system in mood disorders and the antidepressant response, attention is now on characterizing the antidepressant effects of glutamate modulating drugs.

6.0 Targeting the glutamate system in the treatment of MDD

6.1 AMPA potentiators

As previously reported, studies have shown that monoamine modulating antidepressants cause a potentiation of AMPAR mediated transmission. Indeed, preclinical studies were conducted to test whether AMPA potentiators exhibit antidepressant-like effects in behavioral models of depression. For instance, the AMPA potentiators LY392098, and LY451616 have both been shown to reduce immobility time in the forced swim test (FST) and the TST, both of which are behavioral models of depression in rodents [75]. Moreover, Andreasen et al. (2013) showed that the AMPA potentiator (R,R)-N,N-(2,20-[biphenyl-4-40-diyl]bis[propane-2,1-diyl])dimethanesulfonamide (PIMSD) enhanced the effect of MK-801 in the mouse FST, further emphasizing the importance of AMPA receptor activation in mediating an antidepressant response [6]. Although the antidepressant-like effects of AMPA potentiators are preclinically evident, there are currently no placebo-controlled clinical trials assessing the antidepressant effects of AMPA potentiating compounds to date.

6.2 NMDA antagonists

As antidepressants have been shown to modulate NMDAR function, studies are now focused on assessing whether NMDA antagonists induce antidepressant-like effects in behavioral models of depression. Amantadine is an antiviral agent that has been used since 1996 against influenza A viral infections [86]. It is a well-tolerated, noncompetitive, selective NMDA antagonist which has been shown, using patch-and

concentration-clamp techniques, to reduce NMDAR function by 50% when administered at high doses [102]. Preclinical studies have shown that amantadine possesses antidepressant-like properties [131]. Specifically, amantadine administration has been shown to produce a dose-dependent decrease in immobility time in the rat Porsolt or FST. [111]. Clinical studies have also reported the antidepressant-like properties of amantadine in depressed patients as well as in those with Parkinson's disease [126]. Moreover, these clinical investigations have also revealed that amantadine administration in depressed patients with borna disease virus (BDV) infections resulted in a 68% reduction of depressive symptoms in 2.9 weeks [112]. However these finding are confounded by the additional effects of amantadine on DA and 5-HT neurons, making it difficult to delineate glutamate's role in the pathophysiology of MDD [61].

The antidepressant properties of memantine, a derivative of amantadine, were also investigated. Memantine is a low affinity, noncompetitive, open channel NMDA antagonist. Preclinical studies describe a dose-dependent decrease in immobility time in the FST following the administration of memantine [111]. In 2006, an 8 week double blind placebo controlled trial involving 32 patients failed to show a statistically significant effect of memantine in the treatments of depressive symptoms [147]. These findings were replicated in 2012, where no significant improvement was observed in patients with late-life depression administered memantine for 12 weeks [71]. This lack of effect was attributed to the low affinity of memantine to the NMDAR [71]. Henceforth, attempts focused on assessing the antidepressant properties of NMDA antagonists with higher affinity to the NMDA receptors, including ketamine.

7.0 Ketamine

7.1 Background

During the 1950s, phencyclidine (PCP), produced by Parke-Davis, was the primary anesthetic used during surgical procedures. There were concerns regarding its use; primarily its potent dissociative and hallucinogenic effects. Ketamine, a chiral arylcyclohexylamine (RS)-2-(2-chlorophenyl)-2-methylaminocyclo-hexanone, is a derivative of PCP that was synthesized by the same company in 1963 as a safer alternative to PCP. Ketamine was first administered to a human subject in 1964 [110], but was not approved for general use until 1970. The anesthetic state produced by ketamine is characterized by normal or slightly enhanced muscular tone, cardiovascular stimulation and an occasional and transient respiratory depression. Anesthesia is achieved by an intravenous (IV) bolus at a dose of 1-3 mg/kg [67].

Ketamine is an FDA-approved anesthetic and analgesic. It is classified as a non-competitive NMDA antagonist [65].It is important to note that the pharmacological profile of ketamine is complex as it exhibits affinities to numerous receptors [37, 142], [121, 139]. Ketamine is metabolized into two main metabolites, norketamine, the primary and active metabolite, and dehydronorketamine, an inactive metabolite [86]. Once ketamine is absorbed, its elimination half-life is 2.5 hours [124]. As opposed to memantine, ketamine has a higher affinity for the PCP site within the ionotropic channel [65]. The primary mechanism of action of ketamine is the blockade of NMDAR at the PCP site within the ionotropic channel [65]. It imposes its blockade via a trapping block mechanism, as opposed to partial trapping that is exhibited by memantine. A trapping

block mechanism allows for a relatively robust blockade of channel, ultimately leading to a more efficacious blockade of influx of the secondary messenger Ca^{2+} into the cell [7, 81].

7.2 Antidepressant properties of ketamine

Similar to amantadine and memantine, the antidepressant-like properties of ketamine were displayed in multiple behavioral studies. A subanesthetic dose of ketamine in rats (10 mg/kg i.p) resulted in a decrease in latency of escapes in the learned helplessness test and a decrease in immobility times in the FST [34]. Despite these preclinical findings, it was not until the turn of the century that a clinical trial aimed at assessing the antidepressant properties of ketamine in depressed patients was conducted [17]. This was a small randomized, double-blind study that was conducted on seven treatment-resistant patients (TRD). Within 72 hours of a single infusion of ketamine at the subanesthetic dose of 0.5 mg/kg (i.v.) over 40 minutes, patients displayed significant improvements in depressive symptoms.

Several years later, a larger double-blind study with cross-over design was conducted to replicate previous findings. Eighteen treatment resistant patients with recurring MDD received intravenous infusions of saline solution and ketamine hydrochloride (0.5 mg/kg, over 40 minutes). Significant improvement in the 21-item Hamilton Depression Rating Scale (HDRS) was noted at every time point from 110 minutes through seven days. On the day after the infusion, 12 of the 17 patients (71%) met response criteria and five of seventeen (29%) met remission criteria [82].

Although the aforementioned study provided a much needed insight into the clinical efficacy of ketamine in the treatment of MDD, an active placebo control

condition is required to mitigate the influence of nonspecific factors on the antidepressant outcome of ketamine. To address this, Murrough, et al. (2013) conducted a larger two-site trial involving 73 treatment-resistant patients with moderate-to-severe and persistent depressive symptoms, using midazolam to control for the psychotomimetic effects of ketamine [97]. In this study, they report that a single low dose of ketamine was associated with a rapid-onset of antidepressant effect when compared to the psychoactive placebo control medication [97]. The ketamine responders maintained the gains for several days following the initial infusion. However, no statistically significant differences between groups were observed 7 days following the infusion [97].

The fact that ketamine can exert a rapid antidepressant action is clinically remarkable in that it induces antidepressant effects, even in MDD patients who are treatment-resistant. Furthermore, the antidepressant action of ketamine occurs within a day of a single subanesthetic dose [23, 148]. The response rate (a 50% decrease of signs and symptoms) following ketamine infusion at day 1 is comparable to the response rates obtained with standard antidepressants in non-resistant patients after 8 weeks. For example, the response rates involving non-treatment-resistant MDD patients were 63% for SSRI, 62% for bupropion and 65% for venlafaxine at week 8 [48, 134]. The public health implications of these findings are enormous and warrant the funding for larger scale studies.

8.0 Limitations of ketamine

8.1 Transient AD effects

Although the aforementioned clinical studies were met with great enthusiasm, it is important to recognize that the use of ketamine as an MDD treatment strategy is not

without its limitations. Indeed, one major limitation of ketamine is its transient effects on alleviating depressive symptoms. Studies that followed participants until relapse report that about one third of patients relapsed within 3 days, a third relapsed within a week, and the last third relapsed within 10-20 days [65]. Nonetheless, patients who have relapsed following ketamine infusion do not show any signs of tolerance and exhibit signs of remission following additional infusions of ketamine [133]. Consequently, studies have evaluated the therapeutic benefit of repeated ketamine infusion. In one such study six open label intravenous ketamine infusions were administered over 12 days [35]. This study found that ketamine was associated with an initial and sustained antidepressant effect. Post-ketamine, 8 of 9 patients relapsed, on average 19 days following the final infusion. One patient exhibited minimal depressive symptoms for 3 months without additional antidepressant interventions following the infusion. Although this study provides insights into the beneficial effects of repeated infusions, double-blind placebo controlled studies with larger sample sizes are required before any conclusions can be accurately drawn.

8.2 Safety concerns

Despite the encouraging outcomes and positive data, it is crucial that the beneficial effects of ketamine use are weighted against the negative aspects of its administration in order to effectively determine the viability of such a treatment. It is important to note that ketamine has been widely used as an anesthetic agent for over 40 years. Patients receiving infusions of ketamine have reported dissociative symptoms, perceptual disturbances, and elevations in blood pressure, euphoria and dizziness. Luckily these effects are mild, and are only transient when they do occur (symptoms

cease within 80 minutes of the infusion) [148]. In 833 infusions of a subanesthetic dose of ketamine in healthy volunteers, no serious adverse events have been reported [23, 105]. Moreover, when a subanesthetic dose of ketamine was administered to 369 subjects with refractory pain, no serious adverse effects were reported [103].

It is crucial to recognize that ketamine is used recreationally due to the dissociative effects users experience following administration. It is a drug with addictive potential and hence it should be noted that users might present themselves, under false pretenses, as depressed or suicidal patients in order to get access to the drug. Therefore clinicians must be vigilant of drug seeking behaviors when evaluating whether a patient is a candidate for infusion. Ideally, infusions of ketamine should be limited to patients with no history of drug abuse or dependence.

9.0 Neurobiology of ketamine

Preclinical and clinical studies provided very encouraging data indicating that ketamine produces a comparable antidepressant response in patients with MDD but in a fraction of the time, when compared to conventional antidepressants [82]. These findings are made even more remarkable by the fact the infusions were performed on TRD patients. Moreover, this effect of ketamine can be sustained for up to a week in some cases, and even longer in others [82]. Building on this remarkable data, studies are now exploring the mechanism by which ketamine produces these effects. It has been postulated that the antidepressant effects of ketamine seen in the clinic are due to two distinct processes. The rapid antidepressant effects of ketamine that occur within 60 minutes following the infusion cannot be explained by neuroplastic changes, as such

changes do not occur in such a time frame. Rather, such rapid antidepressant effects are a consequence of increased AMPA glutamatergic output, relative to NMDA [82]. The sustained effects of ketamine seen days following the infusion however are most likely due to changes in neuroplasticity [34].

