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The Effects of Ketamine Administration on Novel Object Placement and Hippocampal AMPA Receptor Expression in the Male Long Evan Rats

Ruchael McNair
Montclair State University

Abstract

Many psychiatric and neurological disorders such as major depressive disorder (MDD) and mild cognitive impairment (MCI) present with deficits across many cognitive domains including spatial memory and structural dysfunction of the hippocampus. The dorsal hippocampus is indicated to play a special role in spatial memory. Synaptic plasticity mechanisms are also critical in memory function in the hippocampus. Long-term potentiation (LTP) is one type of synaptic plasticity that is directly associated with memory function. Mechanisms of LTP increase α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors through n-methyl-d-aspartate (NMDA) receptor activation. Ketamine is an NMDA antagonist that has recently gained attention for its rapid and sustained effects on synaptic plasticity, improvements in depressive symptoms and improvements in cognitive performance. The extant literature suggests that ketamine increases AMPA receptor expression, LTP and other mechanisms in the hippocampus that govern cognitive performance. Furthermore, the literature suggests that ketamine administered 24 hours prior to cognitive tasks improves some aspects of memory. The mechanisms through which ketamine may produce changes in synaptic plasticity remains unknown. We sought to investigate ketamine's effects on AMPA receptor expression in the dorsal hippocampus. We hypothesized that ketamine would produce dose-dependent improvements in spatial memory performance in the NOP task, and increases in AMPA receptor binding in the dorsal hippocampus. We found that ketamine had no effect on spatial memory performance or AMPA receptor binding, which does not support some of the previous findings in the literature. Further research is important in determining the mechanism underlying ketamine effects on synaptic mechanisms.

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The Effects of Ketamine Administration on Novel Object Placement and Hippocampal AMPA Receptor Expression in the Male Long Evan Rats

by

Ruchael McNair

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Thesis Committee:

[redacted]

Dr. Alan Pehrson

Thesis Sponsor

[redacted]

Dr. Jennifer Pardo

Committee Member

[redacted]

Dr. Erin Kang

Committee Member

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The cognitive deficits associated with many psychiatric and neurological disorders play a major role in functional disability and affect patient quality of life. Major Depressive Disorder (MDD) is one example of a psychiatric illness that is greatly impacted by the associated cognitive impairments. MDD is mainly defined as experiencing depressed mood and/or anhedonia or apathy (American Psychiatric Association, 2013). While MDD is historically characterized by its mood symptoms, recent studies suggest the disorder is associated with cognitive dysfunction as well. Cognitive functioning is impaired across many domains including spatial ability (Porter et al., 2003; Gould et al., 2007; Rose & Ebmeir 2006), psychomotor speed (Lampe et al., 2004; Kampf-Sherf et al., 2004; Rose & Ebmeir, 2006), and memory (Kampf-Sherf et al., 2004; Harvey et al., 2004; Rose & Ebmeir, 2006). These impairments are not specific to individuals in a current depressive state. Studies suggest remitted major depressive patients also show impairments in immediate memory (Baune et al., 2010), executive function (Paelecke-Habermann & Leplow 2005), and spatial working memory (Weiland-Fiedler et al., 2004). This evidence suggests cognitive impairments not only exist in patients with current depressive mood, but they persist even after their moods are stabilized. Ultimately, this indicates the prominent role cognitive impairment plays in individuals with MDD.

Because of the cognitive deficits associated with MDD, many patients report impairments in life functioning. Jaeger et al. (2006) suggest deficits in neurocognitive domains such as visuo-spatial ability, attention, ideational fluency/executive functioning, and learning were strongly associated with life functioning disability. While Baune et al. (2010) did not find a significant relationship between cognitive function and life impairment, they did find that current and

remitted persons with MDD who were currently unemployed did significantly worse on all cognitive tasks compared to healthy individuals. Furthermore, unemployment status in current and remitted depressed individuals was significantly associated with cognitive dysfunction. Quality of life in patients with remitted MDD was significantly decreased in domains including physical health, psychological health, and social relationships compared to healthy controls. (Bo et al., 2019). 63.8% of adults with MDD report impaired life functioning in regard to home management, work, close relationships with others, and social life. Additionally, 70.8% of adolescent persons with MDD report life functioning impairments in regard to chores at home, school or work, close relationships with family, and social life (McCance-Katz et al., 2019)

Considering these findings, it should not come as a surprise that MDD has major socioeconomic effects. It is estimated that the incremental economic burden of individuals with MDD is around $210.5 billion. The direct costs of medical expenses are about $98.8 million, and the workplace costs are about $102 million (Greenberg, Fournier, Sisitsky, Pike, Kessler, 2015). Finally, MDD is considered the second leading cause of global disability (Vos et al., 2015). In totality, these findings imply that targeting cognitive deficits associated with MDD is of critical importance in order to effectively and completely treat these patients.

Mild Cognitive Impairment (MCI) is a cognitive state between normal cognitive functioning and dementia that is characterized by cognitive deficits that are greater than expected for one's age (Gauthier et al., 2006). The diagnosis of MCI is currently classified by two types: amnestic mild cognitive impairment (aMCI) and non-amnestic mild cognitive impairment (naMCI). Individuals with aMCI suffer from significant memory impairment, but do not meet the criteria for dementia. On the other hand, individuals with naMCI have intact memory but have impairments in areas like language, information processing, attention, and executive

functioning (Petersen et al., 2014). Patients with aMCI are impaired in many domains across memory including visual recognition memory (De Anna et al., 2014), recall memory (Barbeau et al., 2008), and spatial reference memory (Lee et al., 2014).

The cognitive deficits that individuals with MCI suffer from negatively impact their quality of life (Teng, Tassniyom, Lu et al., 2012 & Bárrios et al., 2013). Farias et al. (2006) found patients with MCI demonstrated functional deficits in many aspects of everyday living including impairments in everyday memory and visual spatial skills. Furthermore, everyday activities that require memory were the most commonly reported deficits. Ismail et al. (2017) did a systematic review and meta-analysis of 57 studies assessing depression in patients with MCI and found a high prevalence of depression amongst this group of individuals. Additionally, a diagnosis of MCI is correlated with a later diagnosis of dementia (Bruscoli & Lovestone, 2004 & DeCarli et al., 2004); that often leads to a more severe diagnosis of Alzheimer's Disease (Wahlund, Pihlstrand, Jönhagen, 2003 & Jicha et al., 2006). Although the prevalence of MCI has ranged greatly in the literature due to changes and inconsistencies in diagnostic criteria (Petersen, 2016), it is estimated that MCI has a 16% prevalence amongst individuals ranging from 70-89 years old (Petersen et al., 2010). Considering the life-changing effects of the cognitive impairments associated with neurological and psychological disorders, it is important to focus on developing novel treatments that could potentially improve the quality of life for these individuals.

The Hippocampus

The hippocampus is a limbic system structure that plays a role in affective processes such as anxiety and is critically responsible for cognitive functions such as spatial memory (Bannerman et al., 2004). The hippocampus consists of three primary regions, including the dentate gyrus (DG), the Cornu Ammonis (CA), and the subiculum. In rodents, the CA region is

divided into three subregions: CA1-CA3. The hippocampus is also divided into two subregions: the dorsal and ventral hippocampus. Studies of the anatomy of this hippocampus show that these subregions are separated along the septo-temporal axis and play crucial roles in different functions (Tanti & Belzung, 2013). The dorsal hippocampus is specifically shown to play a crucial role in spatial memory. Moser M.B., Moser E.l., Forrest, Anderson, and Morris (1993) showed whole lesions in the hippocampus of rats impaired performance in the Morris water maze (MWM). These authors also found lesions to the dorsal hippocampus of rats produced no difference in performance compared to the whole hippocampal lesion group, while rats in the ventral hippocampal lesion group did not show these impairments. Richmond et al. (1999) investigated the role of both the dorsal and ventral hippocampal lesions on spatial memory in the MWM and fear in the contextual fear conditioning task. This group found whole hippocampal lesions impaired performance in both tasks. The ventral hippocampal lesion group showed no impairments in MWM performance but showed impairments similar to the whole hippocampal lesion group in the contextual fear conditioning task. The animals in the dorsal hippocampus lesion showed there were no impairments in the contextual fear conditioning task. An overview of this literature can be found in Table 1.

The sensory information that the dorsal hippocampus receives from the cortex may be the reason why it is so important in spatial memory. On the other hand, the ventral hippocampus has connections to the prefrontal cortex and other subcortical regions like the amygdala, which may be the reason why the ventral hippocampus is crucial in anxiety (Bannerman et al., 2004).

Circuitry of the Hippocampus

The hippocampus has two primary inputs, which are well-characterized. The first of these inputs is an indirect pathway commonly known as the trisynaptic loop, which is

important for hippocampal learning and memory functions (Basu & Siegelbaum, 2015). The trisynaptic circuit consists of the signals that project from the entorhinal cortex, which is the hippocampus' primary input structure, to the dentate gyrus via the perforant path. From the dentate gyrus, signals are sent to the CA3 region via mossy fibers, and then from CA3 to CA1 via Schaffer collaterals. In addition to this pathway, information can come to CA1 from the entorhinal cortex via the direct pathway. From CA1, information is primarily sent to the subiculum, which is the hippocampus' primary output structure (Freund & Buszaki, 1996). In each of these regions, there are interneurons and principal cells that play major roles in regulating the signaling properties within these regions. Figure 1 presents a schematic depiction of the trisynaptic circuit.

The dentate gyrus. The principal cells in the dentate gyrus are excitatory glutamatergic cells called granule cells. The granule cells in the DG are the target of excitatory axon collaterals that project from stellate cells in the entorhinal cortex. The axons of granule cells are called mossy fibers. Mossy fibers are the main output axons of the dentate gyrus that synapse onto the principal cells and inhibitory cells in CA3.

Additionally, mossy fibers send axons to the hilar region of the DG where another prominent glutamatergic cell type called mossy cells are found. Mossy cells send projections to granule cells and interneurons. Although mossy cell axons are excitatory, their overall effect on granule cells is inhibitory because they can exert strong disynaptic feedforward inhibition (Senzai, 2019).

There are also many inhibitory interneurons in the DG. The most studied interneurons in the DG are classified as fast-spiking parvalbumin-positive perisomatic inhibitory cells. These cells include basket cells and axo-axonic (chandelier) cells. The basket cells and the axo-axonic

cells in the DG receive excitatory input from the perforant pathway and local granule cells. This excitatory input causes the interneurons to inhibit granule cells (Yuan et al., 2017). These interneurons also send inhibitory signals to pyramidal cells in CA3. (Senzai, 2019). There are also other types of inhibitory interneurons in the DG that are far less studied including Regular-spiking cholecystokinin-expressing basket cells, hilar interneuron with perforant path associated (HIPP) cells, molecular layer perforant path associated cells (MOPP), hilar commissural associated path (HICAP) cells, and interneuron selective cells (ISC) (Senzai, 2019).

The Cornu Ammonis regions. The principal cells in the CA1 and CA3 regions are glutamatergic pyramidal cells. The pyramidal cells in CA3 are the main target of mossy fibers from the DG¬¬¬–and also can receive input directly from the entorhinal cortex. The axons of CA3 pyramidal cells are called Schaffer collaterals and they are the main output of the region. These collaterals mainly extend to CA1 pyramidal neurons, but they also are sent to the hilar subregion of the DG as well (Freund & Buszaki, 1996). CA1 pyramidal neurons are the main output of the trisynpatic circuit. Their axons mainly extend to the subiculum and the entorhinal cortex. They can also target other areas including the limbic cortical areas, lateral septum, nucleus accumbens, and olfactory bulb (Freund & Buszaki, (1996). The CA3 and CA1 region of the hippocampus are also heavily regulated by inhibitory interneurons. The main interneurons in these regions are the same as the DG and work similarly. The fast-spiking parvalbumin positive GABAergic basket cells and axo-axonic cells receive excitatory input which then exerts inhibitory signals onto pyramidal cells. CA1 and CA3 also have many other interneurons that are less studied including regular spiking cholecystokinin basket cells and many different dendritic inhibitory interneurons (Booker & Vida, 2018).

