EFFECTS OF D-SERINE ON VISUAL WORKING MEMORY IN MACAQUE MONKEYS

by

Jaishri Manjunath

A thesis submitted to the Centre for Neuroscience Studies
In conformity with the requirements for
the degree of Master of Science

Queen’s University
Kingston, Ontario, Canada
(September, 2013)

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Abstract

Schizophrenia is characterized by positive and negative symptoms along with cognitive symptoms that include impairment in working memory (WM). WM is the storage of relevant information for short intervals of time to guide thoughts and actions. The neural correlate of WM is thought to be the persistent activity exhibited during the retention interval of WM tasks. Persistent activity is hypothesized to be mediated by the activation of NMDA receptors (NMDAR) within recurrent neuronal circuits.

Consistent with this hypothesis, studies with healthy humans and monkeys have shown that the administration of the NMDAR antagonist ketamine induces memory-load dependent deficits in WM, along with increasing response time. In parallel to this, the pathophysiology of schizophrenia has been hypothesized to rest on the hypofunction of NMDAR. Previous studies in humans indicate that blockade of NMDAR induces schizophrenia-like symptoms. In addition, symptoms of schizophrenia patients are alleviated with sub-chronic treatments focusing on the activation of the NMDAR co-agonist site. Based on these observations, I tested the hypothesis that increasing the activation of NMDAR with co-agonist stimulation has beneficial effects on WM. D-serine (100mg/kg/day-6 weeks) was orally administered to two female macaque monkeys performing a visual sequential comparison task (VCST), which allows the manipulation of memory load. In this task, the monkeys had to identify the location of a colour change within an array of 2 to 5 coloured stimuli following a retention interval of 1 second. I hypothesized that sub-chronic treatment with D-serine produces a gradual improvement in
the monkeys’ performance on the VSCT. Specifically, I predicted that the improvement would scale with memory load due to increased demands on WM resources at higher loads.

Contrary to my hypothesis, D-serine produced minute changes in response accuracy, which were not memory load-dependent. Also, the response latency of the monkeys was found to increase, which is commonly observed following NMDAR antagonist treatments. These findings suggest that D-serine has a limited role in increasing the activation of NMDARs to improve WM per se. The beneficial effects reported by NMDAR co-agonists in schizophrenic patients could be a general reduction in cognitive symptoms, not specifically related to WM.
Co-Authorship

Dr. Martin Paré was the principal investigator and supervisor for the studies described in this thesis. Dr. Paré was responsible for the planning and implementation of research protocols. I was responsible for the majority of the data collection and data analysis. I composed this thesis in its entirety, with constructive criticism and editing provided by Dr. Martin Paré.
Acknowledgements

I would like to thank Dr. Martin Paré, for his continued support and never ending patience while guiding me for the past two years. Being an international student, I came here with much apprehension and his constant support motivated me to pursue this degree with confidence.

I want to thank all the members of the Paré lab for their continuous scientific and personal support, whenever I needed them. I want to thank Valérie Barrette and the entire veterinary and ACS staff for their outstanding expertise and help with the animal care. I would also like to thank Clara Li and Valérie Barrette for their valuable feedback during the iterations of this work.

I want to thank all my friends and my family for their continued support.
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<tbody>
<tr>
<td>BPRS</td>
<td>Brief Psychiatric Rating Scale</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<td>DAO</td>
<td>D-Amino acid Oxidase</td>
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<td>DLPFC</td>
<td>Dorsolateral Pre-frontal cortex</td>
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<td>ERP</td>
<td>Evoked Response Potential</td>
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<tr>
<td>GlyT1</td>
<td>Glycine Transporter 1</td>
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<tr>
<td>GlyT1-I</td>
<td>Glycine Transporter 1 - Inhibitor</td>
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<td>MATRICS</td>
<td>Measurement and Treatment Research to Improve Cognition in Schizophrenia</td>
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<td>NMDAR</td>
<td>NMDA receptors</td>
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<td>PANSS</td>
<td>Positive and Negative Syndrome Scale</td>
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<tr>
<td>PCP</td>
<td>Phencyclidine</td>
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<td>PFC</td>
<td>Pre-Frontal Cortex</td>
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<td>PhMRI</td>
<td>Pharmacological fMRI</td>
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<tr>
<td>PPC</td>
<td>Posterior Parietal Cortex</td>
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<td>PT</td>
<td>Post-test</td>
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<tr>
<td>SANS</td>
<td>Scale of Assessment of Negative Symptoms</td>
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<td>SS2-SS5</td>
<td>Set Sizes 2 to 5</td>
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<tr>
<td>T</td>
<td>Test</td>
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<tr>
<td>VCST</td>
<td>Visual Sequential Comparison Task</td>
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<td>WM</td>
<td>Working memory</td>
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Chapter 1