The antagonistic effects of ketamine on NMDAR have been well established. In addition to its postsynaptic effects on NMDAR, ketamine has also been shown to result in an increase in glutamate release from the presynaptic cleft [82]. Therefore the net effect of ketamine is an increase in AMPAR throughput relative to NMDAR. The importance of AMPAR potentiation to the antidepressant response produced by ketamine has been demonstrated preclinically. When the AMPA antagonists NBQX is solely administered in rodents, no changes in immobility time in the FST are observed [83]. However, pre-treatment with NBQX, abolished the antidepressant-like action of ketamine in the FST [83]. In addition, AMPA potentiators have been shown to have antidepressant-like qualities in animal models [75]. Specifically, administration of the AMPA potentiator LY392098 resulted in a reduced mobility in the FST and TST [75]. AMPA potentiators have also been shown to increase BDNF mRNA and protein expression [70], which is analogous to the increase in BDNF levels observed following escitalopram administration [116]. These findings suggest that, at least in animal models, the antidepressant effects of ketamine are mediated in part by increased AMPA receptor throughput.

The sustained antidepressant effects of ketamine are likely achieved through an initiation of a number of downstream signaling pathways, ultimately leading to enhanced synaptic plasticity and neurogenesis [34]. Those include the ubiquitous mammalian target

of rapamycin (mTOR) pathway which regulates translation and long-lasting synaptic plasticity. mTOR protein is a multi-effector serine-threonine protein kinase, whose dysregulation has been implicated in several psychiatric disorders, [59]. Li et al. (2010) demonstrated that ketamine administration leads to a transient increase in the levels of phosphorylation of eukaryotic initiation factor 4E binding protein 1 (4E-BP1), P706 kinase (P706K), and mTOR, all members of the mTOR signaling pathway [73]. Activation of this pathway leads to increased expression of synaptic signaling proteins and a subsequent increase in synaptic plasticity, and spine formation and maturation in the PFC [74].

Increased expression of brain-derived neurotrophic factor (BDNF) is also thought to play an important role in the sustained effects of ketamine. BDNF, a member of the neurotrophin family, is present in both the central and the peripheral nervous system [78, 79] and has been previously been implicated in depression and the antidepressant response. Specifically, several classes of antidepressants, including escitalopram, have been shown to elevate BDNF levels in patients at the advent of the antidepressant response [116]. Recently, it has been shown that levels of BDNF were elevated in the hippocampus of mice 30 minutes following ketamine administration. Specifically, antagonism of NMDAR has been shown to deactivate eukaryotic elongation factor 2 (eEF2) kinase, leading to a decreased phosphorylation of eEF2 and increased BDNF translation [9]. Moreover, activation of AMPA receptors via the AMPA potentiator LY392098 has also been shown to increase mRNA and protein levels of BDNF *in vitro* [70]. These effects of LY392098 were abolished by the AMPA antagonist NBQX [70]. The role of BDNF in the ketamine-induced antidepressant effects was further

strengthened by findings showing that eEF2 inhibitors result in decreased immobility times in the FST [9]. Another study examining the antidepressant-like effects of ketamine in knock-in mice with the inactive val66Met polymorphism; which in turn downregulates BDNF expression, has shown a loss of synaptogenic and antidepressant actions of low-dose ketamine in the PFC. However it is important to note that the role of BDNF in ketamine-induced antidepressant effects remains controversial. For instance, Garcia et al. (2009) show that acute and chronic administration of ketamine does not alter hippocampal BDNF protein levels in the HPC of stressed rats [51]. Moreover, studies have shown that despite ketamine producing antidepressant-like responses in the FST, it did not influence BDNF levels in the HPC in heterozygous BDNF knockout mice, suggesting that BDNF signaling may not be pivotal in the antidepressant effects of ketamine [77].

Glycogen synthase kinase 3 (GSK-3) is a serine threonine protein kinase whose inhibition is believed to play an important role in mediating the antidepressant-like effects of ketamine [20]. Specifically, studies have shown that administration of ketamine in mice resulted in an inhibition of brain GSK-3[20]. This observed inhibition is mediated through an increase in serine-phosphorylation of both α, and β isoforms of the enzyme [76]. The importance of GSK-3 inhibition in ketamine-induced antidepressant-like effects was highlighted in studies revealing that mice with a knock-in mutation that blocks GSK-3 phosphorylation did not respond to ketamine treatment in the learned helplessness test [20]. Like mTOR, the phosphorylation of GSK-3 is mediated by both protein kinase B [38] and P706K [128], and blockade of GSK-3 by pharmacological agents has been shown to increase BDNF levels, implicating GSK-3 synaptogenesis [145].

Taken together, these findings demonstrate the importance of synaptic plasticity and neurotrophic signaling in the ketamine-induced antidepressant response. However it is important to note that although these molecular changes may explain the long lasting effects of ketamine, they are unlikely to account for the immediate antidepressant effects that are observed as early as 30 minutes following intravenous infusions in treatment resistant patients [23]. Specifically, studies have shown a delayed activation of the mTOR pathway, with the expression of synaptic proteins peaking 2-6 hours following ketamine administration. Moreover, increased spine density, as a consequence of increased expression of synapse-associated proteins, is observed 24 hours following ketamine administration [73].

The promptness of the antidepressant response produced by ketamine is its most valuable asset, but the mechanisms behind these effects remain largely unclear. Thus, the present electrophysiological study was aimed at investigating the effects of a single dose of ketamine on monoaminergic neurotransmission and whether changes in monoamine firing, observed shortly following ketamine administration, could help understand its rapid antidepressant effects observed in the clinic. Once the effect of ketamine on monoamine firing is characterized, the potential mechanisms by which ketamine produce these changes were also investigated.

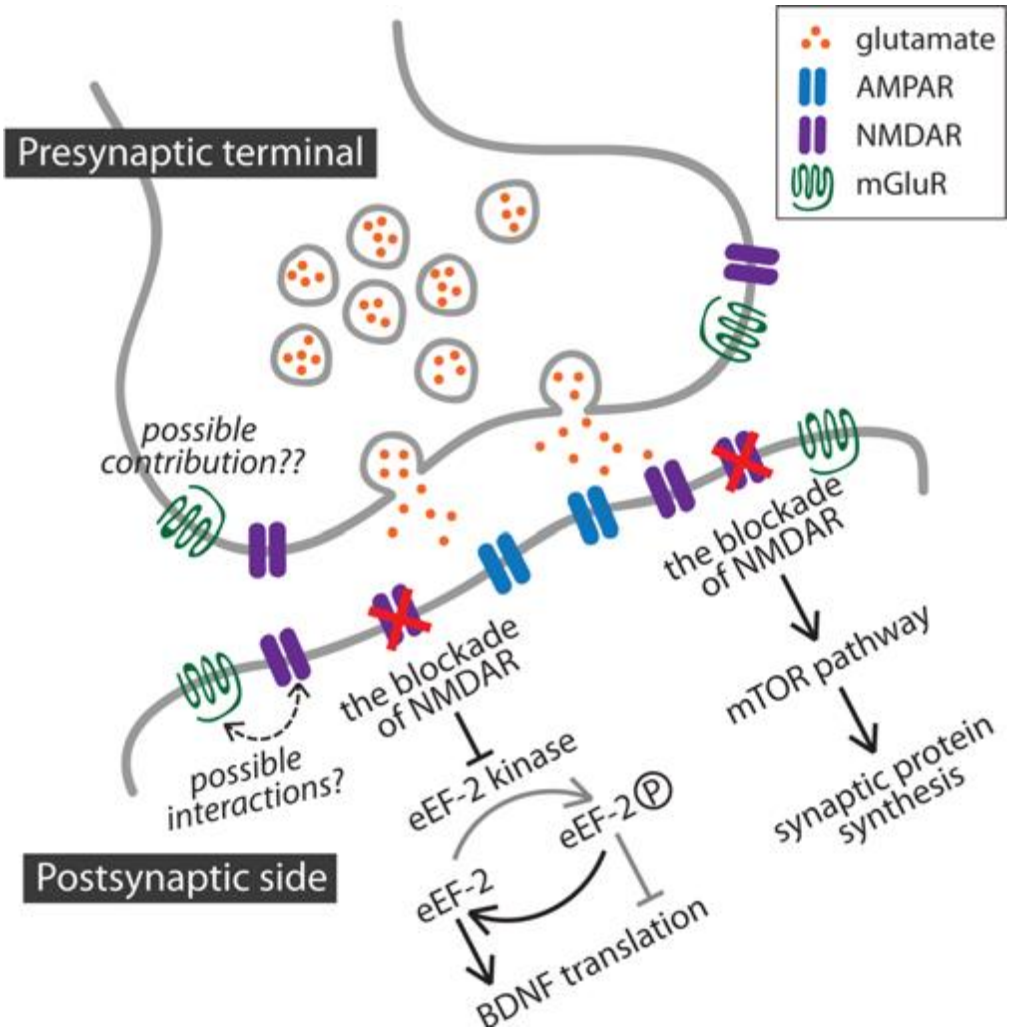

Figure 1. Schematic of a glutamatergic synapse and the proposed effects of ketamine-induced inhibition of NMDAR. Studies suggest that NMDAR antagonism mediated by ketamine has an effect on multiple downstream signaling pathways. Increased BDNF translation may be an effect of a disinhibition of eEF2 and a subsequent increase in eEF2 phosphorylation. Inhibition of NMDAR may also lead to activation of the mTOR pathway, leading to increased translation of regulatory proteins promoting plasticity and synaptogenesis. Adapted from Chung et al. (2012) [34].

MATERIALS AND METHODS

1. Animals

The experiments were carried out on male Sprague-Dawley rats (Charles River, Canada), weighing a minimum of 270-350 g at the time of the experiments. Rats were housed in groups of 2 per cage, under standard laboratory conditions (12:12 h light-dark cycle with access to food and water *ad libitum*). Body temperature was kept at 37°C during surgery and electrophysiological experiments. Animals are 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 protocols.

2. Drug Administration

Ketamine hydrochloride was dissolved in 0.9% aqueous saline solution. For acute experiments, ketamine was administered at a dose of 10 mg/kg and 25 mg/kg intraperitoneally (i.p.) 30 minutes prior to the electrophysiological experiments. In the 2-day administration paradigm, ketamine was administered at a dose of 10 mg/kg for 2 days. An additional third injection was administered on day 3, 30 minutes prior to the electrophysiological experiments. Control rats on the other hand received the vehicle (0.9% aqueous saline solution). A 25 mg/kg dose of ketamine is comparably subanesthetic to the lower dose, and produces a similar behavioral outcome in the FST [52]. The AMPAR antagonist NBQX was also dissolved in 0.9% aqueous saline solution, and administered intraperitoneally at a dose of 10 mg/kg for VTA experiments, and 3 mg/kg for LC experiments, 10 minutes prior to ketamine administration.

3. 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 the anesthetic (100 mg/kg, i.p.) were given to maintain constant anesthesia and prevent any nociceptive reaction to pinching of the hind paws. The body temperature of rats was maintained at constant 37^{o}C by a thermistor-controlled heating pad (Seabrook medican instruments, Saint-Hyacinthe, Quebec, Canada). Extracellular recordings of monoaminergic neurons were performed using single-barrel glass micropipettes (Stoelting, USA) filled with 2 M NaCl solution and an impedance range of 2-4MΩ. 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).