The Role of the Hippocampus in Memory Function

The discovery of the importance of the hippocampus for memory can be traced back to Scoville and Milner (1957) who found lesions to the hippocampus produced severe impairments to recent memory. Since then, researchers have tried to understand the functions associated with the hippocampus and its subregions. In healthy individuals, Travis et al. (2014) showed that CA1-3 and DG volumes within the hippocampal body and tail are correlated with visuospatial memory tasks. Furthermore, studies have shown that reduced hippocampal volume is commonly associated with deficits in cognition and functional disability (Bostrom et al., 2016; Cahn-Weiner et al., 2007). Structural magnetic resonance imaging (MRI) studies suggest that individuals who suffer from MDD (Colla et al., 2007; Barch et al., 2019) and MCI (Moon, Lee, and Choi, 2018; Convit et al., 1997) have reduced hippocampal volumes. Additionally, researchers have investigated the differences in hippocampal subregions. Travis et al. (2014) found that patients with MDD had significantly reduced total hippocampus body volumes and total volume of DG within the hippocampus body—although, these authors did not find differences in global hippocampal volume. The authors also found the duration of illness negatively correlated with hippocampal body DG volumes. There is some evidence that hippocampal volumes can be used as a diagnostic marker for MCI and its progression to other diseases with dementia (Jack et al., 1999). Kantarci et al. (2016) found that individuals with aMCI were more likely to have hippocampal atrophy and were more likely to progress to a later diagnosis of AD. Yavuz et al. (2007) aimed to investigate the hippocampal volumes of individuals with MCI, AD. The authors found that patients with MCI had significantly lower hippocampal volumes compared to healthy controls. Additionally, individuals diagnosed with AD had significantly lower hippocampal volumes than both MCI and control subjects. Interestingly, MRI studies in older adults without neurological or psychiatric disease also suggest reduced hippocampal volumes are associated

with cognitive deficits, especially in episodic and working memory domains (O'Shea, Cohen, Porges, Nissim, and Woods, 2016; Hardcastle et al., 2020).

Spatial memory is a form of memory that is responsible for encoding information about an organism's surroundings, allowing the organism to identify and navigate within its environment. The hippocampus plays a crucial role in spatial memory functions (King, Burgess, Hartley, Vargha-Khadem, and O'Keefe, 2002; Kessels, de Haan, Kappelle, and Postma, 2001). Hippocampal lesions in animal models produce impairments in performance in many tasks assessing spatial memory, like the MWM (Broadbent, Squire, & Clark, 2004; Clark, Broadbent, & Squire, 2005; Logue, Paylor, & Wehner, 1997) and the novel object placement (NOP) task (Barker & Warburton, 2011; Mumby, Gaskin, Glenn, Schramek, & Lehmann, 2002). Clark et al. (2005) did a study where they examined the effects of hippocampal thermocoagulation lesions on different tasks assessing spatial memory in three different experiments. These tasks included the water maze, oasis maze, and the annular water maze. The researchers separated the animals with lesions into two groups: those with dorsal and ventral hippocampal lesions and those with only dorsal hippocampal lesions. Clark et al. (2005) found that animals with hippocampal lesions showed impaired performance on all spatial memory tasks. Additionally, the animals with only dorsal hippocampus lesions showed similar levels of impaired performance to the animals with both dorsal and ventral lesions. Furthermore, Eijkenboom & Van Der Staay (1999) used vincristine, a neurotoxin that blocks assembly of microtubules and impairs cellular transport processes, to induce lesions in the dorsal hippocampus of rats. They found that lesions in this area are associated with impaired performance in the MWM. Melendez, Nordquist, Vanderschuren, and van der Staay (2020) did a similar experiment and found that vincristine-induced lesions in the dorsal hippocampus of rats with bilateral lesions presented with impaired

performance in working memory and reference memory in the conefield spatial discrimination task.

Studies have also been done to further understand the role different hippocampal subregions play in aspects of spatial memory performance. Lee, Hunsaker, and Kesner (2005) studied the effect of lesions induced by the microtubule toxin colchicine in the DG, CA1, and CA3 regions of the dorsal hippocampus separately on performance in an open platform object placement task. The researchers found that hippocampal lesions in the DG and CA3 significantly impaired the animals' ability to detect novel placement. The animals with lesions in CA1 showed minimal impairment in the spatial memory task. Additionally, Stupien, Florian, and Roullet (2003) investigated the effect of blocking the CA3 region of the dorsal hippocampus on memory acquisition, consolidation, and recall in the spatial open field task and the object recognition task. They did this by performing focal injections of diethyldithiocarbamate (DDC) to temporarily block the activity in CA3 regions. The researchers found that animals with DDC treatment showed significant reductions in exploration time of displaced objects during memory acquisition and memory consolidation trials in the spatial memory task. Interestingly, the researchers did not see any significant difference between control and treatment groups during recall. The treatment also had no effect on performance in the object recognition trial, which suggests CA3 may specifically play a crucial role in spatial memory tasks. Florian and Roullet (2004) conducted a similar experiment but with the spatial and non-spatial (all cues are left out except a single proximal cue) versions of the MWM. They found that DDC treatment into the dorsal hippocampus impaired memory acquisition and consolidation, but not recall. Additionally, the DDC-treated animals showed no impairment in the non-spatial MWM task. Although there is a great amount of evidence that supports the role the dorsal hippocampus plays in spatial

memory performance, these studies have limitations because some lesions extend past the targeted area. Nevertheless, these data show the significant role the dorsal hippocampus plays in spatial memory performance and the possibility for CA3 to be crucial as well.

Studies in humans with hippocampal lesions also suggest the hippocampus is important in spatial memory functions. Kessels et al. (2001) did a meta-analysis of 27 studies that investigated patients with hippocampal lesions' performance on spatial memory tasks such as maze learning and object-location memory tasks. Although the researchers found that patients with hippocampal damage had impaired performance on spatial memory tasks, their analysis has limitations as most patients had damage that extended in regions beyond their hippocampus¬.

The Role of Synaptic Plasticity in Memory Function

Synaptic Plasticity. Synaptic plasticity is the modification of the strength and efficacy of synaptic transmission. These modifications can cause either an enhancement or depression of synaptic transmission. Additionally, synaptic plasticity can produce short-term or long-term changes. Short-term synaptic plasticity produces changes that last from milliseconds up to minutes. Meanwhile, long-term synaptic plasticity creates changes that last for long periods of time. Synaptic plasticity is considered to have a critical role in memory function (Whitlock, Heyen, Shuler, & Bear, 2006; Gruart, Muñoz, & Delgado-Garciaet, 2006; Zubareva et al., 2020).

Long-term potentiation (LTP). One example of long-term synaptic plasticity is long-term potentiation (LTP; Citri & Malenka, 2008). LTP is a plasticity phenomenon that is defined as long-lasting activity-dependent changes in neurotransmission that strengthen the strength of a synaptic connection, which is an indicator of learning and memory when seen in the hippocampus (Bliss & Collingridge, 1993). Bliss and Lomo (1973) found that repetitive stimulation into the perforant pathway produces long-lasting changes in hippocampal synaptic

transmission. Hippocampal LTP plays a role in memory function in many ways. Kim et al. (2007) showed that stress impaired MWM task performance in animal models and induced impairments of hippocampal LTP. Increased LTP in the hippocampus enhances memory function (Huo et al., 2020), including in hippocampally-dependent memory tasks. Zhang, Xing, Wang, Tao, and Cheng (2015) showed high frequency-repetitive transcranial magnetic stimulation (HF-rTMS) treatment in animal models of dementia improved learning and memory deficits in the MWM. Ma et al. (2019) also used HF-rTMS to induce LTP in aged animal models for 14 consecutive days prior to performing the MWM task. The authors not only found that HF-rTMS improved performance in MWM, but that 5Hz of HF-rTMS increased synaptic plasticity-related proteins like synaptophysin (SYN), postsynaptic density (PSD)-95, and brain-derived neurotrophic factor (BDNF), and the phosphorylated form of the cyclic adenosine monophosphate response element binding protein (pCREB). Huang et al. (2017) found that low-frequency rTMS treatment improved performance in AD animal models.

Glutamate neurotransmission. Glutamate receptors play an important role in synaptic plasticity mechanisms of the hippocampus that regulate learning and memory functions. Glutamate is the most abundant excitatory neurotransmitter in the nervous system (Zhou & Danbolt, 2014). There are two types of glutamate receptors: ionotropic and metabotropic receptors. Ionotropic receptors are ligand-gated and require a ligand to bind for the receptor to become activated. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptors are two of the main ionotropic glutamate receptors.

AMPA receptors are tetrameric structures and made up of subunits ranging from GluA1-GluA4 that are expressed in different combinations. The most abundant type of AMPA receptors expressed in the hippocampus are made up of GluA1 and GluA2 subunits. AMPA receptors with

GluA2 and GluA3 subunits are also expressed in the hippocampus but at a smaller amount. AMPA receptors are ligand-gated, and once activated, AMPA receptors are permeable to Ca2+ and Na+ (Czondor & Thoumine, 2013).

NMDA receptors are also tetrameric structures and comprised of subunits GluN1-GluN3. Functional NMDA receptors are heterotetramers and express two mandatory GluN1 subunits and either two GluN2 or two GluN3 subunits (Vyklicky et al., 2014). NMDA receptors are unique because they are both ligand-gated and voltage-gated¬¬¬¬. For NMDA receptors to become activated, glycine needs to bind to GluN1 and glutamate needs to bind to GluN2/3. The binding of the ligands allows the ion channel to open but it is blocked by a magnesium ion. The magnesium blockade is removed if the cell membrane around the receptor is sufficiently depolarized. Once the ion channel is cleared, it is permeable to Ca2+ and Na+.

Mechanisms of LTP are heavily dependent on the activation of NMDA receptors and increased AMPA receptor expression at the synapse (Lüscher & Malenka, 2012). Tocco, Maren, Shors, Baundry, and Thompson (1992) found LTP increased AMPA receptor binding in the hippocampus. Additionally, Penn et al. (2017) showed that LTP increased AMPA receptor surface diffusion, and immobilization of AMPA receptor surface diffusion impaired synaptic potentiation in the hippocampus–which was associated with impaired performance on a learning task.

Similar to LTP, dendritic arborization is important in memory function. Chen et al. (2010) showed stress-induced dendritic spine loss was associated with impairments in LTP and performance on memory tasks. Additionally, Mahmmoud et al. (2015) showed that dendritic spine density was increased in the hippocampus of animals trained in the radial arm maze–a spatial memory task.

Ketamine

Ketamine was introduced to clinical practice in the 1970s after being synthesized in the 60s as an anesthetic agent with less severe side effects than the ones that were used at the time. Ketamine is made up of two enantiomers, named R-ketamine and S-ketamine. The chemical and physical properties of the two enantiomers are similar, but they have slightly different shapes that can change some aspects of their affinities for neurotransmitter receptors. Ketamine has binding sites on many receptor types including glutamate receptors, opioid receptors, nicotinic and muscarinic acetylcholine receptors, and GABA receptors (Sinner & Graf, 2008) Ketamine is widely known for its effects as a noncompetitive NMDA receptor antagonist, so named because it blocks the ion channel of activated NMDA receptors. S-ketamine has a three to four times higher affinity for the noncompetitive NMDA receptor binding site than R-ketamine. (Geisslinger et al., 1993). Due to the binding site's placement behind the Mg+ block, the cell must be depolarized for ketamine to bind the NMDA receptor.

Ketamine is water and lipid-soluble so it can be administered in a variety of ways. Most optimally, ketamine is administered intravenously. Other ways ketamine has been administered are per os (i.e. orally), intramuscularly, and intranasally (Sinner & Graf, 2008). Although ketamine has historically been used as anesthesia, it has several other clinical uses. Ketamine is used for pain management and sedation during non-operative procedures. (Gao, Rejaei, and Liu, 2016)

Recently, S-ketamine (also known as esketamine) as a nasal spray has also been approved to treat Treatment-Resistant Depression (TRD). Berman et al. (2000) and Zarate et al. (2006) were the two major clinical trials that influenced the investigation of subanesthetic doses of ketamine to treat TRD and the mechanisms in which it produces rapid and sustained antidepressant effects. Berman et al. (2000) was the first clinical study that investigated

ketamine's antidepressant effects in a group of seven patients with MDD. The authors found that 0.5 mg/kg dose of ketamine given intravenously over a 40 min period significantly reduced self-reported depressive symptoms on the Hamilton Depression Rating Scale (HDRS) and the Beck Depression Inventory (BDI) starting at 4 hours post-treatment and lasting at least 72 hours during their 3-day follow up. The reduced depressive symptoms from ketamine returned to baseline around 1-2 weeks post-treatment. The authors also reported that patients with ketamine treatment showed worse performance on the Brief Psychiatric Rating Scale (BPRS) and the Visual Analog Scales score for intoxication "high" (VAS-high) immediately after injection and lasting for up to 120 minutes after the injection. The purpose of these tests was to examine psychiatric and psychedelic-like symptoms like euphoria and dissociation. Zarate et al. (2006) did a similar clinical study investigating and replicating the rapid and sustained antidepressant effects of ketamine in 17 patients with treatment-resistant MDD.