Introduction

1.1 Working Memory

Working memory (WM) is a fundamental cognitive function by which items are retained for a very short time interval and used to guide thoughts and actions (Baddeley, 1992; Goldman-Rakic, 1995). An example of working memory is remembering to pick up an item while shopping at the store or remembering the names of people who were just introduced. Baddeley and Hitch (1974) proposed a model of working memory, which comprises of three components: a first component for storing visual images, a second component for storing verbal information and a third main component called the ‘central executive’ that integrates information from the former two systems. Working memory is different from long-term memory in which the memory of an item is permanently stored. Instead WM acts as a link between long-term memory, perception and action (Baddeley, 2003)

One of the key features of WM is that items are retained for a very small time interval, during which the information is relevant. In the first example of working memory mentioned above, the item is remembered only until it has been picked up from the store, after which it becomes irrelevant and is no longer retained. In fact, the previous item might be updated to the next item that has to be picked up. Hence, WM is the maintenance of only relevant information, in real-time. Baddeley (1992) describes it to be similar to a ‘mental sketchpad’, in which items are remembered until they are important and afterwards
are erased. Another key property of working memory is that only a few items can be retained at a time. This was shown in humans using sequential comparison tasks, in which they had to compare and indicate if an initial array of stimuli was different from another closely matching array of stimuli presented over a retention interval (e.g., Sperling, 1960; Luck and Vogel, 1997). The arrays to be compared comprised of letters, numbers (e.g., Sperling, 1960), items of different colours, orientations (e.g., Luck and Vogel, 1997) or patterns (Pashler, 1988). During these tasks it was observed that as the number of items to be compared increased, the performance of the subjects decreased (e.g., Sperling, 1960). Miller (1956) suggested that humans can retain up to seven items at a time. Sperling (1960), being one of the initial few to demonstrate both the key features of WM i.e., limited capacity and short-time span, reported that subjects can retain around four items in memory and increasing the retention interval between the initial presentation of the stimulus and response resulted in a reduction in task performance.

Studying WM in animal models helps to understand its neural basis. Animal models such as non-human primates have been used for several years. To study WM, we have implemented the visual sequential comparison task (VSCT) in monkeys and have demonstrated that they are capable of retaining more than one item in their memory (Heyselaar et al., 2011). In this task, the monkeys were required to compare two arrays of stimulus, presented on a visual display one after the other with a brief retention interval in between. The arrays were composed of 2 to 5 coloured squares and differed from each other by one coloured square in the second array. The monkeys have to first identify the colour change in the second array by comparing it with the first array stored in their
working memory and then indicate this change by making a saccade towards the location of the changed stimulus. A retention interval of one second was used in this study. The task used by Heyselaar et al. (2011) was based on the VSCT used by Luck and Vogel (1997) in humans, in which they estimated that humans have a capacity limit of approximately 3 to 4 items. Heyselaar et al. (2011) identified that monkeys’ task performance was similar to that of humans and that they could retain more than one item in working memory. Subsequent studies have reported a decrease in the monkeys’ task performance with increasing retention intervals, suggesting that the monkeys were using WM to do the task (Heyselaar et al., 2009; Oemisch, 2011). These findings support the use of monkeys as an animal model to study the neural mechanisms underlying WM.

1.2 Neural Basis of WM

Persistent activity is hypothesized to be the neural correlate of WM. This is based on neural recordings made in monkeys while performing delayed response tasks (e.g., Fuster and Alexander, 1971; Kubota and Niki, 1971). In these tasks, the monkeys are seated in a chair placed in front of wooden blocks which they can reach out to. The wooden blocks have two identical wells, one of which was baited and covered by the experimenter in full view of the monkey. After the retention interval, the monkey is signaled to retrieve the bait and if the monkey chooses the baited well, it receives a reward. As the baited well is randomized, the monkey has to update its memory of the item for each trial, allowing working memory to be studied. Neuronal recordings from these studies showed persistent activity in the pre-frontal cortex (PFC), during the entire retention interval until the response was executed. This activity was hypothesized to be the neural correlate of the
memoranda. Further studies using oculomotor delayed response task, another variant of the classic delayed response task, attempted to characterize the features of persistent activity (e.g., Funahashi et al., 1989). In this task, the monkey has to fixate on a central light presented on a display during which a cue is presented in its periphery. The monkey has to remember the location of this cue and, following the removal of the cue and the fixation point, make a saccade towards the cue location. Using this task, it was shown that the persistent activity exhibited by neurons was directional in nature, such that they had specific “memory fields” which produced increased firing for certain cue locations around the fixation point (Funahashi et al., 1989). In addition, persistent activity was prolonged if the retention interval was increased. One limitation of using the classic delayed response task, or its variants, is that it is difficult to isolate whether the neuronal activity generated represents the mnemonic event or the planning of a motor response to be executed. Using a task in which monkeys were required to make a saccade opposite to the direction of the stimulus location, Funahashi et al. (1993) showed that the neurons of the PFC are specific to the location of the remembered stimulus. Another study in which the monkey was required to execute one response when the cue appeared in one location and an alternate response when the cue appeared in a different location, suggested that persistent activity also predicted the occurrence of errors as it showed a diminished activity for error trials (e.g., Niki and Watanabe, 1976). A similar finding was reported by Sawaguchi and Yamane (1999) in a spatial delayed matching to sample task. This task has two retention intervals, one that follows the sample cue and the other that follows matching cue. During the first retention interval, the monkeys are required to remember the location of the sample cue
and following the second retention interval the monkeys are required to make an appropriate motor response depending on if the second cue matched the sample stimulus or not. Sawaguchi and Yamane (1999) demonstrated that the neurons that showed persistent activity in the PFC during the first retention interval failed to show activity during the second, indicating that these neurons represented the visual memory. In addition, it was observed that this activity disappeared or was reduced when the subjects made errors. These findings strengthen the hypothesis that the persistent activity generated during the retention interval represents the visual memory rather than preparation to execute motor responses. Beyond PFC, neurophysiological recordings from brain regions, including inferior temporal cortex (IT; e.g., Miller et al., 1991, 1993; Miller and Desimone, 1994) and posterior parietal cortex (PPC; e.g., Gnadt and Andersen 1988; Chafee and Goldman Rakic, 1998) also show persistent activity.