3.1 Recording of serotonergic neurons

Single barrel glass electrodes were pulled on a pipette puller (Narishige, Japan) with impedances ranging from 2-4MΩ. Electrodes are positioned 0.9 mm anterior to lambda (λ) 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 are then identified according to the following criteria: a slow (0.5–2.5 Hz), regular firing rate, long duration and a positive action potential [3].

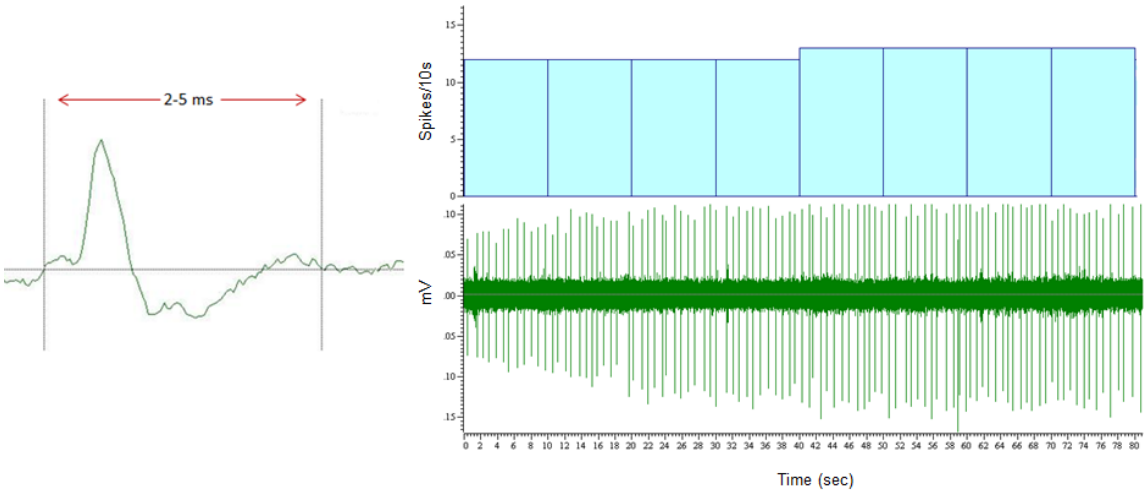

Figure 2. Electrophysiological recording of DRN 5-HT neuron. Single action potential (left) appears as uniform spikes (bottom, right). Frequency of firing activity is slow and regular (upper, right).

3.2 Recording of dopaminergic neurons

Single-barrel glass micropipettes were positioned using the following coordinates (in mm from λ): AP, +3.0 to +3.8; L, 1–0.6; V, 6.5–9. The presumed DA neurons were identified according to the well-established electrophysiological properties in vivo: a typical 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 with slow bursting activity (characterized by spike amplitude decrement) [55, 138]. The onset of a burst was defined as the occurrence of two spikes with an interspike interval shorter than 0.08 s. The termination of a burst was defined as an interspike interval of 0.16 s or longer.

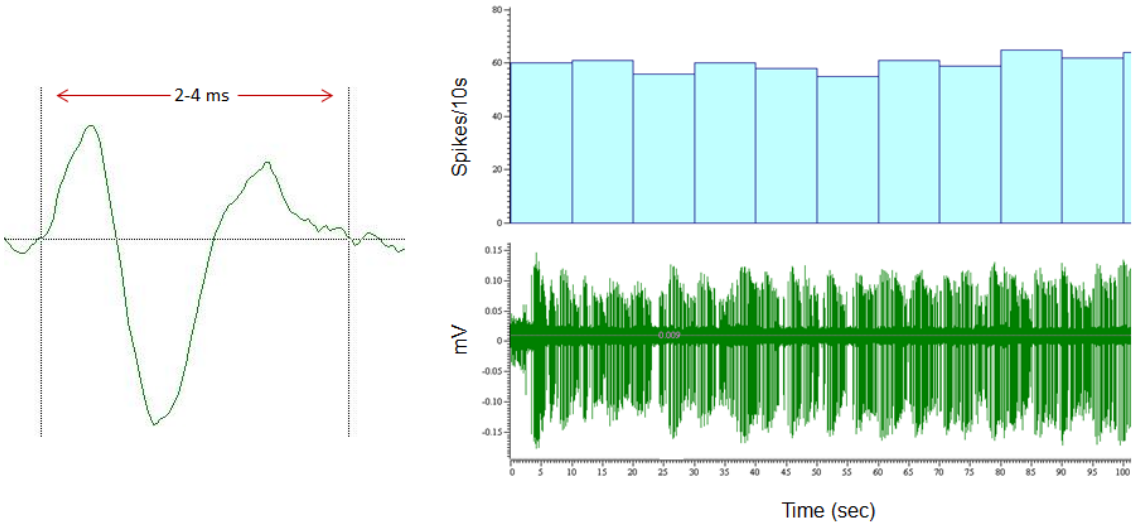

Figure 3. Electrophysiological recording of VTA DA neuron. Single action potential (left) appears as irregular spikes (bottom, right). Neurons often display rapid discharges (upper, right) as well as repeated bursting activity, with the amplitude of spikes decreasing with each discharge within a single burst.

3.3 Recording of noradrenergic neurons

LC NE neurons were recorded with single-barrel glass micropipette positioned at 1.1–1.2 mm posterior to λ and 0.9–1.3 to the midline suture. These neurons were encountered at a depth of 4.5 to 6.0 mm from the surface of the brain. The presumed NE neurons are 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 [2].

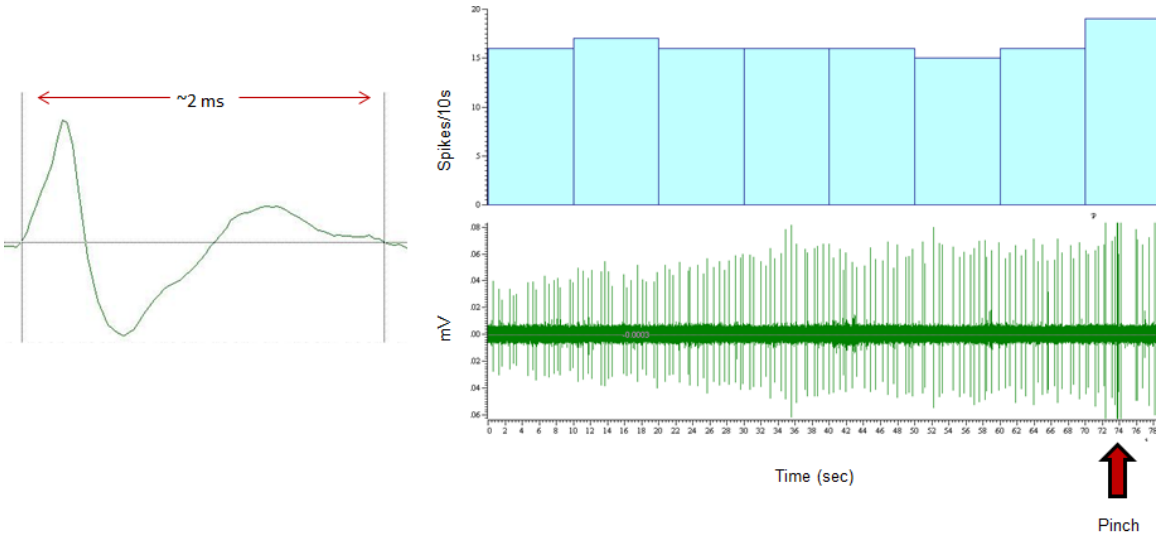

Figure 4. Electrophysiological recording of LC NE neuron. Single action potential (left) appears as uniform spikes (bottom, right). Frequency of firing activity is regular yet higher than 5-HT firing (upper, right). NE neurons display rapid firing of action potentials (arrow) followed by a period of silence when the contralateral hindpaw is pinched.

4. Microiontophoresis and extracellular recording of CA3 dorsal hippocampus neurons

Extracellular recording and microiontophoresis of glutamatergic CA3 pyramidal neurons were carried out with five-barreled glass micropipettes with a tip broken back to 10-12 µm. The central barrel used for the unitary recording was filled with a 2M NaCl solution, and the impedance of these electrodes ranged from 2 to 4 MΩ. The side barrels were filled with the following solutions: AMPA hydrobromide (5 mM in 200 mM NaCl, pH 8), NMDA (10 mM in 200 mM NaCl, pH 8), and 2 M NaCl solution for automatic current balancing. The micropipettes were then lowered into the dorsal CA3 region of the hippocampus using the following coordinates; 4.0 mm anterior to lambda and 4.2 mm lateral [104]. CA3 pyramidal neurons were found at a depth of 4.0 ± 0.5 mm below the surface of the brain. Since pyramidal neurons do not discharge spontaneously in chloral hydrate anesthetized rats, a small current of AMPA (-2 to -5 nA) was constantly applied

to locate activate CA3 pyramidal neurons within their physiological firing range (10 to 15 Hz) [109]. When AMPA and NMDA were not ejected, a retention current of +15 nA was applied to prevent leakage from the barrels. Pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple action potentials, alternating with complex spike discharges [63]. NMDA and AMPA were applied in sequence iontophoretically for 60 seconds. Both the duration and current of AMPA and NMDA for microiontophoresis ejection will remain the same before and after the i.p. injection of ketamine during these experiments.

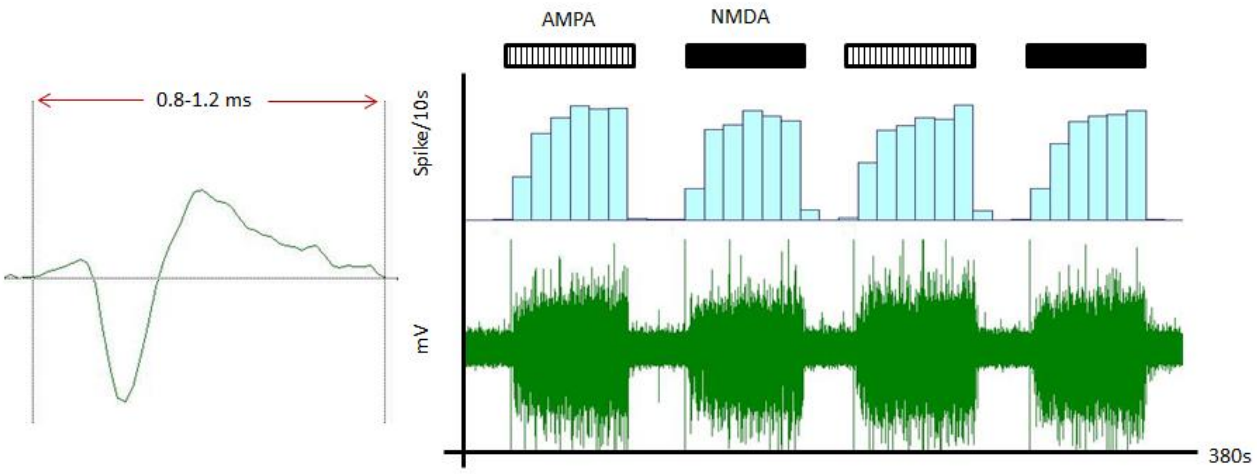

Figure 5. Electrophysiological recording of hippocampus CA3 pyramidal neuron. Single action potential (left) appears as irregular spikes (bottom, right). Rat CA3 neurons are quiescent under chloral hydrate anesthesia and as such are activated to within their physiological range by ejection currents of AMPA and NMDA (upper right). An average ejection current of -2 nA, and -7 nA were used for AMPA and NMDA, respectively.