Similarly, Zarate et al. (2006) showed that ketamine produced rapid and sustained antidepressant effects after 0.5 mg/kg i.v. ketamine injections over a 40-minute period. Patients with ketamine treatment showed improvement in depressive symptoms at 110 minutes on the HDRS and 40 minutes on the BDI. These improvements lasted for up to seven days, which is odd because ketamine has a short half-life of approximately 2 hours in humans (Sinner & Graf, 2008). Also consistent with Berman et al. (2000), these authors found that patients with ketamine treatment had worse scores only at 40 minutes on the BPRS and the Young Mania Rating Scale (YMRS). The authors also report euphoria, dizziness, depersonalization, and derealization as some of the adverse events that were more common in the ketamine group than the placebo group. Additionally, none of these symptoms lasted past 110 minutes for patients.

Although subanesthetic doses of ketamine seem to show rapid and sustained antidepressant effects, it is notable to mention that these experiments have some methodological flaws. The most concerning limitations of these studies are the small sample sizes and the inability for this experiment to be a true double-blind experiment. Considering ketamine produces instant psychomimetic and dissociative effects, it is easy to tell which patients were administered ketamine and which were given the placebo. Ketamine's rapid and sustained antidepressant effects are of great potential importance because the current line of treatments for depression take several weeks to begin working, and only effectively reduce depression scores in about 2/3rds of patients (Trivedi et al, 2006). However, its psychotomimetic and motor-impairing effects, along with its abuse potential severely limit its clinical utility (discussed below).

Ketamine Effects on Synaptic Plasticity

The molecular mechanisms underlying ketamine's effects on cognitive function and depression may be driven by ketamine-induced rapid synaptic plasticity (Zanos & Gould, 2018). Treccani, Ardalan, and Chen (2019) used Flinders Sensitive and Flinders Resistant Line (FSL/FRL) rat models to examine ketamine's effects on dendritic morphology in the CA1 region of the hippocampus. FSL rats are genetically modified to show depressive-like symptoms, whereas FRL rats do not–therefore serving as a control to FSL rats in this study. The researchers found that FSL rats showed significant reductions in dendritic spine length and density. Ketamine improved the deficits in dendritic spine density but not dendritic spine length in FSL treated animals. Tornese et al. (2019) found that chronic mild stress-induced anhedonia and dendritic spine length in the CA3 region of the hippocampus, which were restored by ketamine treatment. Contrary to Treccani et al. (2019), Tornese et al. (2019) found no significant differences in dendritic morphology in the CA1 region of the hippocampus.

A single subanesthetic dose of ketamine also increases hippocampal LTP. Graef et al. (2015) found that intraperitoneal and intravenous ketamine treatment enhanced high-frequency stimulation (HFS) induced hippocampal LTP at 24 hours post-treatment. Yang, Ju, Zhang, and Sun (2018) induced depressive-like symptoms using the chronic social defeat stress (CSDS) model in rodents. Animals exposed to CSDS showed impaired spatial memory performance and HFS induced hippocampal LTP. A subanesthetic dose of ketamine attenuated the impairments in spatial memory and LTP in CSDS exposed animals.

There is also evidence suggesting that ketamine improves synaptic plasticity in the cortex as well (Pryazhnikov et al., 2018). Ng et al. (2018) demonstrated that restraint stress-induced dendritic spine elimination in the frontal cortex of animal models, but ketamine reduced these effects of restraint stress on spine loss. Additionally, these researchers found that ketamine increases parvalbumin interneuron activity in the frontal cortex. Taken together, ketamine appears to increase synaptic plasticity in brain regions that are important for cognitive function and may have applications for treating cognitive impairments in diseases beyond TRD.

Ketamine Effects on Cognitive Function

These changes in glutamatergic synaptic plasticity induced by ketamine could also have applications for improving cognitive function in a variety of disorders featuring cognitive impairment, and there is evidence in the literature that it can improve cognitive function. Ketamine improves the cognitive deficits induced by chronic stress models (Papp, Gruca, Lason-Tyburkiewicz, and Willner et al, 2017; Patton, Lodge, Morilak, and Girotti, 2017). Jett et al. (2016) investigated the effects of ketamine on cognitive function and coping behaviors in chronic unpredictable stress (CUS) animal models. The researchers found that CUS impaired cognitive function in the attentional set-shifting test (AST) and increased immobility in shock-probe

defensive burying (SPDB) test. These impairments were reversed by a subanesthetic dose of ketamine 24 hours prior to tasks. In another study, Paredes, Silva, and Morilak (2018) did a similar study investigating the effects of ketamine on Chronic Intermittent Stress (CIS) induced cognitive impairment. These researchers showed that CIS-induced reversal learning impairments were corrected by a subanesthetic dose of ketamine.

There are also studies in persons with depression that have shown that repeated subanesthetic doses of ketamine improved cognitive function along with its antidepressant effects (Shiroma et al., 2014; Shiroma et al., 2020; Zhou et al., 2018). These findings are contrary to Murrough et al. (2015) findings that patients administered ketamine showed no significantly different improvement in cognition compared to patients administered midazolam– the anesthetic control. Interestingly, Basso et al. (2020) compared the effects of ketamine treatment and electroconvulsive therapy (ECT)-a current standard treatment for MDD– on antidepressant effects and cognitive function, using a naturalistic sample of depressed patients. These researchers found that ketamine and ECT were equally effective, but ketamine-treated individuals showed faster onset of antidepressant effects and improvement in cognitive function. The overall literature on ketamine's effects on cognitive function suggests ketamine may improve many domains of cognitive function, but there is a need for further investigation to fully support its precognitive effects (Gill et al. 2020 & Lee et al. 2016).

Although ketamine produces improvement in depressive symptoms and cognitive impairment, there are many downsides and side-effects that make the drug undesirable. Ketamine has dissociative properties and impairs cognition for a short period of time after administration (Berman et al., 2000; Zarate et al., 2006). Additionally, ketamine is currently administered through a nasal spray that has to be administered at a healthcare provider's office.

Because of the dissociative effects after administration of ketamine, the patient is required to be watched by their healthcare provider for at least 2 hours after. Furthermore, the patient needs to have reliable transportation from the office back to their home. Although ketamine only needs to be administered once per week, this can be a great weekly burden on all parties involved in this process. Ketamine has also gained attention for its gradual increase in prevalence as a drug of abuse around the world (McCambridge, Winstock, Hunt, & Mitcheson, 2007; Liu, Lin, Wu, & Zhou, 2016; World Drug Report, 2010). Despite its reported negative side effects, studies suggest psychomimetic effects are not persistent after administration and that ketamine is well tolerated and safe when used in clinical settings (Wan et al., 2015). Therefore, investigating ketamine as a pharmacological agent to improve cognitive function may be beneficial in further understanding the biological mechanisms underlying improved cognitive function in order to find a drug that has similar pro-cognitive effects as ketamine, but doesn't produce undesirable side effects.

Ketamine As an Antidepressant Treatment - Mechanisms of Action

NMDA receptor mechanisms are not likely to be relevant. Although ketamine is widely known for its non-competitive antagonist effects on NMDA receptors, investigations of the mechanisms underlying the improvements in memory and robust antidepressant effects suggest NMDA receptor antagonism may not be the main mechanisms responsible for these findings. Other NMDA receptor antagonists, such as MK-801, have not shown the same fast-acting acute and sustained antidepressant effects as ketamine (Maeng et al., 2008). Additionally, R-ketamine may show better antidepressant effects than S-ketamine (Fukumoto et al., 2017). Yet, S-ketamine is four times more potent than R-ketamine as an antagonist at NMDA receptors (Zeilhofer, Swandulla, Deissling, & Brune, 1992). Furthermore, studies show that ketamine's metabolite (2R,6R)-hydroxynorketamine (HNK) also works as an antidepressant at

concentrations that do not block NMDA receptors (Lumsden et al., 2019). This evidence suggests that there may be other mechanisms that are prominent in ketamine's effects beyond NMDA receptor antagonism.

A role for AMPA receptors. There is evidence that ketamine produces changes in synaptic plasticity that modulate AMPA receptor function– which is suggested to be the mechanism responsible for ketamine's rapid antidepressant effects. Many studies have found that ketamine-induced changes in synaptic plasticity are blocked by the AMPA receptor antagonist 2,3-dioxo-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) (Zhou et al., 2020; Maeng et al., 2008). Koike, Iijima, and Chaki (2011) showed that acute and sustained ketamine treatment reduced escape failures in the learned helplessness (LH) model and reduced immobility times in the tail suspension test (TST). The acute antidepressant-like effects of ketamine were completely blocked by NBQX, while NBQX only partially blocked ketamine's effects in the sustained condition (administration 72 hours prior to test). Additionally, Koike & Chaki (2014) found that ketamine administered 24 hours prior to FST induced a reduction in immobility time that was blocked by NBQX treatment 30 mins prior to testing.

Additionally, studies have shown that ketamine-induced antidepressant-like behaviors in animals are associated with changes in AMPA receptor expression. Zhang, Yamaki, Wei, Zheng, and Cai (2017) found that ketamine-induced antidepressant-like behaviors in the FST at 30 mins after administration, which lasted for at least 4 hours in animals. Additionally, these researchers found that acute and sustained ketamine treatment increased total AMPA receptor expression in the hippocampus. In another study, Tizabi, Bhatti, Manaye, Das, and Akinfiresoye (2012) found that chronic ketamine treatment (0.5 mg/kg daily for 10 days) significantly increased AMPA

receptor density in the hippocampus. Contrary to the previously mentioned findings, some studies suggest ketamine may not increase total AMPA receptor expression

(Maeng et al., 2008), but may cause increases AMPA receptor surface expression (Nosyreva et al., 2013, Beurel, Grieco, Amadei, Downey, & Jope, 2016)– which is associated with antidepressant-like behaviors in animals. It is important to note that studying ketamine's acute effects at 30 mins post-injection in animal models can cause confounding results due to ketamine's effects on locomotion at that time point (Irifune, Shimizu & Nomoto, 1991). Collectively, the evidence on the mechanisms underlying ketamine-induced changes in synaptic plasticity is unclear and needs further investigation.

Brain-derived neurotrophic factor (BDNF). BDNF is a protein that is a part of a family of neurotrophic factors. BDNF is responsible for promoting nerve growth and protection, by activating tyrosine receptor kinase B (TrkB) receptors. BDNF and TrkB signaling mechanisms play a role in LTP and cognitive functions such as learning and memory. (Binder & Scharfman, 2004). There is evidence that BDNF facilitates the induction of LTP (Rex et al., 2007 & Kramer et al., 2004) and that LTP causes the release of BDNF (Rex et al., 2007). Ma et al. (1998) found that LTP induces increases in BDNF levels in hippocampal slices and animals with good memory performance in the inhibitory avoidance learning task showed increases in LTP and BDNF protein levels in the hippocampus. Blocking BDNF activity impaired memory performance and reduced LTP.

There is evidence that suggests ketamine's rapid changes in synaptic plasticity involve increased BDNF activity (Yang C., Hu, Zhou, Zhang, and Yang JJ., 2013). Garcia et al. (2008) showed that a 15 mg/kg dose of ketamine produces rapid increases in BDNF protein activity in the hippocampus. Zhou et al. (2014) also showed that a subanesthetic dose of ketamine acutely

upregulates BDNF expression in the hippocampus and prefrontal cortex. In these studies, the increases in BDNF were associated with decreases in depressive-like behavior in the FST. Furthermore, studies show that ketamine's behavioral effects are dependent on BDNF and TrkB activity (Ma et al., 2017 & Bjorkholma & Monteggia, 2016). When BDNF and TrkB signaling is blocked ketamine's effects on behavior and synaptic mechanisms are blocked as well. Autry et al. (2011) showed that ketamine-induced reduction in depressive-like behavior in mice was blocked in BDNF knockout mice. Moreover, when Yang et al. (2015) coadministered ketamine with a TrkB antagonist in mice, ketamine's improvements on depressive-like behavior were black as well. This suggests BDNF/TrkB signaling is relevant in the mechanisms underlying ketamine's behavioral effects.

Currently, there are three theories that suggest the mechanism in which ketamine increases BDNF/TrkB activity: 1) Disinhibition hypothesis 2) Eukaryotic elongation factor 2 (eEF2), and 3) Glycogen synthase kinase 3β (GSK-3β). Each of these theories is detailed below.

Disinhibition hypothesis. NMDA receptors are present on the surface of the fast-spiking parvalbumin-positive interneurons in the hippocampus. Because fast-spiking interneurons fire at a high frequency, the cellular membrane is often depolarized, and thus it is more likely that the NMDA receptor's voltage requirements are met on this cell population. As a result, the magnesium block that blocks access to the noncompetitive NMDA receptor binding site is often removed, thus allowing ketamine to bind to the NMDA receptor. When ketamine binds to NMDA, it blocks the flow of ions passing through the channel which essentially turns off the receptor's activity. Figure 2 depicts the function of NMDA receptors under normal circumstances and when ketamine is present. The selective decrease in excitatory input from the NMDA receptors on these interneurons reduces their firing rate and reduces their inhibitory input onto

pyramidal cells (Homayoun and Moghaddam, 2007). These effects allow the pyramidal cells to fire more, causing an increase in excitatory signals (Homayoun and Moghaddam, 2007; Moghaddam, Adams, Verma, & Daly, 1997). Figure 3 shows the relationship between interneuron activity and pyramidal cells under normal circumstances and when ketamine is present. These increases in glutamatergic signaling may increase BDNF/TrkB signaling. This is referred to as the disinhibition hypothesis which was reviewed in the context of ketamine's antidepressant effects by Zanos & Gould (2018).