In humans, imaging studies using functional magnetic resonance imaging (fMRI) have shown persistent activity during the performance of WM tasks in regions similar to that of the monkeys. These regions include PFC (e.g., Cohen et al., 1997; Courtney et al., 1997); PPC (e.g., Cohen et al., 1997; Sereno et al., 2001) and IT (Pessoa et al., 2002). In support of the electrophysiological studies in non-humans primates, imaging studies also suggest that the persistent activity representing the memory of the item is task specific. Cohen et al. (1997) conducted fMRI studies in humans while they were engaged in an N-back task to study the effect of memory load and observed that the fMRI signal amplitude increased with increasing memory load, suggesting that the persistent activity is sensitive to memory load. In an N-back task, the subjects are required to remember the letters that
are displayed ‘N’ trials before the present trial. The value of N varies from 0 back, in which they are required to report the letter appearing on the screen, to 1-back, 2-back or 3-back task wherein they are required to report letters that were displayed 1, 2 or 3 trials before. Pessoa et al. (2002) tested humans on a matching task, similar to the one used for monkeys, and observed that the fMRI signal amplitude was decreased on error trials when compared to correct trials. Using a similar matching task in which the memory load could be manipulated, Todd and Marois (2004) demonstrated an increase in the fMRI amplitude as the memory load was increased. This increase occurred until the individual's memory capacity was reached, after which it plateaued at approximately four items.

Electrophysiological studies in humans using event-related potential (ERP; e.g., Klaver et al., 1999; Vogel and Makizawa, 2004; McCullough et al., 2007; Ikkai et al., 2010) complement the imaging studies in humans and single-cell studies in monkeys. Most of the ERP studies make use of lateralized tasks in which the subjects have to remember the items presented on either sides of a display. Patterns of the ERP signal reflects certain features of persistent activity. A large broadly distributed negative slow wave occurs during the entire duration of the memory retention interval and this represents the item being held in memory. Klaver et al. (1999) indicated that as the negative slow wave was of greater magnitude in the hemifield contralateral to the position of the item to be remembered, it was sensitive to the location of the cue. Other studies have shown that the amplitude of contralateral delay activity is reduced for incorrect trials when compared to correct trials (e.g., Vogel and Makizawa, 2004), is memory load dependent (e.g., Ikkai et al., 2010) and reaches a threshold at the individual's WM capacity (e.g., McCullough et al., 2007).
1.3 Neural Basis of Persistent Activity

Single-cell studies have reported simultaneous persistent activity in both PFC and PPC in monkeys performing oculomotor delayed response tasks (Chafee and Goldman-Rakic 1998). Chafee and Goldman-Rakic (2000) demonstrated that inactivation of PPC, through cortical cooling, affected the neural activity in the PFC suggesting that PFC and PPC are interconnected. In addition to PPC, PFC is reciprocally connected to other areas of the brain including IT (For review see Constantinidis and Procyk, 2004). A network model suggests that the persistent activity generated is not a phenomenon localized to a single region of the brain, but that there exists strong reciprocal loops between different areas that are simultaneously activated and are responsible for retaining the memory of an item (Wang, 2001). Consistent with this, imaging studies using PET and fMRI have shown the simultaneous activation of frontal and parietal areas of the brain in humans engaged in working memory tasks (e.g., Jonides et al., 1993; Courtney et al., 1997). Another model of working memory suggests that persistent activity is maintained locally within a cortical area through a reverberatory process in which neurons selectively excite each other through recurrent connections (e.g., Amit, 1995). According to this model, persistent activity is propagated from one group of neurons to another through feed-forward asymmetric connections. Goldman-Rakic (1995) suggested a similar model for explaining the persistent activity within PFC. In this region, the layers of pyramidal neurons are arranged in a columnar fashion, with excitatory connections maintaining persistent activity when the location of the stimulus is in the preferred direction and inhibitory connection providing feedback connection to inhibit the activity when the location of the stimulus is opposite to
the preferred location. Persistent activity can also be maintained by a single neuron through cellular bistability (For review see Durstewitz et al., 2000). A single neuron can function on its own to maintain persistent activity, without relying on pre-existing neuronal connections that might be required to maintain persistent activity as per the former two models suggested. Here, a single neuron has two stable states; one a resting state and the other, a continuous spiking ‘up’ state. The ‘up’ state can be maintained either through voltage or Ca\textsuperscript{2+} gated membrane currents, or by sufficient synaptic drive. However, one limitation of this model is that the activated state of this neuron is prone to noise and distractors.