5. Statistical analysis

All results provided herein are means ± SEM. Data were obtained from 5 rats per treatment group (including controls) unless specified otherwise. The n represents the number of neurons recorded for each treatment group. Comparisons between controls and treated groups were carried out using the two-tailed Student's t test. Analysis of microiontophoresis data was carried out using the one-way repeated measure ANOVA and the Tukey *post hoc* analysis was conducted when significant ANOVA results were obtained. These comparisons were statistically analyzed and graphed using the software Graphpad (Prism software Inc, La Jolla, CA). In all data analysis, statistical significance was taken as * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

6. Drugs

Ketamine hydrochloride was purchased from ERFA Canada INC. (Montreal, QC, Canada) and was dissolved in 0.9% aqueous saline solution. AMPA hydrobromide and NMDA were both purchased from TOCRIS Biosciences (Ellisville, MO, USA) and dissolved in 200 mM saline solution. NBQX was purchased from TOCRIS Biosciences (Ellisville, MO, USA) and dissolved in 0.9% aqueous saline solution.

RESULTS

1. Effects of acute and 2-day administration of ketamine on the firing activity of DRN 5-HT, LC NE and VTA DA neurons

Investigations of the effects of ketamine on 5-HT firing activity were conducted using 2 paradigms. In the first paradigm, firing of 5-HT neurons was assessed preceding and following ketamine administration in the same rat. The use of this paradigm eliminates any observed variability that could occur between rats. In a second paradigm, the firing activity of 5-HT neurons was assessed in dedicated vehicle and ketamine administered rats. For both paradigms, intraperitoneal administration of ketamine (10 mg/kg) acutely yielded no significant changes in the firing rate and the population activity of 5-HT neurons in the DRN, in comparison to vehicle treated rats (Figure 6A and B; Table 1). This lack of effect on the firing activity of 5-HT neurons persisted despite administration of ketamine at a higher dose of 25 mg/kg (Figure 6C and D). Additionally, no changes in the bursting activity of these neurons were observed (Table 1). Hence the firing activity of 5-HT neurons in the DRN remains unaltered following acute administration of ketamine. A 2-day administration of ketamine also yielded no changes in the firing activity of 5-HT neurons in the DRN. This can attributed to a lack of effect of ketamine on the firing rate, population activity and bursting activity of these neurons (Figure 6E and F; Table 1).

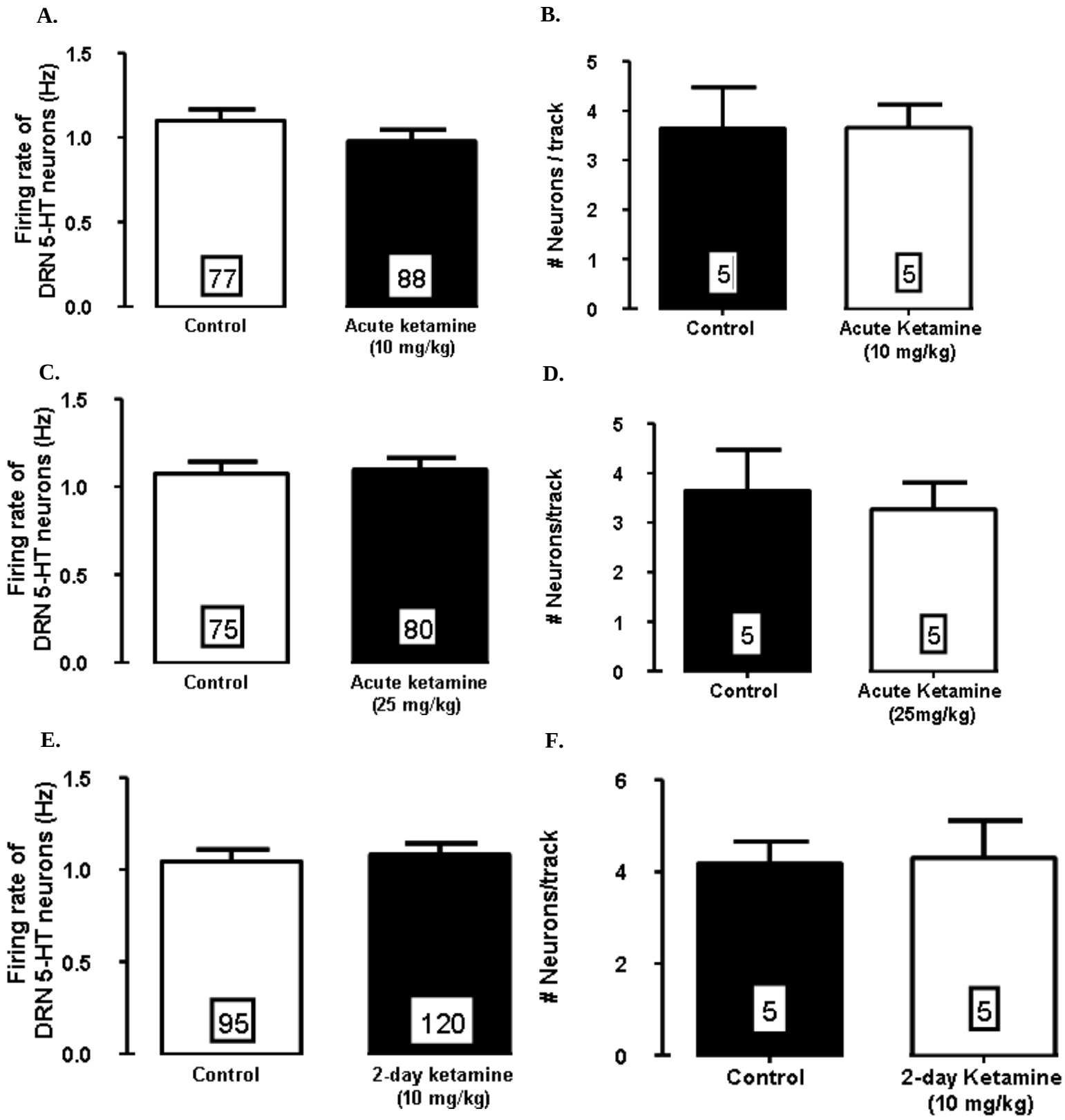

Figure 6. Effects of acute and 2-day ketamine administration on DRN 5-HT firing. Mean (+ SEM) of the firing rate and population activity of DRN 5-HT neurons following acute (A, B, C and D) and 2-day (E and F) administration of vehicle or ketamine at a dose of 10 mg/kg/day and 25 mg/kg/day. The numbers in the boxes correspond to the number of neurons recorded (5 to 6 rats tested per group).

Acute administration of ketamine yielded no significant changes in the firing rate of DA neurons in the VTA, in comparison to vehicle-treated rats (Figure 7A; Table 1). There was also no effect of ketamine on the firing rate of VTA DA neurons following a 2-day administration regimen (Figure 7C; Table 1).

Despite an absence of effect of ketamine administration on the rate of VTA DA neuron firing, the number of neurons recorded per track following acute ketamine administration increased by 113% ($p < 0.01$) compared to vehicle-treated rats (Figure 7B). Administration of the AMPAR antagonist NBQX alone at a dose of 10 mg/kg had no effect on the population activity of DA neurons. However administration of NBQX prior to ketamine abolished the ketamine-induced increase in population activity. The ketamine-induced increase in population activity was not present following a 2-day administration regimen (Figure 7D).

In contrast to the lack of effect of ketamine on the rate of 5-HT and DA neuron firing, acute administration of ketamine produced a modest yet significant elevation in the rate of firing of NE neurons in the LC by 21% ($p<0.05$; Figure 8A; Table 1). A significant increase in the firing rate of LC NE neurons was observed following the sole administration of NBQX at a dose of 10 mg/kg (54% increase; $p<0.001$), and 5 mg/kg (23% increase; $p<0.01$) compared to vehicle-treated rats. Consequently, NBQX was administered at a lower dose of 3 mg/kg in order accurately determine whether the ketamine-induced increases in NE activity were AMPA-dependent. Indeed, the ketamine-induced increase in firing rate was abolished following administration of NBQX prior to ketamine administration (Figure 8A). The ketamine-induced increase in firing rate of LC NE neurons was maintained following a 2-day administration regimen of ketamine, with

a similar increase of 23% ($p<0.001$) in the firing rate of these neurons (Figure 8C; Table 1).

Alongside the increase in firing rate, acute administration of ketamine also led to an increase in the proportion of NE neurons exhibiting bursting activity. Specifically, acute administration lead to a 77% ($p<0.01$) increase in the relative number of neurons exhibiting bursting properties (Figure 8C; Table 1). This ketamine-induced increase in bursting activity was no longer observed in rats administered with the AMPA receptor antagonist NBQX prior to ketamine administration (Figure 8C). Conversely, this ketamine-induced increase in bursting activity was not present following a 2-day administration regimen (Figure 8D; Table 1).

2. Effects of acute ketamine administration on the responsiveness of hippocampus glutamate receptors

Pyramidal neurons displayed a significant increase in responsiveness to iontophoretically-applied AMPA 30 minutes following the administration of ketamine at a dose of 10 mg/kg (Figure 9A), as was demonstrated by the 70% increase in rate of CA3 pyramidal neuron firing ($P<0.05$; Figure 9B). Despite the effects of ketamine on AMPA receptor responsiveness, a 10 mg/kg dose did not produce any change in responsiveness to NMDA, as indicated by a lack of significant changes in pyramidal neuron firing to iontophoretically-applied NMDA (Figure 9C).