Eukaryotic elongation factor 2 (eEF2). Another theory that is proposed about the mechanism in which ketamine increases BDNF/TrkB signaling involves eEF2. eEF2 regulates protein synthesis by determining the translocation step in an intracellular calcium-dependent manner (Kaul, Pattan, & Rafeequi, 2011). While eEF2 is active, it tends to reduce the local translation of proteins within the cell. This process is shown in Figure 4. Ketamine antagonism on NMDA receptors reduces intracellular calcium activity by blocking calcium influx through the NMDA receptor and by reducing voltage-gated calcium channel activity. This reduction in intracellular calcium activity reduces eEF2 phosphorylation (Bjorkholma & Monteggia, 2016), thus suppressing its activity and allowing for local protein translation to continue (Sutton, Taylor, Ito, Pham, & Schuman, 2007). Some theories have suggested that this mechanism increases BDNF/TrkB activity (Kavalali & Monteggia, 2012). However, this concept may be flawed because it once again depends on NMDA receptor antagonism, which does not consistently induce rapid or sustained antidepressant effects.

Glycogen synthase kinase 3β (GSK-3β). A third mechanistic theory that is proposed to explain ketamine effects on BDNF/TrkB signaling is separate from its effects on NMDA receptors. As previously mentioned, there is some evidence suggesting that NMDA receptor

antagonism may not be the primary mechanism responsible for ketamine's antidepressant effects. Ketamine treatment increases phosphorylation of protein kinase B (also known as AKT) which subsequently reduces the activity of GSK-3β by increasing phosphorylation at serine 9. Figure 5 shows how GSK-3β activity is altered after BDNF/TrkB activation, which ultimately increases BDNF. In addition, ketamine's antidepressant effects are potentiated by combination with GSK-3β inhibitors such as lithium (Liu et al. 2013), and its antidepressant effects are dependent on GSK-3β inhibition (Beurel, Song & Jope, 2011; Zhou et al. 2014). These processes are associated with increases in mammalian target of rapamycin (mTOR; Zhou et al., 2014; Beurel et al., 2011) and CREB activity (Wray, Schappi, Singh, Senese, & Rasenic, 2019), which would be expected to increase expression of BDNF and other proteins associated with synaptic plasticity.

BDNF and AMPA receptor activity

There is also a substantial relationship between BNDF and AMPA receptor activity. BDNF increases the expression and membrane insertion of AMPA receptors in the hippocampus (Jourdi & Kabbaj, 2013). Caldeira et al. (2007) demonstrated that BDNF upregulates AMPA receptor protein and mRNA levels, which was blocked by a TrkB receptor inhibitor. Li and Keifer (2008; 2009) demonstrated that bath application of BDNF increases AMPA protein levels. Additionally, these researchers found that learning in a classical conditioning task was associated with an increase in BDNF and AMPA protein levels. These changes detected were all blocked by a TrkB inhibitor. These findings suggest BNDF plays a crucial role in modulating AMPA receptor activity. BDNF also increases glutamatergic neurotransmission in areas other than the hippocampus such as the prefrontal cortex (Chiba et al., 2012) and the nucleus accumbens (NA; Reimers, Loweth, & Wolf, 2014). Increases in AMPA receptor activity also produce increases BDNF activity. Machowiak, O'Neill, Hicks, Bleakman, and Skolnick (2002) showed that an

AMPA receptor potentiator increases BDNF protein and mRNA levels in the hippocampus. The AMPA receptor potentiator increased hippocampal TrkB mRNA expression as well. Legutko and Skolnick (2001) showed AMPA receptor potentiation increased BDNF mRNA and protein levels in the cortex. Additionally, AMPA receptor positive allosteric modulators–which are ligands that increase glutamate-mediated responses of AMPA receptors–increase BDNF release and TrkB activation (Jourdi et al., 2009). Taken together, there is a great amount of evidence that supports the relationship between BDNF and AMPA receptor activity. Because ketamine causes increased BDNF activity, it is plausible that AMPA receptor activity is also increased by ketamine as well.

Rationale

Ketamine produces rapid changes in synaptic plasticity that may promote increases AMPA receptor activity and may have applications for treating cognitive dysfunction. The current literature suggests ketamine may improve cognitive performance and increase AMPA receptor expression, but there is a small amount of evidence that supports these claims and, it is unclear in which areas of the brain ketamine produces changes in synaptic plasticity. Thus, the purpose of this study is to replicate previous findings that suggest ketamine administration can improve function in a hippocampally-dependent cognitive task. In addition, we evaluated whether ketamine administration causes increased AMPA receptor expression within the dorsal hippocampus using ex vivo autoradiography, which allows more regional resolution than the techniques used in previous studies. This investigation will allow for a further understanding of the effects of ketamine after 24 hours in normal animals. We hypothesized that ketamine would produce a dose-dependent improvement in spatial memory in rats, assessed by the NOP task. We also hypothesized that ketamine would produce an increase in AMPA receptor expression in the

dorsal hippocampus, which was assessed by autoradiographic binding experiments using [3H] AMPA.

Methods

Subjects

An a priori power analysis was conducted using G*Power 3.1 in order to estimate the number of animals required for this study. Assuming a one-way ANOVA with four treatment groups, a large effect size, an alpha of 0.05, and power of 0.8¬, this analysis suggested that 60 animals in total, or 15 per group, would be required for this study.

60 adult male Long Evans rats were purchased from Charles River Laboratories (Wilmington, MA). The animals were around 8-9 weeks of age upon arrival. All animals were pair-housed in a temperature and humidity-controlled room which was set at 68-78□F and 30-70% relative humidity. Animals had ad libitum access to food and water while in their cage– and were kept on a 12-hour light and dark cycle that began at 7 a.m. All procedures were conducted in accordance with the Guide to the Care and Use of Laboratory Animals, and were approved by the Montclair State University Institutional Animal Care and Use Committee (IACUC protocol 2020-065).

Apparatus

The novel object placement task and habituation were conducted in an open field apparatus which is shown in Figure 6. The model is a square arena (60 cm x 60 cm x 40 cm), which is constructed of blue Plexiglas (Maze Engineers, Boston, MA). There are black and white shapes placed on each wall of the arena in laminated paper (21.6 cm x 28 cm) that served as spatial cues. Figure 7 shows the objects for this apparatus consist of two opaque white plastic bottles with narrow necks and red plastic tops that are 2.69 inches in base diameter and 7 inches

in height were filled halfway with sterilized sand to reduce the chance of animals moving or knocking over bottle during behavior task. These bottles weighed about 450 grams each. An overhead camera mounted above the apparatus recorded video data and tracked locomotor activity for all trials using SMART video-tracking software (Harvard Apparatus, Holliston, MA). Videos were used to evaluate object exploration and locomotor activity.

Drugs and chemicals

Ketamine Hydrochloride (Ketamine HCl) was purchased from Sigma-Millipore (Burlington, MA). NBQX was purchased from Tocris Bioscience (Bristol, UK). [3H] AMPA was purchased from Perkin-Elmer (Waltham, MA).

Drug Administration

Ketamine HCl was dissolved in saline at a pH between 6 and 7 and was syringe filtered for sterility prior to injection. All injections were administered at a volume of 1 mL/kg of body weight. All animals were randomly assigned to receive vehicle, 3 mg/kg, 10 mg/kg, or 30 mg/kg ketamine HCl intraperitoneally (I.P.) 24 hours prior to the start of the information trial portion of the Novel Object Placement task (see below). Doses refer to the mass of the free base, rather than the salt.

Novel Object Placement Test

Handling. Figure 8 shows a timeline of the behavior procedures. When the animals arrived at the lab, they were allowed to acclimate to the vivarium environment without disruption for one week. The animals were then handled by a trained research assistant for about 5 minutes per day for a week.

Habituation. After the week of handling was completed, the animals were habituated to the open field for four consecutive days. During these habituation sessions, the animals were

allowed to freely explore the open field arena with only spatial cues inside for 10 minutes per day. The day after habituation was complete, vehicle or ketamine was administered in a randomized control manner as previously described.

Information Trial. The bottle placement for the object placement task is shown in Figure 9. Each animal began their information trial (IT) exactly 24 hours after injection with vehicle or ketamine. During this trial, the animals were allowed to explore the arena while the bottles are inside for five minutes. The bottles were placed 10 cm away from each wall of the two lower corners of the arena.

Inter-trial interval. Immediately after the completion of the information trial, rats were placed back in their home cage for a 10-minute inter-trial interval (ITI).

Retention Trial. Upon completion of the inter-trial interval, the retention trial (RT) began. During this trial, one bottle was placed in a novel location. The location and identity of the object in the novel location (i.e. left vs right) was counterbalanced across dose groups in a block-wise fashion. Additionally, the bottles and open field arena were cleaned using Virkon S disinfectant between each behavioral trial in order to eliminate olfactory cues.

Ex Vivo Autoradiography of [^{3}H] AMPA.

The effects of ketamine administration on AMPA receptor expression were investigated using ex vivo autoradiography of [^{3}H] AMPA, which is a tritium-labeled version of the drug for which AMPA receptors were named. AMPA receptor expression was evaluated in animals treated with vehicle or 10 mg/kg ketamine only. The 10 mg/kg ketamine dose was selected because it is the most common dose used in studies of this drug's antidepressant-like properties.

Tissue Preparation. Immediately upon completion of the Novel Object Placement task retention trial, animals were anesthetized using CO2 and killed by decapitation using a sharpened

guillotine. Brains were quickly dissected from the skull, hemisected at the sagittal sulcus, flash-frozen on dry ice, and stored at -20oC until use. Four replicate 12 μm thick coronal tissue sections were collected per brain and thaw mounted on glass microscope slides (SuperFrost Plus, Fisher Scientific, Hampton, NH) using a cryostat (ThermoFisher, Waltham, MA). 5 replicate slides were produced for each brain. Slices were collected from a region of the brain ranging from 2.4 to 2.9 mm posterior to Bregma (Paxinos and Watson, 1982), which is a region of the brain including the dorsal hippocampus. This region was chosen based on evidence that it is critical for the performance of the OP task (Barker & Warburton, 2011). Slides were stored in slide boxes with desiccant pellets at -20oC until use.

Binding Procedures. On the day of the binding experiment, slide boxes were be equilibrated to room temperature prior to opening, in order to ensure that no frost accumulates on tissue slices. Slides were preincubated for 20 minutes at 4oC in a buffer consisting of 30 mM Tris HCl and 2.5 mM CaCl2 (pH = 7.4). Subsequently, slides were dried at room temperature prior to incubation in an assay buffer consisting of 30 mM Tris HCl, 2.5 mM CaCl2, 100 mM KSCN, and 10 nM [^{3}H] AMPA at RT for 60 minutes. Nonspecific binding was determined in a subset of slides by adding 10 μM of the AMPA receptor antagonist NBQX (Ki = 60 nM; Ohmori et al, 1994). After binding is completed, slides were washed three times in cold preincubation buffer (4oC) for 60 seconds each. Slides were dried in a vacuum desiccator overnight before being apposed to a tritium-sensitive phosphor plate (Fuji imaging plate, GE Healthcare, Pittsburgh, PA) for 7 days. Finally, an autoradiographic image was taken using a Typhoon Biomolecular Imager (GE Healthcare, Pittsburgh, PA).

Data Analysis and Statistical Methods

Novel object placement task. Three trained researchers who were blind to treatment conditions evaluated object exploration time for each object from videos. An intraclass correlation (ICC) assuming fixed coders was performed for exploration time scores to measure interrater reliability. The intraclass correlation was high (see results section), so rater scores were averaged to produce the final object exploration times.

Object exploration was defined as facing the object with < 2cm distance, and interacting with or sniffing the object. Sitting next to or on top of the object was not considered exploration. Touching the object with paws while looking away from the object was also not considered exploration.

The primary dependent variable was each animal's preference for the bottle placed in the novel location during the retention trial. The preference score was calculated as follows: (amount of time spent exploring the object in the novel location)/(total amount of time spent exploring both objects) x 100.

We explored two secondary dependent measures from the NOP task: total exploration time (IT only), and distance traveled during the IT and RT trials.