For persistent activity to be maintained through recurrent excitation within specific neurons, the neurons have to be selectively activated. Lisman et al. (1998) suggested that selective activation is mediated by a property of NMDA receptors (NMDAR). These are glutamatergic receptors that require the binding of its agonist site by glutamate and co-agonist sites by glycine/D-serine in order to be activated (Cull-Candy et al., 2001). According to the computational model by Lisman et al. (1998), as NMDAR require the sufficient postsynaptic depolarization in addition to the binding of glutamate, synaptic transmission is conditional and this brings about selective activation. This property of voltage dependency of NMDAR indicates that only those synapses that are depolarized will be activated upon glutamate binding. Further, for this activity to persist even after the input ceases, the synapses should continue to receive inward current. As glutamate remains bound for a longer time period to the NMDAR when compared to other glutamatergic receptors, the receptor remains open for a longer time and exhibits slow kinetics. Wang et
al. (1999) suggested that this slow kinetics of NMDAR (approx. 100 ms) facilitates temporal summation of input signals to maintain persistent activity. Physiological (e.g., Seamans et al., 2003) and pharmacological evidence (e.g., Adler et al., 1998; Lofwall et al., 2006) support the computational studies in describing the role of NMDAR in maintaining persistent activity. The most common strategy employed in these studies is examining the effects caused by blockade of NMDAR by its antagonists such as phencyclidine (PCP), ketamine and MK-81. Seamans et al. (2003) showed that when the pre-limbic cortex in rats was exposed to ketamine in vivo, there was a reduction in persistent activity. Further, in pharmacological studies conducted in humans, exposure to low doses of ketamine impaired their performance in N-back task (e.g., Adler et al., 1998; Morgan et al., 2004; Lofwall et al., 2006).

Similar studies have been conducted in monkeys (e.g., Roberts et al., 2010; Heijseelaar, 2011). Roberts et al. (2010) studied the effects of administering low doses of ketamine (0.1-1.7 mg/kg, i.m) in monkeys performing the delayed response task. They showed impairment in monkeys’ task performance following ketamine injection. In a similar study conducted in our lab, it was shown that low doses of ketamine (<1mg/kg), impaired monkeys’ performance in VSCT (Heijseelaar, 2011). As VSCT allowed the manipulation of memory load, unlike the classic delayed response task, results from this study indicates that impairment caused by ketamine is not only dose-dependent, but is also memory load dependent. Higher doses of ketamine led to increased impairments, and impairments in WM were more pronounced at a higher memory load. Overall, these studies
suggest that NMDAR play a vital role in maintaining persistent activity required for the maintenance of the memory of an item.

### 1.4 Schizophrenia

Schizophrenia is a severe psychiatric disorder that affects approximately 1% of the population. Symptoms of schizophrenia are classified into positive, negative and cognitive symptoms (Schizophrenia Society of Canada, Canadian Psychiatric Association, 2007). Most of the symptoms of schizophrenia are presently diagnosed using the Structured Clinical Interview for the Diagnostic and statistical manual of mental disorder IV criteria (SCID). The symptoms and behaviours that are characteristic of schizophrenia are assessed using different scales. An experienced clinician or a researcher interviews the patient using standard cognitive scales and rates their symptoms based on the disorder severity. This can range from 0, indicating the absence of a disorder, to a higher value like 7, indicating that the patient has severe disorder. Some of the commonly used schizophrenia scales are the Brief Psychiatric rating Scale (BPRS; Overall et al., 1962), Positive and Negative syndrome scale (PANSS; Kay et al., 1987) to assess positive and negative symptoms, Scale of Assessment of Negative Symptoms (SANS; Andreasen 1983), and Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS; Nuechterlein et al., 2008) to assess cognitive symptoms and General psychopathology scale. The positive symptoms associated with schizophrenia include conceptual disorganization, delusions, and hallucinations. Patients with positive symptoms are generally known to respond well to typical antipsychotic drugs. Negative symptoms, under the BPRS scale, include affective and motivational deficits, emotional and social withdrawal, disorganized speech, and
motor retardation. Cognitive symptoms include deficits in executive processes such as learning, attention and working memory (Ross et al., 2006).