As a low dose of ketamine did not produce any change pertaining to NMDA receptors, the responsiveness of AMPA and NMDA receptors were assessed following administration of a higher yet similarly subanesthetic, dose of ketamine (25 mg/kg;

Figure 10A). As was the case with the lower dose of ketamine, iontophoretic application of AMPA also produced a significant increase in the rate of firing of CA3 pyramidal neurons, indicating an increase in AMPA receptor responsiveness. However, these changes occurred more rapidly with an increase in the rate of firing observed as early as 15 minutes (45% increase; $p<0.05$), and persisting after 22 minutes (48% increase; $p<0.01$), and 30 minutes (61% increase; $p<0.01$) following administration of ketamine (Figure 10B). NMDA receptors on the other hand displayed a decrease in responsiveness 7 minutes following ketamine administration, as shown by the decrease in firing rate (45% decrease; $p<0.05$) of pyramidal neurons (Figure 10B)

			Firing activity (Hz ± SEM)		**bursting vs. non-bursting neurons (%)**	
Dose	Area	Treatment	***Vehicle***	***Ketamine***	***Vehicle***	***Ketamine***
10 mg/kg	**DRN 5-HT**	Acute	1.1 ± 0.07	1.0 ± 0.07	22	28
		2-day	1.0 ± 0.06	1.1 ± 0.06	22	33
10 mg/kg	**VTA DA**	Acute	4.0 ± 0.3	3.4 ± 0.2	90	80
		2-day	2.0 ± 0.09	1.3 ± 0.09	81	74
10 mg/kg	**LC NE**	Acute	1.4 ± 0.06	1.8 ± 0.08 *	17	30**
		2-day	1.4 ± 0.06	1.8 ± 0.07 *	17	27

Table 1. Summary table of the effects of acute and 2-day ketamine (10 mg/kg) on the firing and bursting activity of DRN 5-HT, VTA DA, and LC NE neurons. * *p < 0.05*, ** *p < 0.01*.

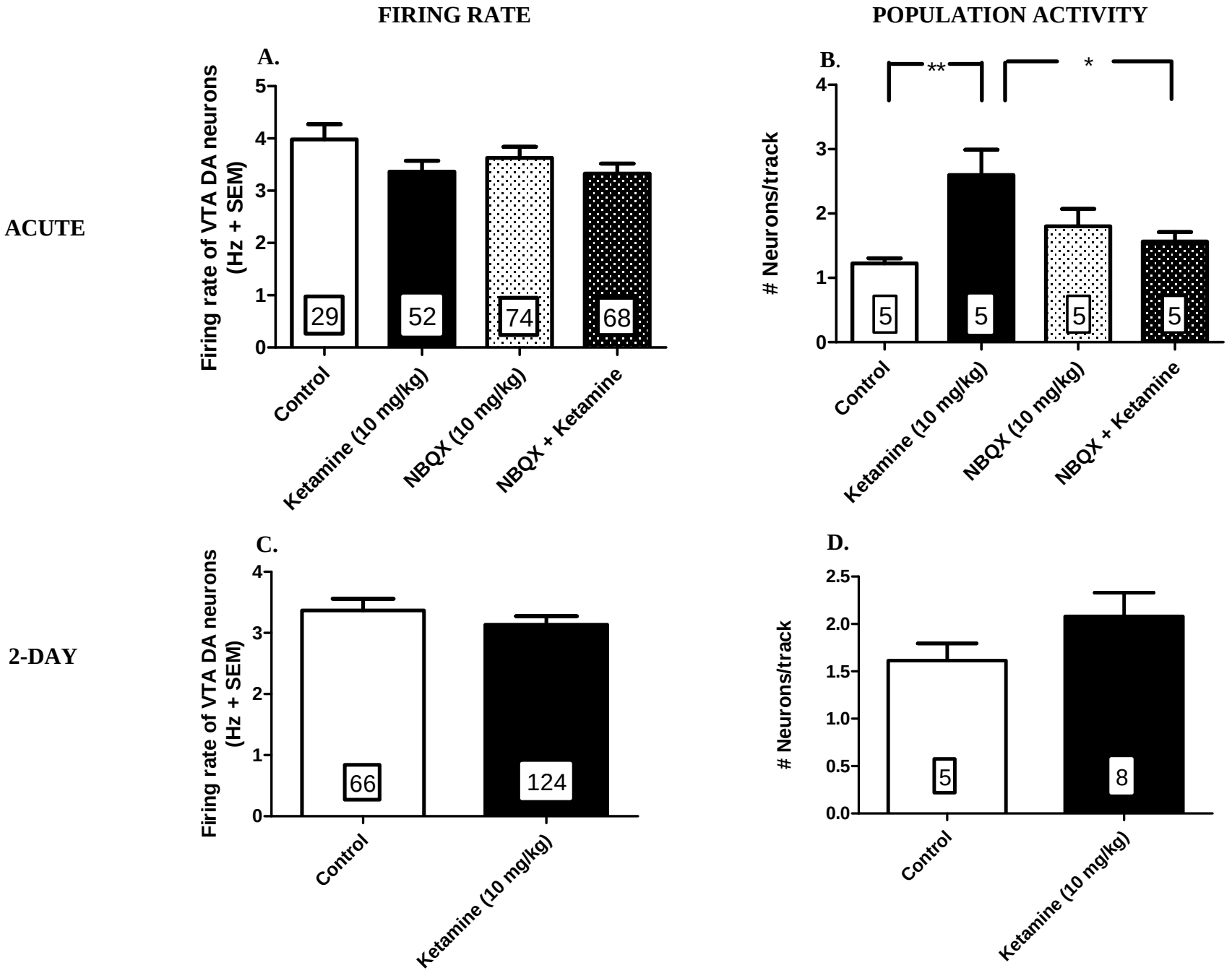

Figure 7. The effects of acute and 2-day ketamine administration on VTA DA firing. Mean (+ SEM) of the firing rate and population activity of VTA DA neurons following acute (A & B) and 2-day (C & D) administration of vehicle or ketamine (10 mg/kg). The numbers in the boxes correspond to either the number of neurons recorded (A & C) or the number of rats rested per group (B & D). $^{**}p < 0.01$ and $^{*}p < 0.05$ in (B) using 1-way repeated measure ANOVA and Tukey *post-hoc*.

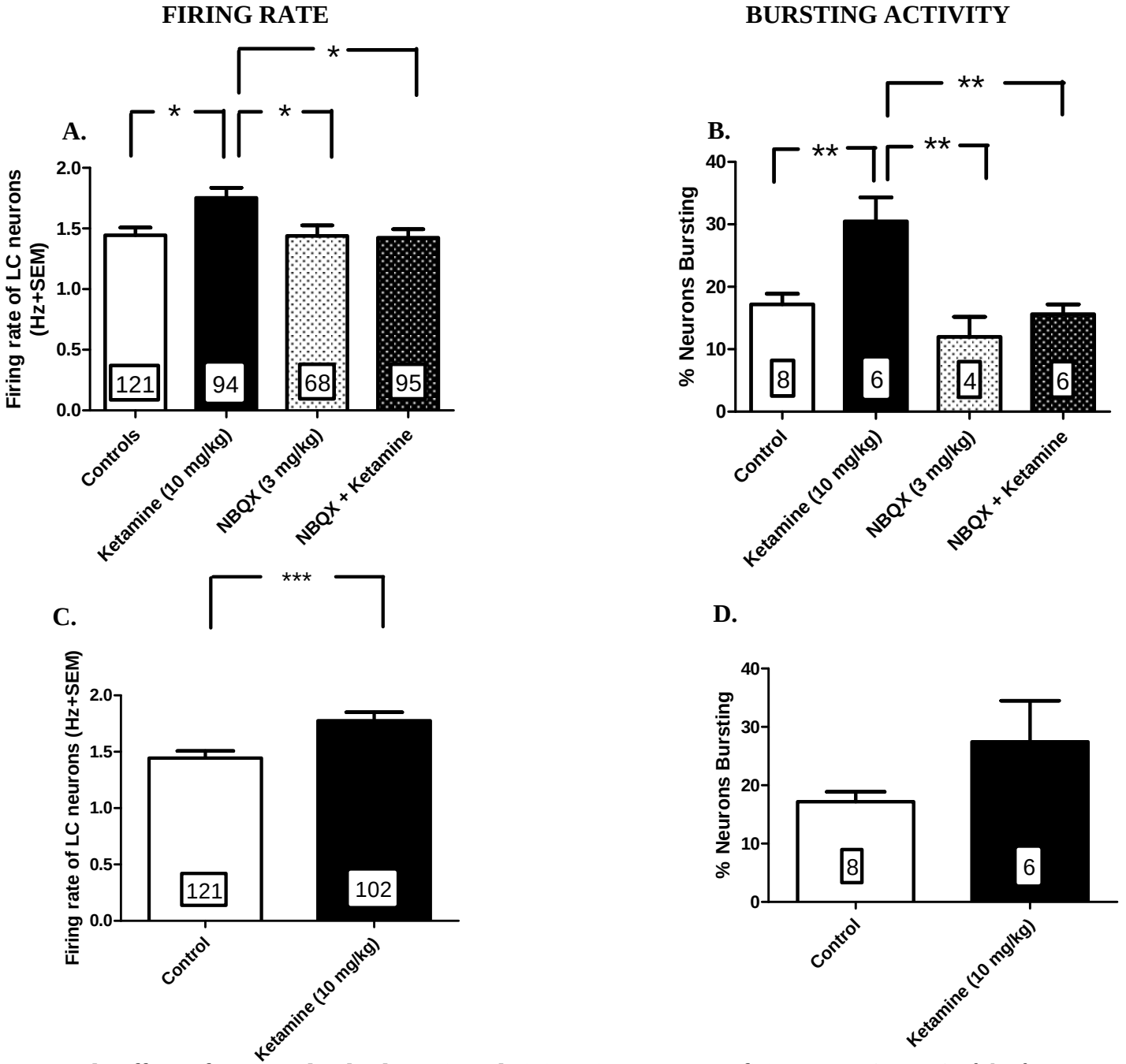

Figure 8. The effects of acute and 2-day ketamine administration on LC NE firing. Mean (+ SEM) of the firing rate and bursting behavior of LC NE neurons bursting following acute (A & B) and 2-day (C & D) administration of vehicle or ketamine (10 mg/kg). The numbers in the boxes correspond to either the number of neurons recorded (A & C) or the number of rats rested per group (B & D). $^{**}p < 0.01$ and $^{*}p < 0.05$ in (A & B), and $^{***}p < 0.001$ in (C) using 1-way repeated measure ANOVA and Tukey *post-hoc,* and unpaired Student's *t*-test, respectively.