Ataxia. Ataxia, which is defined as a loss of coordinated body movement, is a common behavioral side effect of subanesthetic doses of noncompetitive NMDA receptor antagonists. Thus, we recorded the frequency of ataxia as a proximal measure of the acute activity of the ketamine preparation. Ataxia was measured from 0-30 minutes after drug administration for all animals.

AMPA receptor binding. The primary biological dependent measure we evaluated was AMPA receptor binding in the dorsal hippocampus. Optical intensity values were quantified from the dorsal hippocampus using ImageQuant TL software (GE Healthcare, Pittsburgh, PA). The

primary dependent measure for AMPA receptor binding studies was bound radioactivity, measured in units of femtomoles (fmol) of bound radioligand / mg of tissue. A standard curve was generated by linear regression of intensity measurements from tritium standards (American Radiolabeled Chemicals, St. Louis, MO) included in the slide cassette during exposure. Bound radioactivity values for tissue were interpolated from optical intensity values from a standard curve using a method described previously (Pehrson et al, 2018).

Statistical analysis. Prior to inferential analysis, the Kolmogorov–Smirnov test was used to evaluate whether behavioral data deviated from normality, while the Brown-Forsyth test was used to evaluate whether assumptions of homogeneity of variance were violated. In cases where the data were normally distributed and variation was homogenous between groups, we conducted a one-way between-subjects ANOVA for each of the following dependent variables: novel object placement preference, total exploration time, and distance traveled in the NOP task. In cases where the data was not normally distributed, a Kruskal Wallis test was used.

Levine's test was used to test for violations of variance homogeneity in the AMPA receptor binding data. AMPA receptor binding data in animals treated with vehicle or 10 mg/kg ketamine was analyzed using an independent-samples t-test.

We conducted a chi-square test of trend (also known as the Cochran-Armitage test) to examine the relationship between ketamine dose and the frequency of ataxia during a 0–30-minute time period after drug administration.

All statistical analyses other than the Intraclass Correlation (ICC) and Levine's test were conducted using GraphPad Prism 8. Both the ICC and Levene's test were conducted in R (Appendix A). Alpha levels were set at 0.05 for all inferential analyses.

Results

Object Placement Task

Inter-rater reliability. We performed an ICC between three independent coders who rated object exploration in the NOP task using a single-fixed rater model. We found an ICC of 0.945 with the 95% confidence interval ranging from 0.94 to 0.95 for object exploration time. Thus, there was a high level of agreement between the independent coders on the amount of time individual subjects spent exploring the objects.

Omitted animals. One animal in the 10 mg/kg ketamine group was omitted from all further analyses because it received only a partial injection during drug administration. One animal in the vehicle condition was omitted from all analyses for knocking over a bottle in the RT. One animal in the 3 mg/kg group and another from the 30 mg/kg group were removed from all analyses regarding the IT for knocking over the bottle during the IT. Table 2 shows the final number of animals in each group that were used for each analysis.

Novel placement preference. Normality and homogeneity of variance test results for all analyses are shown in Table 3. Novel object placement preference scores are presented in Figure 10. The data in the analysis was normally distributed and variance was homogenous between all groups. A one-way between-subjects ANOVA was conducted to investigate the effect of ketamine on novel object preference 24 hours after drug administration. There was no significant effect of ketamine on novel object preference ($F(3, 54) = 1.980$, n.s., $\square 2 = 0 .099$).

Secondary dependent measures. Behavioral secondary dependent measures for the object placement task, including total exploration times and locomotor activity for the IT and RT, are presented in Figure 10. The data for total exploration and distance traveled in the RT was normally distributed and variance was homogenous between all groups. A one-way between-

subjects ANOVA showed no effect of ketamine on total exploration times in the IT ($F(3, 52) = 1.576$, n.s, $\square 2 = 0.083$). Another one-way between-subjects ANOVA revealed no effect of treatment on distance traveled in the RT ($F(3, 54) = 0.3380$, n.s, $\square 2 = 0.018$). The data collected on distance traveled in the IT did not meet the assumptions for a parametric test, so we conducted a Kruskal Wallis test. This test revealed ketamine had no effect on locomotor activity in the IT ($H(4) = 2.429$, $p = 0.4883$).

The frequency of ataxia was measured as a secondary dependent measure aimed at evaluating whether ketamine doses were acutely active. The data for this analysis was normally distributed and variance was homogenous between all groups. Ataxia frequency data is presented in Figure 11. A chi-square test of trend was performed to examine the relationship between ketamine dose and the frequency of the presence of ataxia from 0-30 mins after dose administration. The relationship between ketamine and ataxia was significant ($X2 (1, N = 59) = 32.05$, $p < 0.05$). There was a linear relationship between ketamine dose and frequency of ataxia.

AMPA Receptor Binding

Omitted animals. Two animals in the vehicle condition were removed from the AMPA receptor binding analysis because of damaged tissue. One animal in the 10 mg/kg ketamine group was omitted for partial injection. The final number of animals in this analysis is shown in Table 2.

AMPA receptor-specific binding. Figure 12 represents [^{3}H]AMPA binding in the dorsal hippocampus of animals treated with vehicle or 10 mg/kg ketamine. The data for this analysis was normally distributed and variance was homogenous between both groups. Two animals were omitted from the vehicle group in this analysis due to damaged tissue. An independent-samples t-

test found no significant difference ($t(26) = 0.01816$, n.s, $g = 0.007$) between vehicle- (M=66.38 fmol/mg; SD = 22.01) and ketamine-treated animals (M=67.28.56 fmol/mg; SD = 28.21).

Discussion

Based on extensive evidence implicating increased synaptic plasticity, AMPA receptor-mediated neurotransmission, and increased hippocampal LTP, we hypothesized that ketamine treatment would induce improvements in a hippocampus-dependent spatial memory task, the novel object placement task. In addition, we hypothesized that ketamine treatment would significantly increase AMPA receptor expression in the dorsal hippocampus. We found no evidence supporting these hypotheses. Ketamine treatment had no effect on novel object preference in the NOP task at 24 hours post drug administration, and there was no difference in AMPA receptor binding in the dorsal hippocampus of animals treated with vehicle or10 mg/kg ketamine. However, we found evidence of a dose-related relationship between ketamine and the presence of ataxia for at least 30 minutes after the injection, which is consistent with the acute activity of a noncompetitive NMDA receptor antagonist. Thus, the lack of observed changes in NOP task performance or AMPA receptor expression is not due to an inactive lot of ketamine or improper formulation.

Ketamine's effect on spatial memory tasks

These data are at odds with the results of several other papers in which the effects of ketamine on cognitive function were assessed. Shi et al. (2021) used animals in a normal cognitive state to investigate the effects of ketamine (20 mg/kg, i.p.) on spatial memory in the MWM. These researchers found that ketamine improved spatial memory performance 24 hours post-injection. Aleksandrova, Wang, and Philips (2019) also found that a subanesthetic dose of ketamine improves performance in a spatial memory task. They used Wistar-Kyoto (WKY) rats–

which are a model of endogenous stress susceptible to depression and cognitive impairments (Aleksandrova et al., 2019)–to investigate whether ketamine (5mg/kg, i.p.) improved performance in the novel object location recognition task (NOLRT) 24 hours post-injection. It is interesting that these researchers found that ketamine did not affect control animals with normal cognitive states. The dose used in Aleksandrova et al. (2019) is small compared to other studies investigating ketamine's effect in animals, though it may be possible that this dose is all that is needed to improve cognitive performance in impaired animals. In another stress model Yang, Weina, Haining, and Li et al. (2018) investigated ketamine (5mg/kg, 24 hr i.p.) effects on spatial memory in the Y maze spontaneous alternation spatial memory task in mice. The chronic social defeat stress model was used on all animals and the animals were grouped into either control or ketamine groups. The animals receiving ketamine treatment showed improved spatial memory performance.

There is also evidence that ketamine can improve cognitive functions that are dependent on the frontal cortex. Jett et al. (2015) used a chronic unpredictable stress (CUS) model, which is a common method to induce depression-like states in rodents. The authors found that CUS impaired animals' performance in the attentional set-shifting task (AST), which is a frontal cortex-dependent task that is analogous to the Wisconsin card sort task in humans. These impairments were reversed by a 10 mg/kg i.p dose of ketamine 24 hours prior to performing the task. Patton et al. (2017) found that chronic intermittent cold (CIC) stress model impaired performance in reversal learning, which is also a cognitive task dependent on the frontal cortex. A 10 mg/kg dose of ketamine reversed impaired performance in stress model animals. Parades et al. (2018) did the same study, but in female animals, and found that ketamine (10 mg/kg, 24 hr,

i.p) reversed impairment in reversal learning just like it did in male animals. In all of these studies, ketamine did not affect animals in the no-stress conditions.

There are some differences between the studies previously mentioned and the study in this paper. In some of the previous work that examines ketamine's effect on spatial memory, the researcher used spatial memory that is heavily dependent on training the animals. There are some possible methodological flaws to consider with memory tasks that require training because it is possible that performance in these tasks can involve other mechanisms beyond memory. Additionally, although our findings suggest ketamine may have no effect on spatial memory after 24 hours, our results are not completely different from previous findings that ketamine has no effect in normal animals, with the exception of Shi et al. (2021). Previous studies also show ketamine may produce its effect on improved cognitive function in a different region from the one we looked at in this study. Additionally, these findings may suggest that ketamine improves cognitive function in impaired animals, but does not affect their controls in normal states, may suggest that ketamine's effects on spatial memory 24 hours prior to task are limited to impaired animal models.

Ketamine's effect on AMPA receptor activity

Tizabi et al. (2012) investigated the effects of ketamine on AMPA receptor expression in the hippocampus of WKY and control rats. These authors used a chronic i.p dose of 0.5mg/kg for 10 days and then assessed AMPA receptor expression 20 hours after the last dose using autoradiography methods. These researchers found increased AMPA receptor binding in WKY rats but did not find any difference in AMPA receptor binding in their controls. These researchers used different dosing than we did. It is possible that chronic administration of a subanesthetic dose of ketamine is needed to make synaptic changes in the hippocampus. These findings are

also interesting because there's evidence that ketamine does not induce substantial occupancy of NMDA receptors at this dose (Fernandes et al., 2015). These findings could lend further support to the idea that ketamine's effects on AMPA expression do not depend on NMDA receptor antagonism. There is also evidence that a subanesthetic dose of ketamine increases glutamatergic activity in the prefrontal cortex (PFC). Li et al. (2010) investigated ketamine (10 mg/kg, i.p) effects on AMPA receptor levels in normal animals. They found that AMPA receptor levels in the medial PFC (mPFC) increased as early as 2 hours and lasted for at least 72 hours post-administration. Yang et al. (2016) used a chronic stress model to investigate ketamine's effects on AMPA receptors. These researchers collected brain tissue eight days after administration and found that ketamine (10 mg/kg, i.p) attenuated the stress-induced reductions in AMPA receptor levels. The researchers all assessed AMPA receptor activity using western blot analyses of the whole area mentioned. These findings are contrary to our findings of ketamine's effects on AMPA receptors. It is possible that ketamine produces changes in synaptic plasticity in the cortex instead of the hippocampus although it is interesting that Yang et al. (2016) found ketamine attenuates the reduction in AMPA receptor levels in the hippocampus of chronic stress animals as well.

However, other published studies have found no differences in AMPA receptor expression in the hippocampus. Maeng et al. (2008) found no differences in AMPA receptor expression in the hippocampus of normal rats treated with ketamine, which is similar to our findings. However, these researchers also found that ketamine reduced phosphorylation at S845 at GluA1 which may increase AMPA receptor activity (Banke et al., 2000) and regulate synaptic plasticity mechanisms (He et al., 2011). Although the findings of no change in AMPA receptor expression align with what we found in our study it is important to highlight some limitations of

this study. The researchers are not clear at which time point they investigate ketamine's effects on AMPA receptor expression, though the behavioral tasks assessing antidepressant-like effects were at 30 mins, 24 hours, and 2 weeks post-drug administration. If the researchers investigated ketamine's effects on AMPA receptor expression at 30 mins post-injection that could be the reason why no difference was detected. Although 30 minutes post-injection is considered an acute timeframe, ketamine induces ataxia, hyperlocomotion, and other dissociative effects at this time point. This group also state that they use 0.5 mg/kg, 2.5 mg/kg, and 10 mg/kg doses but do not say which dose(s) affected AMPA receptor expression. Interestingly, Shen et al. 2018 found that ketamine (10 mg/kg, i.p) increases phosphorylation at S845 on GluA1 receptors in the PFC 24 hours after drug administration in normal animals as well. These findings may also suggest that ketamine's effect on AMPA receptor binding may be limited to impaired animal models.