While the exact cause for schizophrenia is not known, it has been hypothesized that altered connectivity between different cortical areas or an imbalance of neurotransmitters are possible causes of the disorder (e.g., Goldman-Rakic, 1999). Neuroimaging studies conducted while schizophrenia patients were being tested on different cognitive tasks support this hypothesis by showing that several regions of their brain show abnormalities (For review see Niznikiewicz et al., 2003). These abnormalities were more prominent in the frontal and temporal areas of the brain and along with abnormal neuronal connections between these, which may have led to deficits in the performance of patients in cognitive tasks including WM tasks. Park and Holzman (1992) reported that the performance of schizophrenia patients was reduced in the oculomotor delayed response task. In comparison to an electrophysiological study in monkeys, which reported the activation of dorsolateral PFC (DLPFC) during the performance of this task (Funahashi et al., 1989), Park and Holzman (1992) hypothesized that the reduced task performance in schizophrenia patients was due to damage in DLPFC. To support this, subsequent neuroimaging studies have suggested that patients with schizophrenia show reduced activity in the DLPFC when assessed using WM tasks, such as sequential N-back task (e.g., Barch et al., 2002) and show abnormal patterns of activity in the frontal and temporal areas of the brain (e.g., Weinberger et al., 1992; For review see Ragland et al., 2007).

As DLPFC is highly innervated with dopamine receptors, Goldman-Rakic (1995) suggested that alterations in dopamine levels in the PFC might be responsible for the WM
impairments seen in schizophrenia patients. Further, the successful treatment of amphetamine-induced psychotic symptoms (Janowsky and Risch, 1979) with antipsychotics targeting the D2-dopamine receptors (Seeman, 2002) strengthened the dopamine hypothesis of schizophrenia. However, as the antipsychotics were able to reduce positive symptoms of schizophrenia only and showed no effects on the negative and cognitive symptoms of the disorder, the possible role of other neurotransmitters in causing the disorder requires investigation.

1.5 Role of NMDA Receptor Hypofunction in Schizophrenia

The NMDA receptor is a tetramer of two subunits; NR1 and NR2/3. Glutamate normally binds to the NR2 subunit of the receptor and co-agonists, glycine/D-serine binds to the NR1 site of the subunit (Cull-Candy et al., 2001). At resting membrane potential, Mg2+ blocks the receptor channel and this blockade is removed by sufficient depolarization (Nowak et al., 1984). Within the channel is a binding site for antagonists such as ketamine, PCP and MK-801, which causes the blockade of the receptor channel. Complete activation of NMDAR requires the binding of both NR1 and NR2 sites, and sufficient membrane depolarization in order to remove the Mg2+ blockade.

NMDAR hypofunction is thought to underlie the pathophysiology of schizophrenia. Javitt and Zukin (1991) proposed this hypothesis in their meta-analysis in which they analysed the psychomimetic effects caused by the NMDAR antagonist, PCP. The degree of psychomimetic effects produced by PCP was dependent on whom it was administered to: in normal subjects PCP induced psychosis; in schizophrenia patients, PCP worsened symptoms; and in victims of abuse, low doses of PCP induced symptoms that
were very close to the positive symptoms of the schizophrenia. PCP induced negative and
cognitive symptoms as well as positive symptoms similar to those induced by
amphetamines. Further, they noted that the serum concentration of PCP that induced the
symptoms was comparable to the amount required to block the NMDAR. In addition, as
varying concentrations of other NMDAR antagonists also induced symptoms similar to
that induced by PCP (Figure 1), it was hypothesized that NMDA receptor hypofunction
possibly underlies the pathophysiology of schizophrenia. A study conducted by Krystal et
al. (1994) in healthy humans reported that administration of ketamine impaired
performance in tasks such as the Wisconsin card-sorting task and verbal fluency test. As
these tasks required the use of frontal areas of the brain, it was suggested that blockade of
NMDAR possibly altered the function of these regions, leading to a reduced task
performance. In this study, two doses of ketamine (0.1 mg/kg and 0.5 mg/kg) were tested
and the higher dose was shown to induce symptoms. As mentioned before, Adler et al.
(1998) reported that in addition to impairing task performance in the N-back task,
ketamine also impaired thought process and verbal fluency in normal subjects. It was also
reported that these NMDAR antagonists produced certain physiological abnormalities in
normal subjects that were characteristic to schizophrenia. This included altered signal
amplitude in ERP studies, which was correlated with an increased BPRS scores following
ketamine administration (Umbricht et al., 2002; Ahn et al., 2003) and altered blood flow
in frontal areas assessed using neuroimaging techniques such as PET (Vollenweider et al.,
1997; Lahti et al., 2001) and fMRI (Anticevic et al., 2013). In an ERP study (Ahn et al.,
2003), subjects were assessed in a WM task similar to matching task that required them to
Figure 1. Degree of schizophrenia-like symptoms induced by NMDAR antagonists, representing the correlation between the potency of an NMDA antagonist to induce symptoms on par with PCP and their ability to bind to the NMDAR (modified from Javitt and Zukin, 1991)
Acute doses of ketamine produced impairment in several cognitive functions in monkeys performing different WM tasks such as the delayed match-to sample task (e.g., Buccafusco and Terry, 2009), sequential comparison tasks (Heijseelaar, 2011; Blackman et al., 2013) and spatial search task (e.g., Taffe et al., 2002) in a dose-dependent manner. Further, Tsukada et al. (2005) reported that administration of MK-801, an NMDAR antagonist in both acute and chronic doses, produced impairments in monkeys performing an oculomotor delayed response task along with altering dopamine levels in the PFC. Using microdialysis and PET imaging, they showed that MK-801 induced a dose-dependent effect on dopamine levels in the PFC, indicating that NMDAR acts together with the dopamine system to produce WM impairments. In rodents, infusion of acute doses of NMDAR antagonists induced several behavioural impairments, such as locomotor hyperactivity and reduced performance in memory tasks, and chronic exposure led to long-term deficits in cognitive functions (For review see Labrie and Roder, 2010). Rodent models of schizophrenia produced by chronic exposure to antagonists in the early postnatal period or by knockout of the NR1 subunit of the NMDAR also show behavioural impairments, supporting the NMDAR hypofunction model of schizophrenia (Labrie and Roder, 2010). Overall, these findings support the idea that NMDAR hypofunction plays a role in the pathophysiology of schizophrenia.
1.6 Modulation of NMDA Receptors