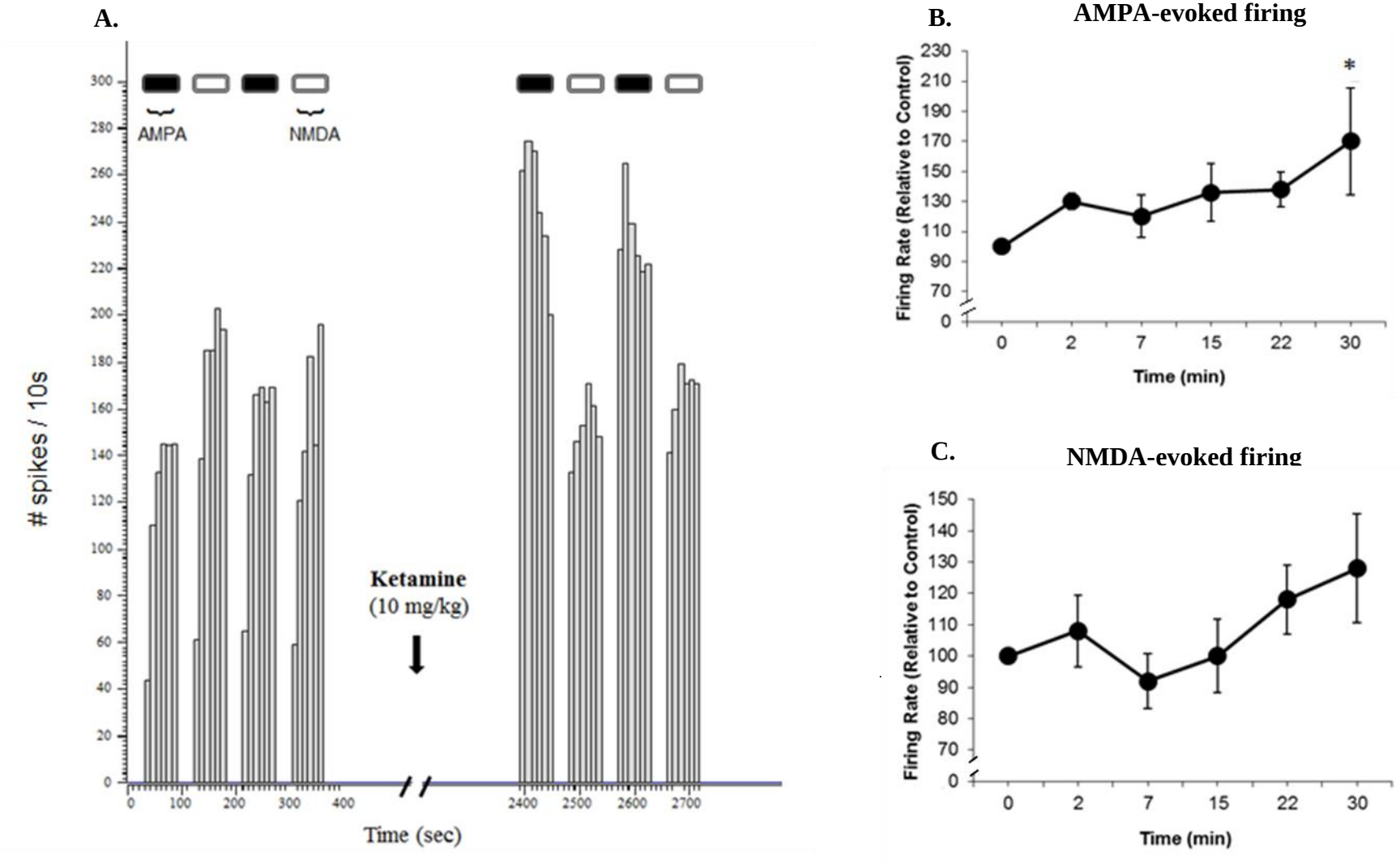

Figure 10. The effects of acute ketamine administration (10 mg/kg) on the responsiveness of AMPA and NMDA receptors. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons showing the effects of ketamine administration (indicated by arrows) at a dose of 10 mg/kg (A). Each bar corresponds to the application of AMPA or NMDA. Ejection currents of -1 nA for AMPA and -8 nA for NMDA were used. The overall changes in the % of baseline firing rate of dorsal hippocampus CA3 pyramidal neurons following administration of ketamine (B & C). $^{**}p < 0.01$ and $^{*}p < 0.05$ in (B & C) using 1-way repeated measure ANOVA and Dunnett's *post-hoc* (5 rats were tested for each dose of ketamine).

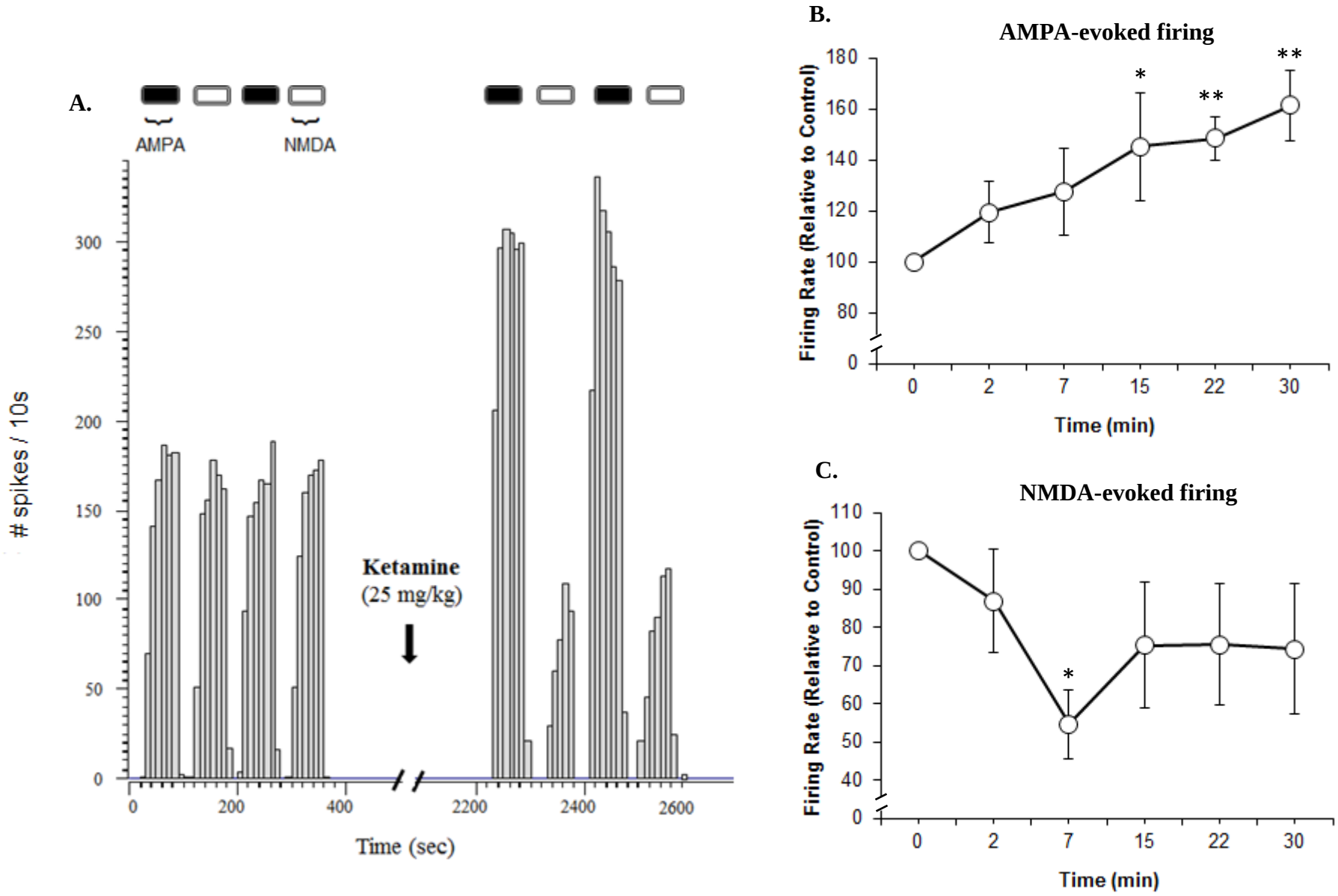

Figure 10. The effects of acute ketamine administration (25 mg/kg) on the responsiveness of AMPA and NMDA receptors. Integrated firing rate histograms of dorsal hippocampus CA3 pyramidal neurons showing the effects of ketamine administration (indicated by arrows) at a dose of 25 mg/kg (A). Each bar corresponds to the application of AMPA or NMDA. Ejection currents of -3 nA for AMPA and -8 nA for NMDA were used. The overall changes in the % of baseline firing rate of dorsal hippocampus CA3 pyramidal neurons following administration of ketamine (B & C). $^{**}p < 0.01$ and $^{*}p < 0.05$ in (B & C) using 1-way repeated measure ANOVA and Dunnett's *post-hoc* (5 rats were tested for each dose of ketamine).

DISCUSSION

While this electrophysiological investigation set out to investigate the possible role of the monoaminergic systems in the immediate antidepressant effects observed in patients clinically following systemic administration of a subanesthetic dose of ketamine, there is little question regarding the effects of NMDA antagonists on monoamine neurotransmission. The effect of ketamine on NE and DA levels was assessed decades ago by Glission et al. [53]. In that study, ketamine administered acutely at a dose of 40 mg/kg caused a marked increase DA levels in the rabbit thalamus and hypothalamus, but not in the midbrain and caudate nucleus [53]. Although higher doses of ketamine were used, this was an elegant proof of concept study demonstrating the ability of ketamine to regulate at least one central monoamine. As a follow up to this study, Kari et al. (1978) set out to investigate the effects ketamine administration on brain monoamine levels in rats [64]. Thirty minutes following the administration of ketamine at a dose of 50 mg/kg, a general decrease in brain NE and DA levels. Although these results contradict findings from the earlier experiments conducted on rabbits, they confirm the ability of systemically administered ketamine to regulate and alter monoamine metabolism. Furthermore, these studies suggest that ketamine produces an effect on the monoaminergic systems that is secondary to its primary hypoglutamatergic effect, mediated through its antagonism of the NMDA receptor.

The aim of this electrophysiological study was to investigate the effects of acute ketamine administration on monoamine firing, and examine the possible mechanisms by which any incurred changes occur. While no effect on the 5-HT system were detected following administration of ketamine at both subanesthetic doses (10 mg/kg and 25

mg/kg), an increase in the firing activity of VTA neurons was observed 30 minutes following ketamine administration, in the form of an increased population activity. No effect of ketamine on DA neurons was observed following a 2-day administration regimen. Moreover, an increase in LC NE neurons was also observed in the form of an increased firing rate as well as an increased bursting activity. This increase in firing was maintained following a 2-day administration regimen of ketamine. The effect of ketamine on both VTA DA neurons and LC NE neurons were reversed when NBQX was preadministered. This study also examined the effects of ketamine on AMPA- and NMDA-evoked response in CA3 pyramidal neurons. At a dose of 10 mg/kg, ketamine induced an increase in AMPA-evoked response, observed 30 minutes following administration. Despite the effects of ketamine on AMPA receptor responsiveness, no change in responsiveness to NMDA was observed. However, at a dose of 25 mg/kg, ketamine administration resulted in a more rapid increase in AMPA-evoked response, as well as a transient decrease in NMDA-evoked response. These effects of ketamine will be discussed in more detail in the following section.