In another experiment, Nosyreva et al. (2013) found that ketamine (5 mg/kg, i.p) increased AMPA receptor surface expression in the hippocampus of normal animals three hours post-injection. These authors used biotinylation methods to examine AMPA receptor surface expression which assesses the whole hippocampus homogenate. Beurel, Grieco, Amadei, Downey, and Jope (2016) also found that ketamine (10 mg/kg, i.p) increases surface expression of AMPA receptors in normal animals at 30 minutes post-injection. It is interesting to note that Nosyreva et al. (2013) and Beurel et al. (2016) were able to detect changes in animals that are not impaired.

The previously mentioned findings in other studies looking at AMPA receptor activity after a subanesthetic dose of ketamine may differ from our study because of the difference in methods used to assess AMPA receptor activity. In this study, we used autoradiography methods which only allow for the detection of increased overall expression, not changes in surface

expression. It is possible that this may be the way ketamine affects AMPA receptors in the hippocampus. Additionally, in previous studies, the whole hippocampus was homogenized to assess AMPA receptor binding, which differs from our study that looks specifically at the dorsal region of the hippocampus. Another difference between our study is the time timepoint at which AMPA receptors were assessed. Nosyreva et al. (2013) looked at ketamine's effect 3 hours post-injection, which may be a more relevant timepoint where changes in AMPA receptors can be detected compared to 24 hours. Although it should be noted that some studies looked at ketamine's effect on AMPA receptors at 30 mins post-injection. As stated previously, ketamine induces ataxia, hyperlocomotion, and other dissociative effects at this time point. It is crucial that the mechanisms important for ketamine's effects on cognitive function are separated from its mechanisms in which it produces its undesirable effects upon administration.

Limitations

There are some limitations of this study that should be noted. One of the first limitations of our study is that we did not use animal models of depression or aging. Although many studies investigating ketamine's rapid and sustained effects commonly used animals in a normal state, it is possible that ketamine's effects are limited to improving impaired synaptic mechanisms. Another limitation of our study is our ability to detect changes in AMPA receptor expression. The autoradiography methods we used to detect total changes in AMPA receptor expression or changes in AMPA receptor affinity. These methods will not detect changes in surface expression. Our methods of detecting AMPA receptor expression also differ from many other studies that use western blot analysis because these studies examine the whole hippocampus while we only looked at the dorsal region of the hippocampus. Although this is a limitation of these findings, these methods are useful for differentiating the area(s) in the hippocampus that may be involved

in ketamine's effect. Our study is also limited to detecting changes in the hippocampus. There is a strong amount of evidence that ketamine's rapid changes in synaptic plasticity occur in the prefrontal cortex. It is possible that changes in AMPA receptor expression happen in the cortex. Lastly, another limitation of this study was the sample size. We expected ketamine's effect to have a large effect size, but since the effect size is very small, we did not have enough animals to detect a difference if there was one.

Future Directions

Currently, we have ongoing studies looking at ketamine's effects on AMPA receptor binding and phosphorylation states of subunits in the hippocampus–which may control surface expression of AMPA receptors. Future studies in this lab will investigate ketamine's effect on animal models that show depressive-like behaviors or cognitive impairments in chronic stress models– which is the most common biological model of depression and cognitive impairment in use. We will also investigate the effect of subanesthetic doses of ketamine on AMPA receptor expression in the frontal cortex and on frontal cortex-dependent behavior tasks. Additionally, considering BDNF's effects on AMPA activity and its relationship with ketamine, future studies in the lab will also investigate the expression of BDNF after ketamine treatment. Lastly, based on our current findings, we will power future studies investigating ketamine's effect for a small effect size.

Conclusion

All in all, we found no effect of ketamine on hippocampal spatial memory performance and hippocampal AMPA receptor binding. These findings are at odds with some of the literature on the effects of a subanesthetic dose of ketamine. There are a few limitations of this study that we plan to address in future studies. There is a need for further investigation into

ketamine's behavioral effects and the mechanisms underlying them in order to find treatments that are effective and efficient for individuals who suffer from cognitive impairments.

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Table 1

Overview of Specific Roles of the Dorsal and Ventral Hippocampus

Lesion Area	**Behavioral Task**	**Results**	**Manuscript**
Whole hippocampus	Spatial Memory	Impaired performance	Moser et al. (1993)
	Spatial Memory	Impaired performance	Richmond et al. (1999)
	Fear Conditioning	Impaired performance	
Ventral Hippocampus	Spatial Memory	Intact performance.	Moser et al. (1993)
	Spatial Memory	Intact performance	Richmond et al. (1999)
	Fear Conditioning	Impaired performance	
Dorsal Hippocamps	Spatial Memory	Impaired performance	Moser et al. (1993)
	Spatial Memory	Impaired performance	Richmond et al. (1999)
	Fear conditioning	Intact performance	

Note. This table is an overview of the data on the different roles the dorsal and ventral hippocampus play in cognitive functions. Lesions to both regions impair spatial memory and fear conditioning performance in animals. Lesions to the dorsal hippocampus impair spatial memory performance in animals, while lesions to the ventral hippocampus impairs fear conditioning performance in animals. These results show the specificity of the two regions in the hippocampus for different cognitive tasks.

Table 2

Final Sample Sizes by Experimental Group and Dependent Variable

Analysis	Group			
	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Novel object preference	14	15	14	15
Exploration time	14	14	14	14
Distanced traveled (IT)	14	14	14	14
Distance Traveled (RT)	14	15	14	15
Ataxia	15	15	14	15
AMPA receptor binding	13	N/A	14	N/A

Note. This table displays the final number of animals in each group per analysis.

Table 3

Results for Tests of Normality and Homogeneity of Variance.

Variable	Test	Statistic	*p*-value
Novel object preference	Kolmogorov–Smirnov	0.07	0.1
	Brown-Forsythe test	0.06	0.979
Exploration time	Kolmogorov–Smirnov	0.07	0.1
	Brown-Forsythe test	0.91	0.44
Distanced traveled (IT)	Kolmogorov–Smirnov	0.21	< 0.05*
	Brown-Forsythe test	0.84	0.48
Distanced traveled (RT)	Kolmogorov–Smirnov	0.06	0.1
	Brown-Forsythe test	0.62	0.61
AMPA receptor binding	Kolmogorov–Smirnov	0.14	0.1
	Levene's test	0.43	0.52

Note. This table displays the results from the normality tests that we ran before running our parametric and non-parametric tests. * Indicates significant value. Information trial (IT). Retention trial (RT)

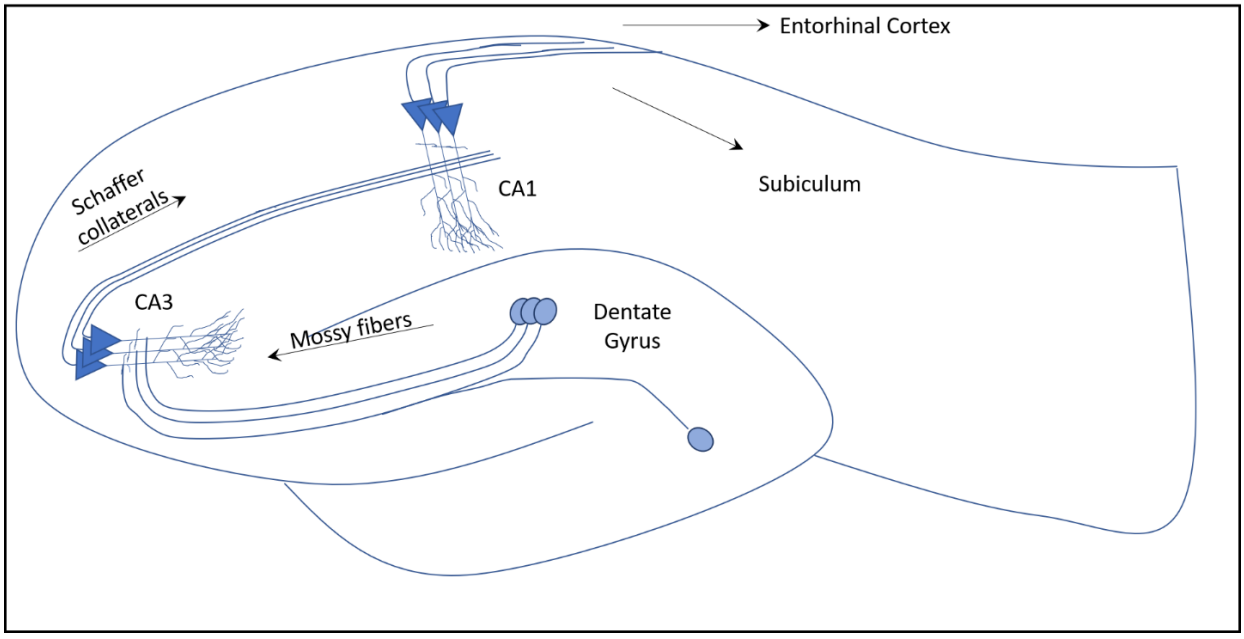

Figure 1. The Circuitry of the Hippocampus. The trysynaptic loop consists of the entorhinal cortex, dentate gyrus, and the *Cornu Ammonis (CA).* The dentate gyrus sends mossy fibers to the CA3 region. The CA3 region sends Schaffer collaterals to the CA1 region. Axons from the CA1 regions project to the entorhinal cortex and subiculum.

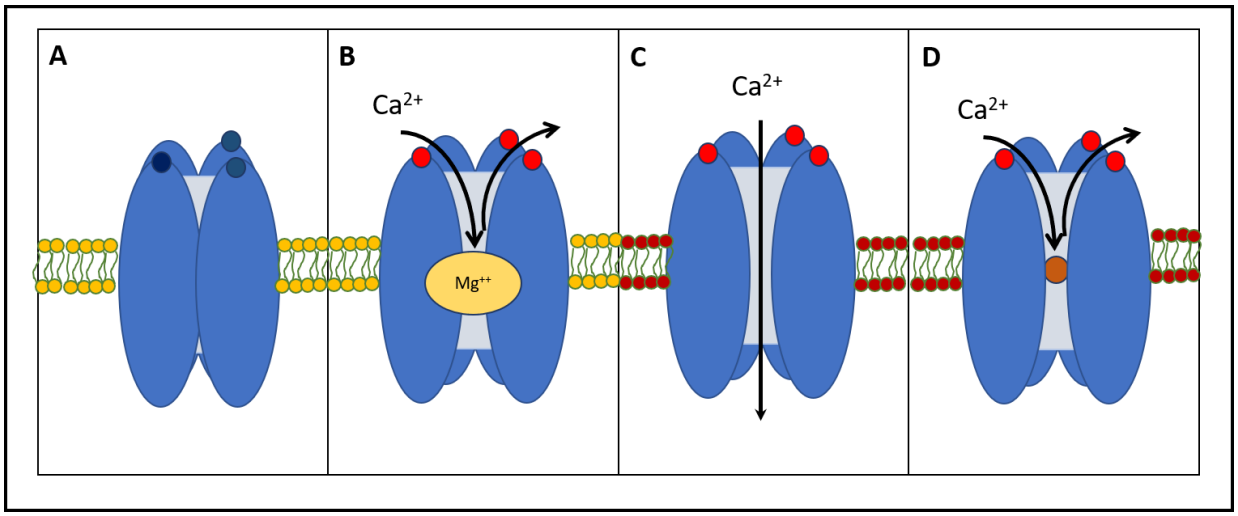

Figure 2. N-methyl-D-aspartate (NMDA) Receptor Activation and Ion Flow Properties. **A.** The NMDA receptor is closed until glycine and glutamate bind to their respective binding sites. **B.** Once the channel is open, there is a voltage-gated magnesium block that blocks the flow of ions in and out of the cell. **C.** When the cell is depolarized the magnesium block is removed and ions are able to flow in and out of the cell. **D.** Ketamine's binding site is behind the magnessium block. When ketamine is bound to the receptor it blocks the flow of ions.