NMDAR play a crucial role in the maintenance of persistent activity that is required for the maintenance of WM and its hypofunction is possibly contributes to WM deficits in schizophrenia. This suggests that increasing the activation of these receptors may be beneficial to WM. NMDAR are activated by the simultaneous binding of the agonist site by glutamate and the co-agonist site by glycine/D-serine. Using glutamate to increase the activation of receptors may not be an ideal option as it may cause excessive receptor activation, leading to neurotoxicity and neuronal loss (Coyle, 2006). Alternatively, agents targeting the co-agonist site of the receptor, such as glycine, D-serine, D-cycloserine or glycine transporter (GlyT1) inhibitors/sarcosine, could be used. On their own, these co-agonist site modulating agents do not directly increase the activation of NMDAR, but increase the chances of activation of those NMDAR that are already bound by glutamate. Targeting co-agonist sites is a possible option as neurophysiological studies have shown that exogenous administration of glycine/D-serine evoked NMDAR mediated responses in several brain regions including the PFC both in vivo and in vitro (e.g., Chen et al., 2003; Fossat et al., 2011).

Several pharmacological studies have been conducted in schizophrenia patients to test the effects of D-cycloserine, glycine, D-serine and sarcosine. D-cycloserine is a partial co-agonist of the glycine site of the NMDAR. In his review, Coyle (2006) suggested that D-cycloserine was not efficient at reducing the symptoms of the disorder, based on studies which tested chronic doses (50mg/kg) of D-cycloserine in schizophrenia patients (e.g., Goff et al., 1999, 2005). While Goff et al. (1999) reported that D-cycloserine was able to
produce amelioration of negative symptoms only. A later study done by this group (Goff et al., 2005) noted that D-cycloserine did not produce any improvement in the negative symptom either. Since D-cycloserine is only a partial co-agonist of the glycine site, its efficacy is reduced (by almost 50%) when compared to full agonists such as glycine and D-serine. Several clinical studies suggest that a high dose of glycine (30-60 g/day) has proved to be beneficial in reducing some of the negative (e.g., Javitt et al., 1994, 2001; Leiderman et al., 1996) and cognitive impairments (Heresco-Levy et al., 1999) in patients with chronic schizophrenia already receiving typical antipsychotics (For review see Labrie and Roder, 2010). However, a multi-center double blind study that examined the potential role of glycine and D-cycloserine found that both these agents failed to produce improvements in cognitive and negative symptoms assessed using different scales. The results from this study suggest that neither glycine nor D-cycloserine is effective in reducing the symptoms of schizophrenia (Buchanan et al., 2007).

An alternative and indirect method of modulating NMDAR is by using GlyT1 inhibitors (GlyT1-I). The level of glycine is tightly regulated by GlyT1. GlyT1-I act by increasing the extra synaptic levels of glycine by binding to the GlyT1. Previous studies have shown that high affinity GlyT1-I can potentiate NMDAR-mediated neurotransmission in animal models relevant to schizophrenia (For review see Javitt, 2012). One such study conducted by Roberts et al. (2010), tested the ability of GlyT1-I administration (0.01, 0.05 and 0.17 mg/kg; subcutaneously) in reversing ketamine induced working memory impairments in monkeys. In this study, the monkeys performed a spatial delayed response task after administration of GlyT1-I in two conditions: with or without
acute doses of ketamine. It was observed that pretreatment with GlyT1-I was effective in reversing the impairment in the delayed response task performance caused by ketamine. The study also tested the effects of GlyT1-I alone without a following injection with ketamine. GlyT1-I alone failed to increase the task performance of monkeys suggesting that an underlying impairment, caused by ketamine, might be required for GlyT-I to show its effects. Additionally, Roberts et al. used a delayed response task in their study. As this task involves the retrieval of a treat from a baited well, it may involve the preparation of the animal to perform the task, in addition to WM. Thus this study is limited in isolating the effect of GlyT1-I on WM alone.