1.0 Effects of ketamine on 5-HT firing in the DRN

The effects of NMDA antagonists on the serotonergic system are much less understood than those of antidepressants, and the effects of such compounds on 5-HT firing had yet to be fully investigated. Although to our knowledge, this study is the first electrophysiological investigation of its kind to attempt to investigate the effects of acutely administered ketamine on the firing of DRN 5-HT neurons, the importance of the 5-HT system in mediating the antidepressant-like effects has previously been demonstrated. Specifically, the antidepressant-like effect of ketamine in the FST was

abolished when 5-HT was depleted with PCPA, suggesting a pertinent role of 5-HT in ketamine-induced antidepressant effects [52]. The present study showed that acute administration of a subanesthetic dose of ketamine has no effect on the firing activity of 5-HT neurons in the DRN. This lack of effect cannot be attributed to the low dose of ketamine used in this study as a higher dose produced a similar lack of effect. While no prior study has investigated the effect of acute ketamine on 5-HT firing, it is important to note that the NMDA antagonist MK-801 has been shown to produce an increase in firing rate of 5-HT neurons in the DRN. Whilst both MK-801 and ketamine are non-competitive antagonists of NMDAR that produce behaviorally comparable results in the FST [9], they have different affinities and binding kinetics, leading to slight differences in the manner to which they bind to the NMDAR [85]. For example, MK-801 is more selective to the NMDAR when compared to ketamine [85]. Furthermore ketamine binds to NMDAR faster than MK-801 and hence reaches binding equilibrium more rapidly [85]. Although minor, these dissimilarities can account for the differences in effects of ketamine and MK-801 administration on the firing rate of 5-HT neurons. Indeed, differences between the effects of ketamine and MK-801 have been previously reported in the literature, including the failure of MK-801 to produce an analogous blockade of long term potentiation in the dentate gyrus when compared to ketamine [85].

Whilst the lack of change in the firing rate of 5-HT neurons suggests that it is unlikely that ketamine has an effect on the somatodendritic 5-HT_{1A} autoreceptors, it does not rule out the possibility that other components of the 5-HT system are affected. Indeed, Callado et al. (2000) have shown that administration of MK-801 results in a significant increase in 5-HT release, as well as an increase in the half time of 5-HT

reuptake [28]. These effects are not specific to MK-801 as Tso et al. (2004) also report a similar enhancement of 5-HT efflux and as well as an increase in half time of 5-HT reuptake following administration of either enantiomer of ketamine [137]. Shortly after, this was confirmed by Amargós et al. (2005) when they demonstrated that ketamine at a dose of 25 mg/kg produced a marked increase in the efflux of 5-HT in the PFC [91].

It has been postulated that a potentiation of 5-HT release along with an attenuation of reuptake is not mediated through antagonism of NMDAR, but rather by inhibition of 5-HT reuptake sites [62]. The effect of ketamine on SERT was investigated *in vivo* recently in a study where conscious monkeys were administered subanesthetic doses of ketamine in a paradigm that is highly analogous to ketamine infusions performed on patients in the clinic [144]. This study has shown that ketamine results in a global reduction of [^{11}C]DASB radioligand binding to SERT during the 40 minute infusion. This effect was however lost 24 hours following the infusion. Moreover, a microdialysis analysis indicated an increase in extracellular 5-HT levels in the PFC [144]. Collectively, these findings suggest that a subanesthetic dose of ketamine enhances serotonergic transmission through the inhibition of SERT activity, findings that coincide with previous studies demonstrating an increase in release and an attenuation of reuptake of 5-HT following administration of either MK-801 or ketamine [28, 137].

If antagonism of the NMDAR results in an increase in extracellular 5-HT levels, it would be expected that administration of such agents would ultimately result in a decrease in the firing rate of DRN 5-HT neurons, due to an activation of the inhibitory 5-HT_{1A} autoreceptors. However in the current study, no changes in the firing rate of DRN 5-HT were observed. This lack of change following ketamine administration could be

explained by influences from glutamatergic afferents. Monoaminergic neurons, including 5-HT neurons in the DRN, are controlled by corticofugal glutamatergic afferents (Figure 11; [29, 32, 117]). These neurons can act as accelerators when they synapse with 5-HT neurons directly, or they can act as brakes via GABAergic interneurons (Figure 11; [29]. Although there appears to be a balance between the accelerator and brake influences on the monoaminergic systems, there seems to be a slight bias towards braking [29]. Hence, an increased transmission of glutamate afferents from the cortex imposes an overall inhibitory effect on postsynaptic monoaminergic neurons in the brainstem. With this bias, it is possible that a hypoglutamatergic effect of ketamine mediated via its inhibitory effects on NMDAR relieves the inhibitory effects of cortical afferents on 5-HT neurons (Figure 11; [29]). This stimulatory effect of ketamine on 5-HT neurons may be counterbalanced the inhibitory effect of 5-HT_{1A} autoreceptor activation, explaining the absence of change in firing rate.

Despite the reported increase in extracellular 5-HT levels in the PFC following subanesthetic administration of ketamine, a more reliable method to investigate whether ketamine causes a net increase in 5-HT transmission to projection areas is to assess tonic activation. The degree of activation of the inhibitory postsynaptic 5-HT_{1A} receptors in pyramidal neurons in the CA3 layer of the HPC can be measured by administration of the 5-HT_{1A} antagonist WAY 100635. Agents that exhibit antidepressant-like effects typically enhance the tonic activation of forebrain postsynaptic 5-HT_{1A} receptors [46, 57]. It is also important to note that an effect of ketamine on 5-HT_{1B} autoreceptors could contribute to the reported increases in extracellular levels of 5-HT. These receptors play an important role in regulating the release of 5-HT from the synaptic bouton, and a desensitization of

these receptors would ultimately result in a potentiation of 5-HT release. The sensitivity of 5-HT_{1B} autoreceptors can be assessed using a stimulation paradigm. If the 5-HT_{1B} autoreceptor is desensitized following acute administration of ketamine, the difference between the duration of suppression (DOS) values observed following stimulation at 1 and 5 Hz is expected to be significantly reduced. Glutamatergic agents that exhibit antidepressant-like effects, including lamotrogine and carisbamate, have been shown to cause a significant increase in tonic activation that was attributable a desensitization of the 5-HT_{1B} autoreceptor, despite their inhibitory effect on 5-HT firing following 2- and 14-day administration regimens [117].

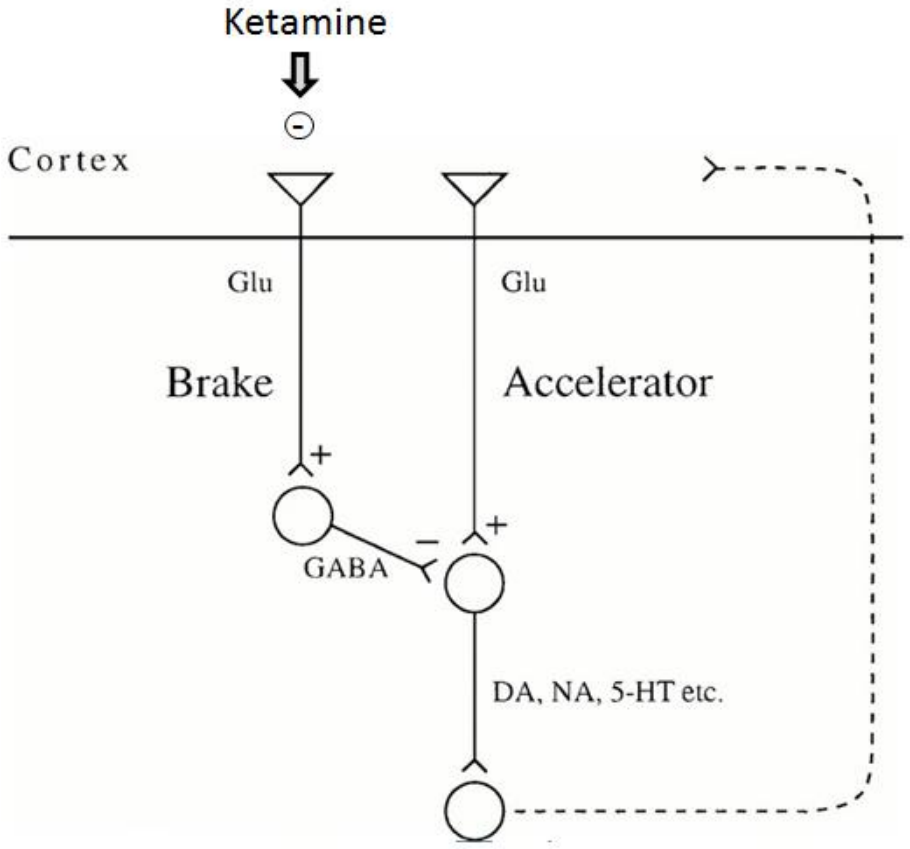

Figure 11. A model describing cortical regulation of brainstem monoamine systems. Glutamatergic efferent from the cortex regulate the activity of monoaminergic brainstem neurons directly (accelerator) or via an indirect glutamate/GABA pathway (brake). Adapted from Carlsson et al. [29].

2.0 Effects of ketamine on DA firing in the VTA

Despite the very recent emergence of ketamine and other glutamatergic agents as possible alternatives to conventional antidepressants, a multitude of studies have shown the effects of the non-competitive NMDA antagonist MK-801 on DA activity. This focus on the DA system was due to the discovery of the schizophrenomimetic effects of NMDA antagonists, leading to their subsequent use to understand brain impairments in schizophrenia. Such studies have shown that MK-801 administered systemically results in an increased firing rate of DA neurons recorded in the VTA [96]. In the current study, no changes in the firing rate of DA neurons in the VTA were observed. However an increase in firing activity manifested as an increase in population activity following administration of a subanesthetic does of ketamine. This effect of ketamine was reversed by preadministration of the AMPA antagonist NBQX, highlighting the importance of AMPA receptors in mediating this effect. Interestingly, an increase in the population activity of DA neurons has been previously shown following chronic administration of antidepressant including agomelatine, highlighting the potential importance of increased DA activity in mediating the antidepressant effects of ketamine [33].

The increased number of spontaneously firing DA neurons is not unexpected as studies have revealed that glutamatergic afferents play an important role in regulating VTA population activity [49]. Stimulation of the hippocampal ventral subiculum (vSUB) activates glutamate afferents to the NAc. As the NAc sends predominantly inhibitory GABAergic projections to the ventral pallidum (VP), such an activation of the NAc would result in an inhibition of these neurons. Consequently, the tonic inhibition of VTA neurons by the VP is removed, leading to an increase in spontaneous DA firing (Figure

12) [49]. As acute administration of ketamine has been shown to result in an increase in glutamate activity in the hippocampus, this pathway provides a possible mechanism explaining the elevation of population activity in the VTA that is observed following acute ketamine administration [82].