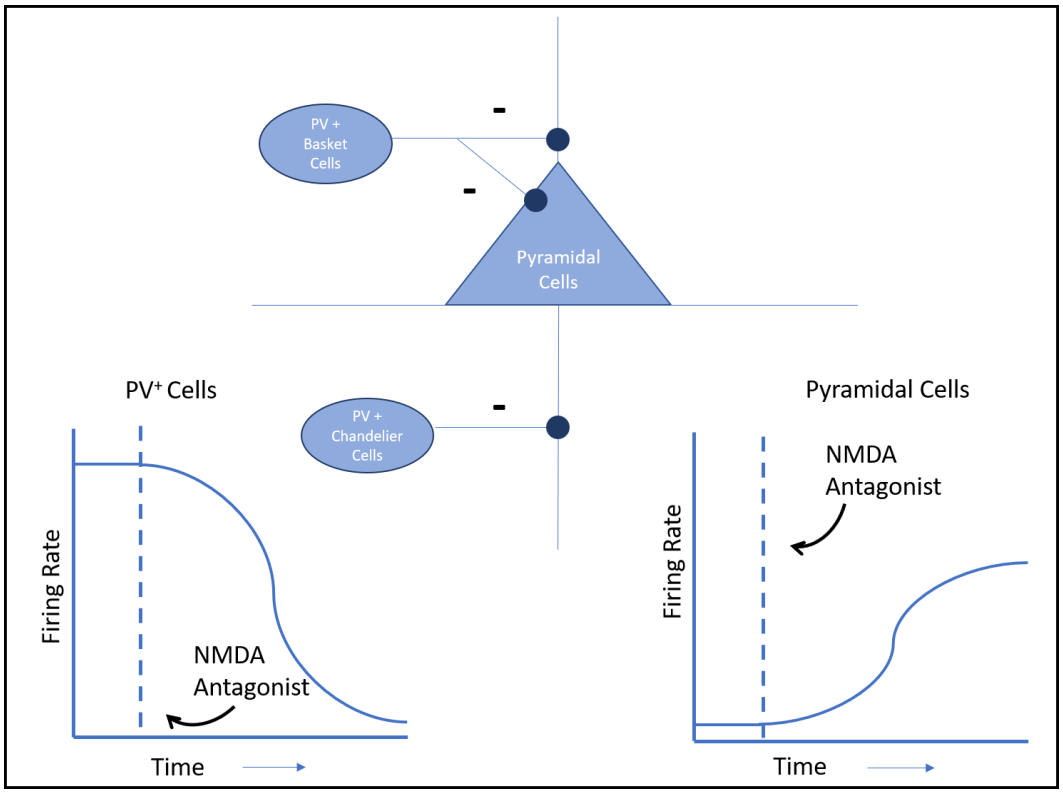

Figure 3. Pyramidal Cell Disinhibition by Noncompetitive Antagonists of N-methyl-D-aspartate (NMDA) receptors. Fast-spiking parvalbumin-positive interneurons inhibit the firing of hippocampal pyramidal cells and express NMDA receptors. When a noncompetitive NMDA receptor antagonist such as ketamine binds to the NMDA receptor, the firing rate of the interneurons is reduced, which causes a downstream increase in pyramidal cell firing.

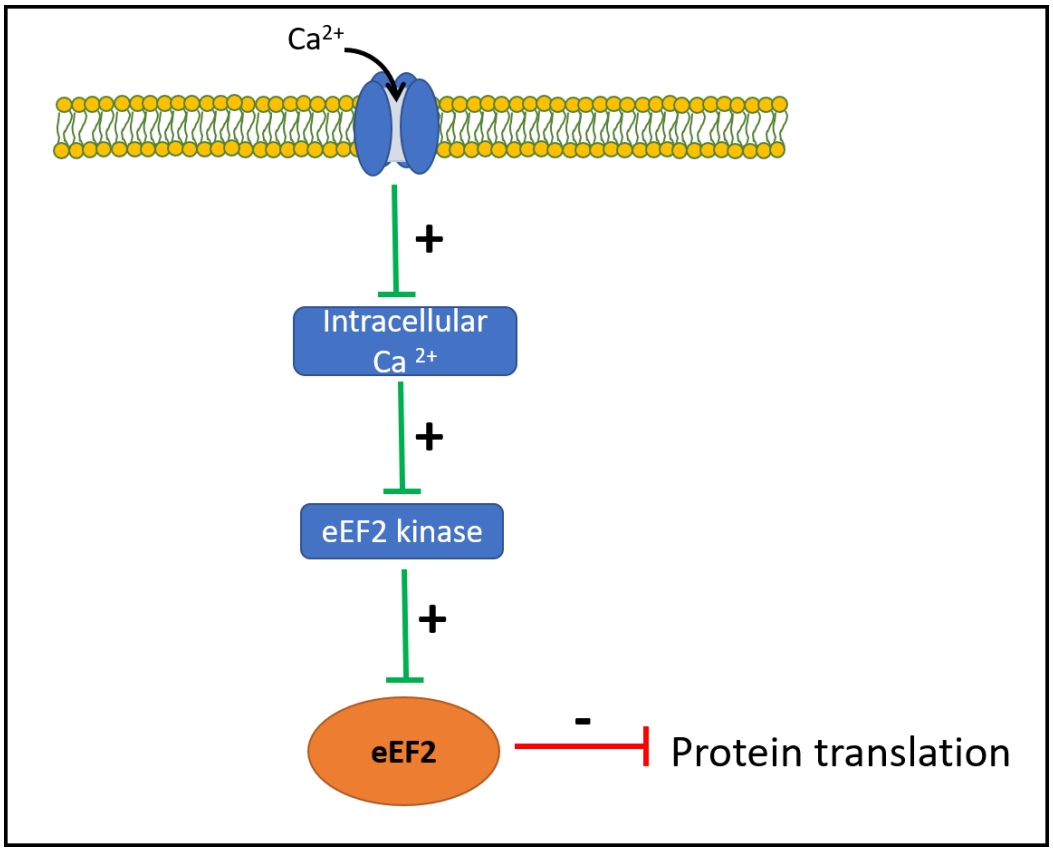

Figure 4. Eukaryotic elongation factor 2 (eEF2) Regulation of Protein Synthesis. Activation of ionotropic receptors such as the *N*-methyl-D-aspartate (NMDA) receptor increases intracellular calcium concentrations. This increase in calcium causes an increase in eEF2 kinase activity, and finally increases eEF2 activity. When eEF2 is active, local protein translation is suppressed. Ketamine blocks calcium from entering the cell via NMDA receptors which reduces the cell's intracellular calcium-ultimately increasing protein translation.

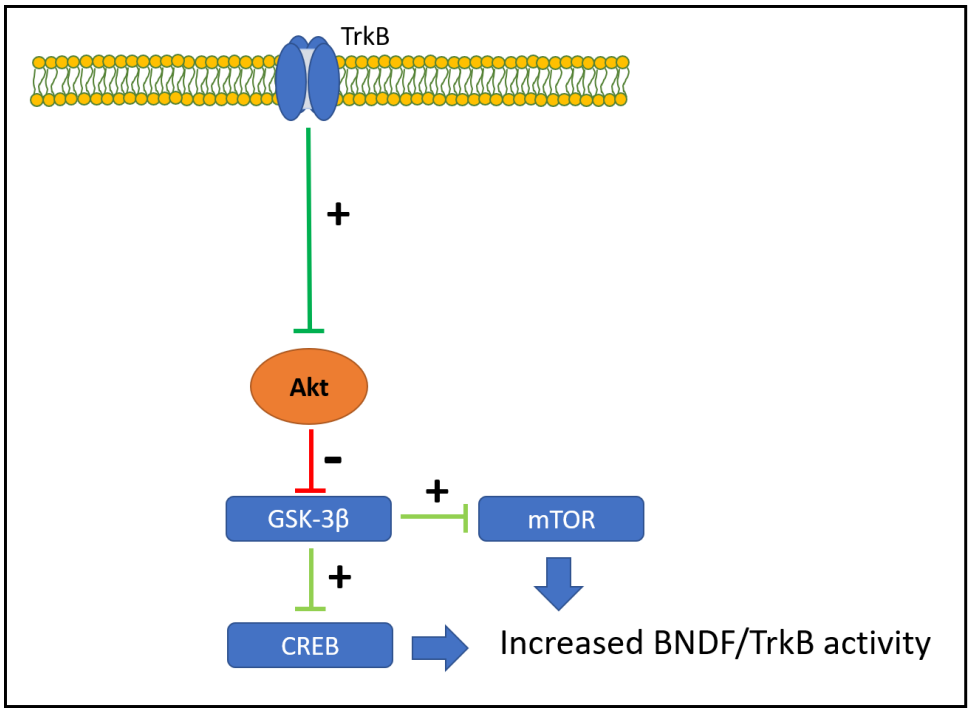

Figure 5. Glycogen synthase kinase 3 beta (GSK-3B) Regulation BDNF/TrkB Activity. When BDNF binds to TrkB the phosphorylation of protein kinase B (Akt) is increased. This in turn increases the phosphorylation of GSK-3B, which reduces its activity. The reduction in GSK-3B activity causes increases in the activity of mTOR and CREB, which increases BDNF/TrkB activity.

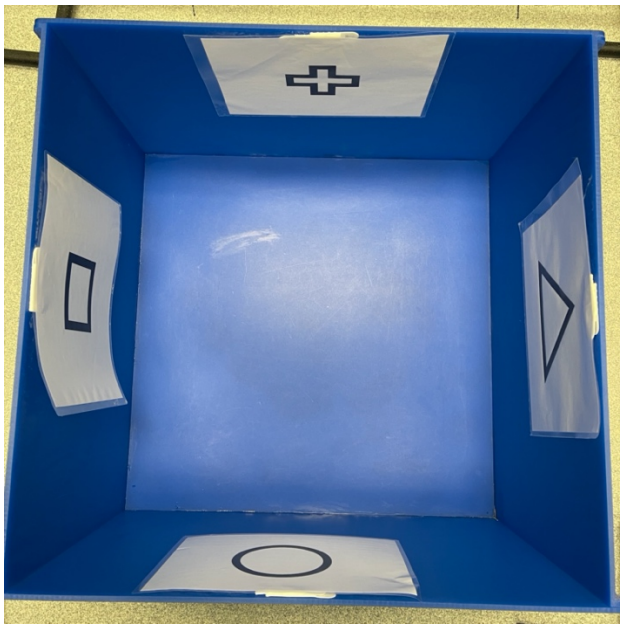

Figure 6. Open Field Apparatus. The open field arena is 60 cm x 60 cm x 40 cm and constructed of blue Plexiglas. The arena consists of black and white shapes placed on each wall of the arena in laminated paper (21.6 cm x 28 cm) that served as spatial cues.

Figure 7. Novel Object Placement (NOP) Task Bottles. These identical bottles were used in the NOP task during the IT and the RT. They are made of plastic and are 2.69 inches in base diameter and 7 inches in height. Once they were filled with sterilized sand, they weighed 450 grams. The bottles on the left were used for the IT and the bottles on the right were used for the RT. We the same two bottles for the trials they were assigned to, to prevent rats from scent-marking the objects.

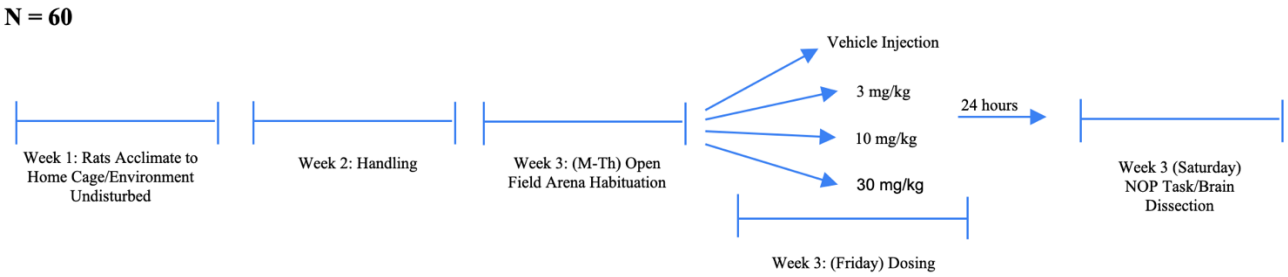

Figure 8. Timeline of study procedures. All animals were habituated to their environment, handled, and habituated in the open field arena upon arriving to the lab. Animals were then dosed 24 hours prior to performing the NOP task and brain dissection.

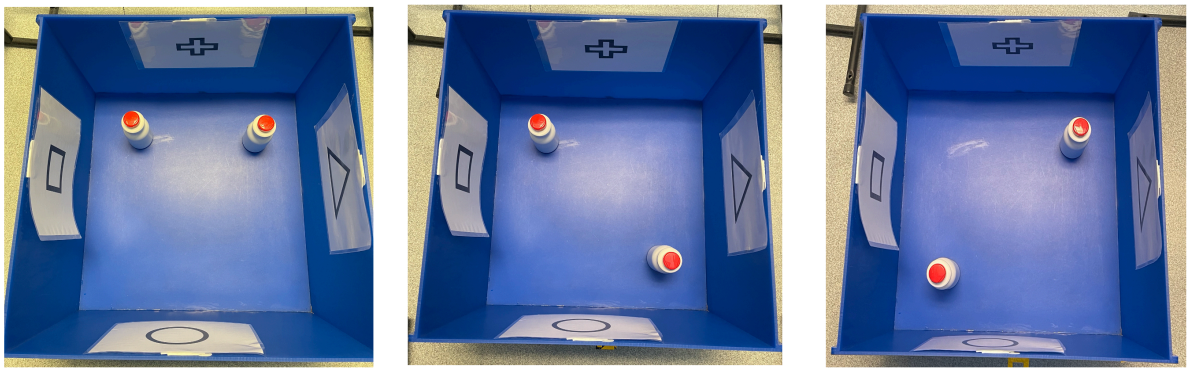

Figure 9. Novel Object Placement Task. These pictures depict the bottle placement in the open field arena during the IT and RT in the NOP task. The picture on the far left shows the IT of the task. The middle picture and the picture on the far right show the RT of the task. Each animal did one IT and one RT. The novel object placement was counterbalanced in the RT so that half of the animals performed the RT where the right bottle was moved, and the other half of the animals performed the RT where the left bottle was moved.