1.7 Modulation of NMDA Receptors using D-Serine

Another suitable method of modulating the co-agonist site of the NMDAR is by using D-serine. In their review, Labrie and Roder (2010) suggested that, unlike D-cycloserine and glycine, D-serine may be more effective in reducing the symptoms of schizophrenia. A crystallographic study describing the activation of NMDAR suggests that the binding of D-serine is tighter than glycine and D-cycloserine at the co-agonist site (Figure 2; Furukawa and Gouaux, 2003), making D-serine a more suitable option. Further, electrophysiological studies indicated that depletion of D-serine by D-amino acid oxidase (DAO), the catabolic enzyme for D-serine, attenuated NMDAR activity more so than a depletion of glycine by its catabolic enzyme, glycine oxidase (e.g., Panatier et al., 2006; Fossat et al., 2011). This suggests that D-serine, rather than glycine, is the dominant ligand for the D-serine/glycine site of the NMDAR. In addition, D-serine increased the NMDAR cell signalling in several in vitro studies examining NMDAR evoked excitatory responses.
in regions such as the PFC (e.g., Chen et al., 2003; Fossat et al., 2011), indicating that D-serine is capable of regulating NMDAR activation. Fossat et al. (2011) reported that exogenous administration of D-serine, and not glycine, increased the NMDAR mediated
Figure 2. Binding properties of the co-agonist site of the NMDAR assessed in displacement experiments. A glycine site antagonist was used and the ability of different agonists- D-serine, glycine and D-cycloserine to replace the antagonist was determined. In comparison to glycine, D-serine had more affinity to the receptor as it formed three additional hydrogen bonds (modified from Furukawa and Gouaux, 2003).
Figure 3. Increase in NMDAR mediated EPSC amplitude by co-agonists. NMDAR glycine sites are not completely saturated and exogenous application of D-serine (100μM) and not glycine (100μM) were shown to increase NMDAR mediated EPSC in vitro. Sarcosine (GlyT1-I 0.5mm) produced a very slow rise in NMDAR current **p<0.01, ***p<0.001 student t test. (Taken from Fossat et al., 2011)
EPSCs significantly (Figure 3). Given that the impairment of WM is one of the main symptoms of schizophrenia, and that this disorder is hypothesized to be caused by hypofunction of NMDAR, using D-serine may be a suitable treatment option in alleviating those symptoms. In their meta-analysis, Tsai and Lin (2010) have documented the beneficial effects of chronic treatment with D-serine (30 mg/kg for 6 weeks) in reducing the total psychopharmacology, negative and cognitive symptoms of schizophrenia. D-serine reduced these symptoms in schizophrenia patients when used in conjunction with adjuvants or atypical antipsychotics (Tsai et al., 1998; Heresco-Levy, 2005). However, it was not effective in acutely ill patients (Lane et al., 2005) or when treated in conjunction with clozapine (Tsai et al., 1999). Kantrowitz et al. (2010) studied the effects of chronic administration of higher doses of D-serine (30 mg/kg, 60 mg/kg and 120 mg/kg for 4 weeks) in schizophrenia patients. In this study, they assessed positive and negative symptoms using PANSS, and cognitive symptoms using MATRICS battery, before and after the four week treatment and conclude that D-serine (>30 mg/kg) treatment ameliorated their symptoms. Additional evidence of D-serine in reducing the symptoms of schizophrenia was provided by the observation that D-serine levels are reduced in the serum (e.g., Hashimoto et al., 2003; Calcia et al., 2012) and CSF (Hashimoto et al., 2005) of patients with schizophrenia. In addition, Kantrowitz et al. (2010) reported that the basal levels of D-serine were increased in patients following the 4-week treatment, suggesting that an accumulation of D-serine would have occurred in the system (Figure 4). Also, the increase was dose dependent and corresponded to the degree of amelioration reported in patients. This suggested that chronic administration of D-serine was beneficial in treating
Figure 4. Plasma level of D-serine after chronic administration. Mean plasma level was assessed 24 hours after administration of acute and chronic D-serine during week 1 (filled bars) and week 4 (open bar). Plasma levels were determined using liquid chromatography with fluorescence detection. The serum level of the dosage of 30mg/kg after 4 weeks was not determined (modified from Furukawa and Gouaux, 2003).
the symptoms of the disorder in patients. Though Kantrowitz et al. (2010) reported an amelioration of most of the cognitive symptoms; D-serine did not have an impact on WM. In conclusion, most of the clinical studies conducted till now studied the effect of D-serine on cognitive symptoms, including WM. As most studies discussed above use scales to rate the severity of the disorder, they provide limited information regarding the improvement of WM specifically with D-serine treatment.