Although this remains to be validated, the increase in DA population activity observed in this study could produce a subsequent increase in DA transmission to projection areas. Indeed, stimulation of the vSUB has been shown to increase DA levels in the nAC, suggesting that an increase in population activity plays an important role in mediating this effect [49]. Moreover, PCP and ketamine (30 mg/kg) have also been previously shown to increase DA levels in the nucleus accumbens [89, 95]. Since the antidepressant effects of ketamine are dose-dependent, the effects of a lower dose of ketamine on DA release in projection areas should be investigated. This can be achieved by means of microdialysis to measure extracellular levels of DA in the cortex and the nucleus accumbens shortly following ketamine administration.

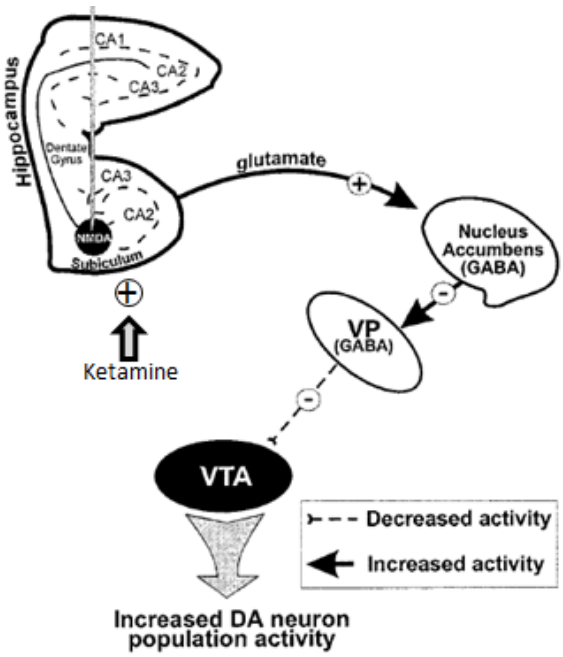

Figure 12. A model describing vSUB control over VTA DA firing. The vSUB sends stimulatory projections to the NAc. This in turn leads to an activation of NAc GABAergic efferents imposing an inhibitory effect on the VP. As a result, the tonic inhibitory influence of the VP on the VTA is removed. Adapted from Floresco et al. (2001) [49].

3.0 Effects of ketamine on NE firing in the LC

Contrary to the serotonergic and dopaminergic systems, no study to date has investigated the effects of ketamine or other non-competitive NMDA antagonists on the firing activity of NE neurons using electrophysiological techniques. The present study has shown that administration of ketamine results in an increase in firing activity, as evidenced by the increase in firing rate. Moreover, an increase in the number of neurons exhibiting burst activity was also observed. These effects of ketamine were reversed by preadministration of the AMPA antagonist NBQX, highlighting the importance of AMPA receptors in mediating these effects. Like 5-HT neurons, NE neurons in the LC are controlled by corticofugal glutamatergic afferents [29]. These glutamatergic afferents

impose an inhibitory effect on LC NE neurons [29]. Hence it is possible that a hypoglutamatergic effect of ketamine mediated via its inhibitory effects on NMDAR relieves the inhibitory effects of cortical afferents on NE neurons, resulting in an overall increase in noradrenergic firing activity in the LC (Figure 11; [29]).

It is important to note that the NE neurons of the LC receive other glutamatergic inputs from the nucleus paragigantocellularis (nPGi) in the rostral ventrolateral medulla [47]. Indeed, this afferent pathway was characterized by Ennis et al. (1988) in a study where they show that electrical stimulation of this nucleus results in a predominantly excitatory effect on LC neurons [47]. Although the effects of ketamine on the glutamatergic neurons of the nPGi are yet to be investigated, it is possible that ketamine exerts a direct effect on this nucleus, contributing to the observed increase in LC NE activity.

Previous studies have shown that bursting activity of NE neurons results in an increase in NE neurotransmitter release for the same number of action potentials discharged [58]. Thusly, we hypothesize that this increase in bursting activity, coupled with the increase in firing rate of LC NE neurons results in an increase in NE neurotransmitter release and ultimately increased NE transmission. Whilst the net effect of ketamine administration on noradrenergic transmission remains to be fully investigated, previous studies suggest that antagonism of NMDAR results in an increase in extracellular NE levels. Namely, Callado et al. (2000) have shown that MK-801 significantly increased NE neurotransmitter release and half time of NE reuptake in the locus coeruleus, ultimately leading to an increase in extracellular NE levels [28].

Although our findings suggest that blockade of NMDAR results in an increased NE transmission to projection areas, assessment of the effects of acute ketamine administration on NE transmission in the forebrain is required in order to confirm these findings. This can be elucidated by assessing the degree of tonic activation of postsynaptic α1 and α2-adrenergic autoreceptors on pyramidal neurons in the CA3 layer of the HPC, through the use of the selective antagonists, idazoxan and prazosin, respectively. Treatment with compounds that result in an increase in NE neurotransmission, including bupropion, results in a significant disinhibition of CA3 pyramidal neuronal firing rate in the HPC, indicating an increase in tonic activation of the α1 and α2-adrenergic autoreceptors [45]. If acute administration of ketamine were to increase net NE transmission to LC projection areas, a similar disinhibition of CA3 pyramidal neuronal firing is to be expected following systemic administration of the adrenergic receptor antagonists, idazoxan and prazosin.

4.0 Effects of ketamine on AMPA/NMDA-evoked response in HPC CA3

In the present study *in vivo* electrophysiological means were utilized to investigate whether acute administration of ketamine produces a potentiation of AMPA response in the CA3 layer of the HPC. To our knowledge, this is the first *in vivo* electrophysiological assessment of AMPA receptor response following acute ketamine administration. At a dose of 10 mg/kg ketamine administration produced an enhancement of AMPA-evoked response, as evidenced by the increased number of spikes induced per nanoampere (nA) following iontophoretic application of AMPA. However, no changes in NMDA-evoked response were observed at this dose. Following administration of a higher, yet similarly

subanesthetic dose of ketamine (25 mg/kg), a significant yet transient decrease in NMDA-evoked response in the HPC was observed. This reduced response to NMDA corroborates previous findings showing that systemic administration of the uncompetitive antagonist memantine produces a decrease in NMDA-evoked firing of pyramidal neurons in the CA1 region of the HPC [132]. At this elevated dose of ketamine, a comparable yet more rapidly induced increase in AMPA-evoked response was observed when compared to the lower dose.

An increase in AMPA throughput, relative to NMDA, is the predominant theory suggested to explain the antidepressant effects of ketamine. Specifically, this theory suggests that a ketamine-induced increase in AMPA receptor activation is an indirect consequence of its blockade of NMDAR, as well as the increase in glutamate release into the synapse. In the present study, our findings suggest that both a decrease in NMDAR response, as well as an increase in AMPAR response occurs, which is consistent with the aforementioned theory. However both of these effects of ketamine occurred at different, non-overlapping time points (Figure 10). Hence, the effects of ketamine on these glutamate receptors may be separate events, and ketamine may have a direct effect on AMPAR.

We hypothesize that the increase in AMPA-evoked response in the CA3 layer of the hippocampus is due to a ketamine-induced increase AMPAR expression in the HPC. Indeed, Tizabi et al. (2012) have shown that chronic administration of ketamine at the low dose of 0.5 mg/kg resulted in an increased AMPA/NMDA receptor density [135]. A similar increase in AMPAR density has also been previously been implicated in the actions of classic antidepressants [13]. Specifically, studies have shown that chronic

treatments with fluoxetine, reboxetine and desipramine induce a positive upregulation of AMPA receptor surface expression by altering the phosphorylation levels of specific AMPA receptor subunits [13, 22]. Moreover, anticonvulsants that display antidepressant effects, including lamotrogine, produce a significant enhancement of surface expression levels of GluR1 and GluR2 in cultured hippocampus neurons [43]. Additionally, treatment with these anticonvulsants produced an increased phosphorylation of GluR1 at the protein kinase A (PKA) site S845, leading to an increased expression of AMPA receptors at the surface [43].

Although administration of ketamine has been shown to alter the phosphorylation levels of GluR1 S845 [83], a direct assessment of the surface expression of AMPA receptors following acute ketamine administration is yet to be done. Using biotinylation followed by a western blot analysis, the surface expression levels of GluR1 and GluR2 can be assessed 30 minutes following treatment of cultured hippocampus neurons with a low dose of ketamine. If increases in the number of AMPA receptors on the neuronal surface area are observed, the functional consequence of such changes could be elucidated by assessing membrane depolarization in cultured hippocampus neurons following ketamine treatment. An increase in AMPA receptor surface expression should correspond with an increase in AMPA-induced membrane depolarization [43].

5.0 Concluding remarks

Although there is a wide array of antidepressants available for the treatment of patients, these monoamine-focused medications are not without their glaring limitations. Despite efforts to maximize patient responses following such treatments, including the use of augmentation strategies, the delayed remission rates remain well below optimal

levels. These limitations have led to the search of mechanistically novel antidepressants, with ketamine being its most exciting breakthrough. Not only does ketamine provide physicians with a rapid and effective tool to help their patients overcome the adversities of their illness, but it is also a crucial tool for researchers in the quest to understand and ultimately develop rapidly acting, highly effective antidepressant treatments.

Discovery of the rapid antidepressant actions of ketamine and efforts to elucidate the mechanisms underlying these effects has provided a platform for research aiming to identify agents that exhibit a similar efficacy and rapidity to ketamine, but without the commonly associated psychotomimetic effects. For instance, in a randomized, placebo-controlled, double-blind study, the NR2B subunit-selective NMDA antagonist CP-101,606 was shown to produce a significant antidepressant effect in patients with MDD 5 days following the infusion without producing dissociative effects [107]. The lack of psyotomimetic effects was attributed to the more selective nature of CP-101,606. More recently, Zarate et al. (2012) carried out a randomized, double-blind, placebo-controlled study to assess the antidepressant effects of the low-trapping, nonselective NMDA channel blocker AZD6765 [149]. A single intravenous infusion produced rapid antidepressant effect in TRD patients without inducing psychotomimetic effects [149]. However the antidepressant effects of AZD6765 were not as robust as those observed with ketamine. In a second multidose placebo-controlled trial, 152 patients received lower doses of AZD6765 or a saline solution three times a week for three weeks. This study reported antidepressant effects observed as early as one week following drug administration [41, 114]. This rapid effect of AZD6765 was made even more impressive by the fact that the antidepressant effects persisted in some patients for weeks following

treatment cessation. The most serious side effects were mild and transient dizziness and headaches, with no signs of psychosis [41]. These 2 studies demonstrate that targeting the NMDA receptor complex is a viable treatment option that brings about antidepressant effects in MDD patients.

The present study has provided insight into an additional mechanism that might play an important role in the rapid antidepressant effects of ketamine that are observed in the clinic. Specifically, these antidepressant effects could be due in part to a dopaminergic and noradrenergic involvement, although the role of the 5-HT system remains to be fully investigated. Moreover, the present study corroborates the importance of AMPAR in mediating the therapeutic effects of ketamine. The potential of ketamine to promote cellular plasticity and cell survival, as well as its positive catecholaminergic effects makes it a valuable future target for the treatment of depression.

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