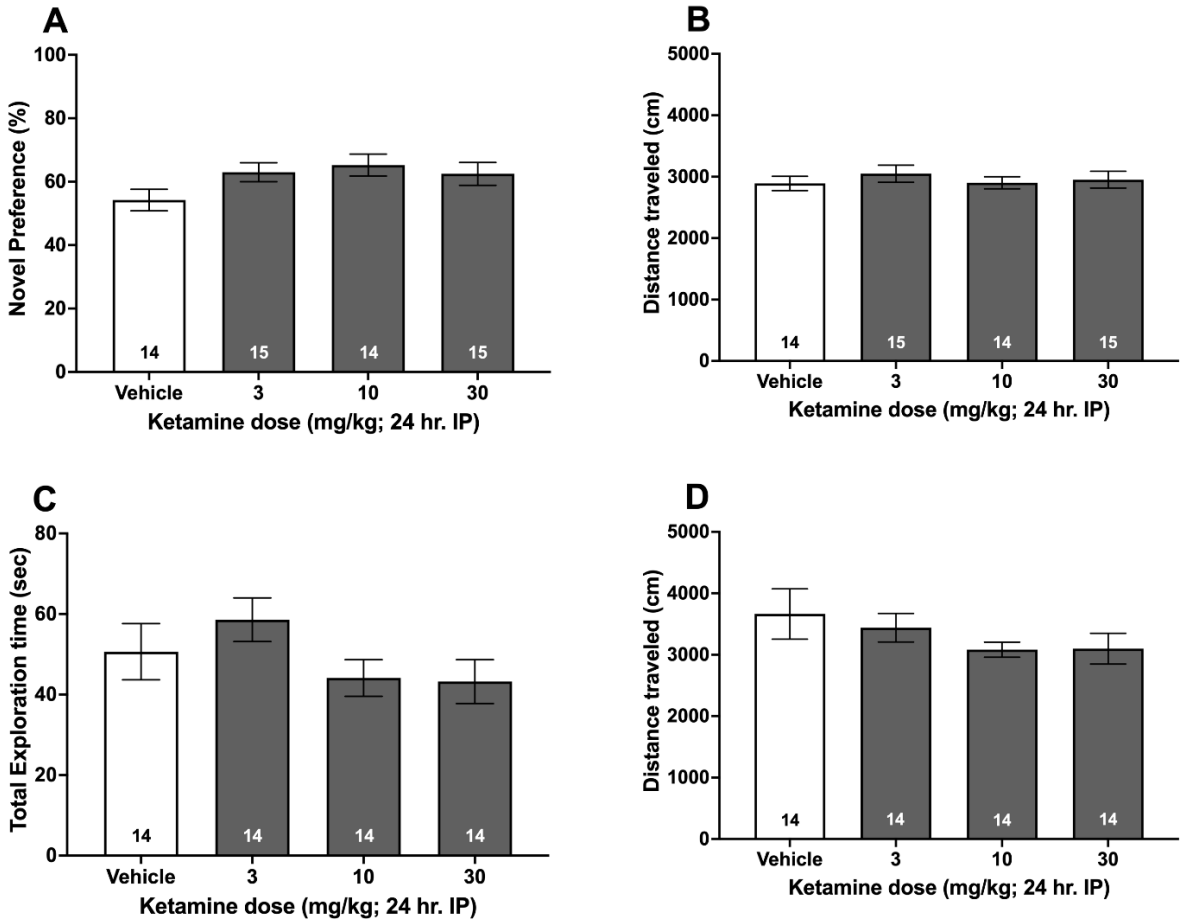

Figure 10. The Effects of Ketamine Treatment on Novel Placement Preference Scores, Locomotor Activity, and Total Exploration in the Novel Object Placement Task 24 Hours After Administration. Data are represented as Mean ± SEM. **A.** Ketamine treatment had no significant effect on novel object preference scores. **B.** Ketamine treatment has no effect on locomotor activity in the RT. **C.** Ketamine treatment had no significant effect in total exploration times in the IT. **D.** Ketamine treatment had no effect on locomotor activity in the IT.

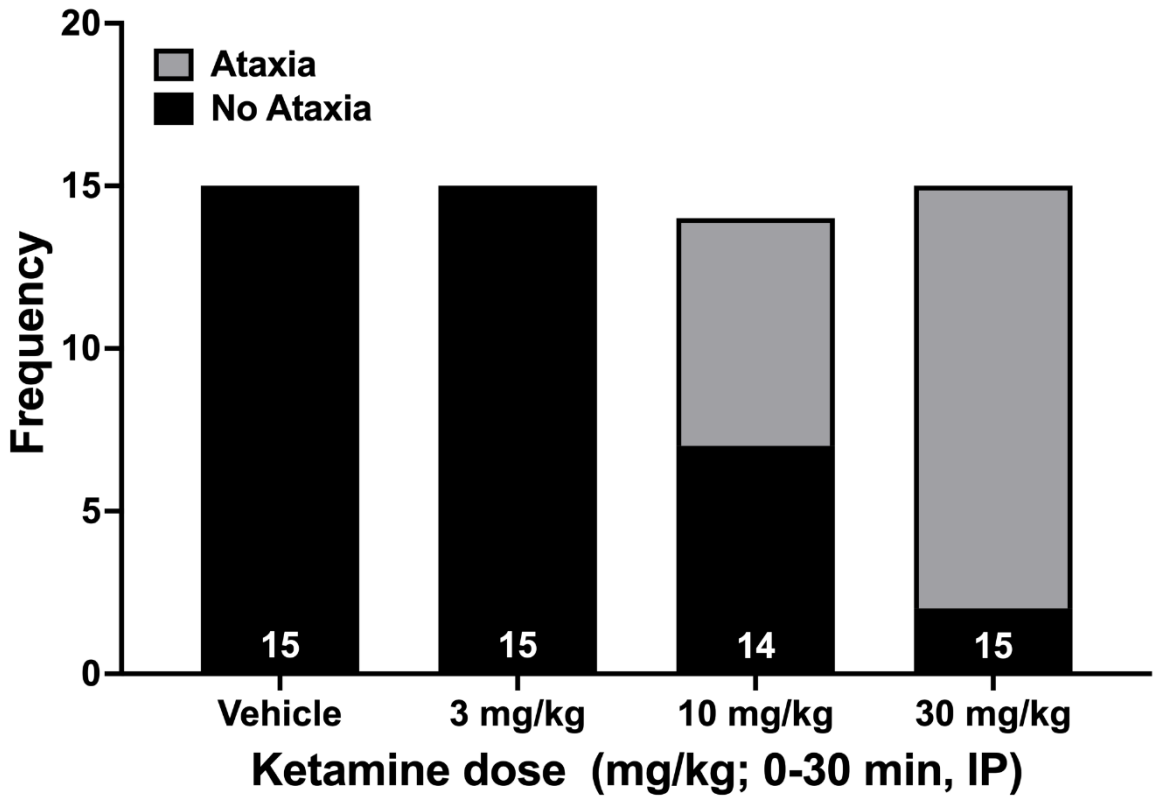

Figure 11. The Effects of Ketamine Treatment on the Frequency of Ataxia from 0-30 Minutes Post-Administration. Data are represented as the observed frequency of ataxia or no ataxia. Ketamine treatment caused a dose-dependent increase in the frequency of ataxia present in rats at the timeframe of 0-30 minutes post-injection. One animal was removed from the 10 mg/kg dose group because it only received a partial dose.

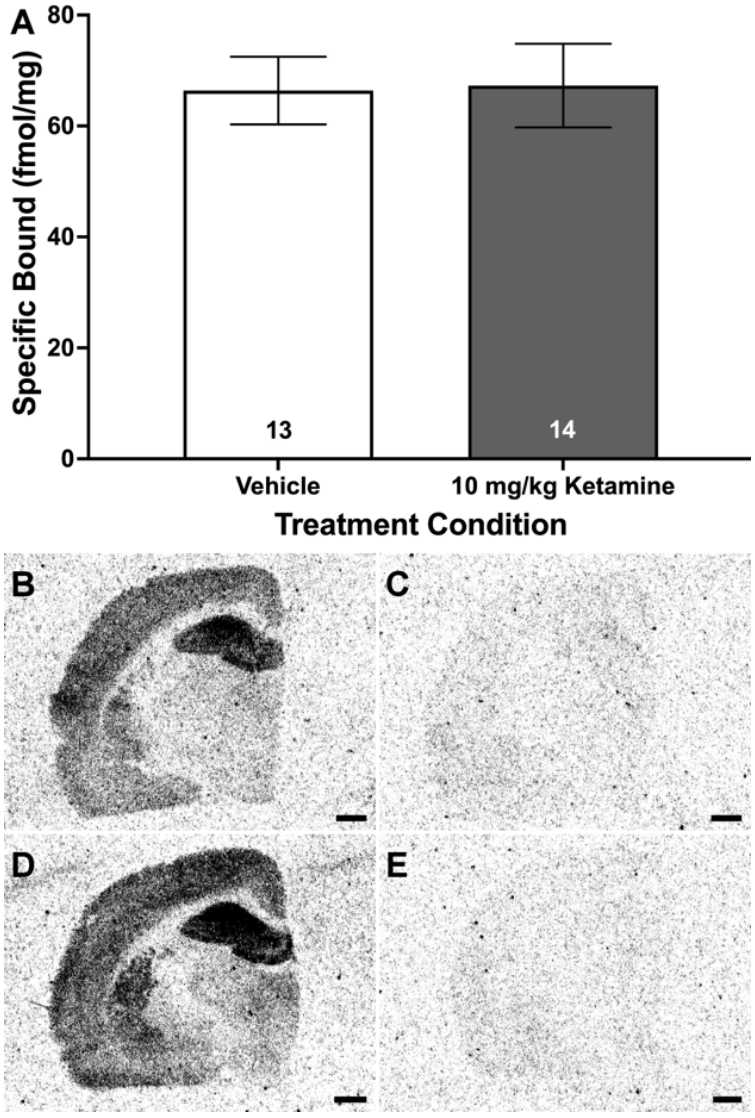

Figure 12. The Effects of Ketamine Treatment on [^{3}H] AMPA Binding in the Hippocampus 24 Hours Post Administration. **A.** Data are represented as Mean ± SEM. Ketamine treatment produced no significant difference in AMPA receptor binding between the vehicle- and ketamine-treated animals. **B.** Total binding vehicle animal. **C.** Non-specific biding vehicle animal. **D.** Total Binding 10 mg/kg animal. **E.** Non-specific binding 10 mg/kg animal. Scale bars in panels B-E represent 1 mm.

APPENDIX A

R Code for Levene's test and Intraclass Correlation Coefficient

```
R code for Levene's test (homogeneity of variance for AMPA binding)
library(car)

data <- read.csv(file.choose())

leveneTest(AMPA_binding ~ Condition, data=data)

# Levene's Test for Homogeneity of Variance (center = median)
#       Df      F value  Pr(>F)
# group  1,25  0.4345  0.5158

R code for ICC data
# This analysis will examine initial interrater reliability in the object placement assay.

# Dependencies
library(tidyverse)
library(psych)

Interrater_data <- read_csv("Ketamine op data formatted for ICC.csv")

data_only <- Interrater_data %>% select('Coder1', 'Coder2', 'Coder3')

data_complete <- data_only %>% drop_na()

ICC(data_complete)

# using the single fixed raters model, the ICC score is 0.95, with the 95% CI
# ranging from 0.93-0.96

# Intraclass correlation coefficients
# type  ICC  F df1 df2        p lower bound upper bound
# Single_raters_absolute   ICC1 0.92 38 233 468 9.1e-212        0.91        0.94
# Single_random_raters     ICC2 0.93 53 233 466 7.5e-244        0.87        0.95
# Single_fixed_raters      ICC3 0.95 53 233 466 7.5e-244        0.94        0.95
# Average_raters_absolute ICC1k 0.97 38 233 468 9.1e-212        0.97        0.98
# Average_random_raters   ICC2k 0.97 53 233 466 7.5e-244        0.95        0.98
# Average_fixed_raters    ICC3k 0.98 53 233 466 7.5e-244        0.98        0.98
#
# Number of subjects = 234     Number of Judges =  3
```