Several studies involving rodent models of schizophrenia also suggest that the behavioural abnormalities induced by NMDAR antagonists can be reversed using D-serine (Labrie and Roder, 2010). One study in mice tested the effects of sub-chronic administration of D-serine in the delayed alternation task. In this task, mice had to remember to alternate from trial to trial between two arms. They observed that the group which received D-serine performed better than the control group. A better performance in this task was taken to reflect an improvement in WM (Figure 5; Bado et al., 2010). However, performance in the delayed alternation task may not exclusively reflect WM as a constant retention interval cannot be maintained between trials and other components, such as odour cues, might be involved in guiding the mice. In addition, one of the most important limitations of this task is that memory load cannot be manipulated. Thus, to date, there is very limited evidence that increased activation of NMDAR improves WM and we wish to test this hypothesis in monkeys.

1.8 Scope of the Study

As discussed in earlier sections, several studies have indicated that NMDAR are important for the maintenance of persistent activity and maintenance of WM. Blockade of
Figure 5. Performance of mice in T-maze task. Percent accuracy (mean ± s.e.m) of mice in T-maze alternation task. Circles represent mice treated with D-serine (50mg/kg); squares denote mice treated with saline. The horizontal bar above the graph represents the habituation and test schedule. *p<0.05 indicates a significant difference between groups on day 8 testing (modified from Bado et al., 2010)
NMDAR using ketamine decreased WM in a dose and memory load dependent manner in monkeys (Heijseelaar, 2011). Also, previous studies in schizophrenia patients have suggested that the disorder might be related to a hypofunction of NMDAR and that sub-chronic administration of D-serine (30 mg/kg) for 6 weeks may have beneficial effects in reducing certain impairments (Tsai and Lin., 2010). Together, these findings suggest that increasing the activation of NMDAR, by modulating their glycine/D-serine sites, might improve WM.

My hypothesis is that sub-chronic administration of D-serine for 6 weeks in monkeys should alter their performance in a visual sequential comparison task. Here sub-chronic refers to the duration of D-serine administration, which is a repeated administration for six weeks. This is different from acute administration, which is a single exposure to the drug and chronic administration, which is repeated administration of the drug for a several weeks or months together. As opposed to an acute treatment, a sub-chronic treatment for 6 weeks will give an idea regarding the effect of constantly stimulating the NMDAR via D-serine. I predict that there will be a gradual improvement in the performance of monkeys for all the set sizes tested, by the end of six weeks of D-serine administration as a result of drug accumulation. In addition, I predict that enhanced task performance would be more pronounced at higher memory loads due to increased demands on WM resources at higher loads (Figure 6). Besides response accuracy, another measure of task performance is response latency. In contrast to previous studies that have indicated that NMDAR antagonism leads to increase in response latency in a dose-dependent manner, I predict that
Figure 6. Hypothesis D-serine administration for 6 weeks will produce a time-dependent improvement in the working memory task performance of the monkeys because of accumulation of the drug in the system. The improvement will be memory load dependent with a higher degree of improvement for higher memory load.
NMDAR activation by D-serine will decrease the response latency of monkeys attributable to an improved processing caused by D-serine administration. Decrease in response latency will be more visible by the end of six weeks of D-serine administration, again due to the accumulation of the drug. Lastly, to examine if the effects produced by D-serine was due to altered motivation, the proportion of trials the monkey aborted was studied.
Chapter 2

Methods

2.1 Subjects and Apparatus

Data were collected from two female rhesus monkeys (*Macaca mulatta*, 6.0-8.0 kg, 13-15 years old). All animal care and experimental protocols were approved by Queen’s University Animal Care Committee and were in accordance with the Canadian Council on Animal Care guidelines. Animals had been prepared for experiments by undergoing surgery, in which a head restraint and subconjunctival coils for monitoring eye position were implanted. Surgical procedures have been described previously (Shen and Paré, 2006). Monkeys were housed in large enclosures (Clarence et al., 2006) and received both antibiotics and analgesic medications during the post-surgery recovery period. Post recovery they were trained with operant conditioning and positive reinforcement to perform fixation and saccade tasks for a liquid reward until satiation. In order to ensure animal’s motivation, fluid intake was controlled during training and experimental sessions; unrestricted access to monkey chow was available and daily treats consisted of fresh fruit and vegetable. The experimenters, the animal care staff, and the university veterinarians closely monitored the animal’s fluid intake, weight and health.

The behavioral paradigms, visual displays and data acquisition were controlled by QNX-based Real Time Experimentation (REX) system (Hays et al., 1982). Visual stimuli were generated by a display program using Matlab and the Psychophysics toolbox (Brainard, 1997) running on a Power Mac G4 computer, and presented on a 37” monitor.
(Mitsubishi XC-3730C Mega-view Pro 37 or NEC MultiSync XP37 plus, 60-Hz non-interlaced, 800x600 resolution, 32 bit color depth) at a viewing distance of 57 cm. Eye positions were monitored using magnetic search coil technique (Robinson, 1963). Field coils around the animals generated opposing horizontal and vertical magnetic fields, which allowed the recording of the voltage proportional to the horizontal and vertical angular eye position generated from the scleral search coil.

The stimulus array consisted of two to five colored squares, each measuring $1.2^\circ \times 1.2^\circ$ positioned at an eccentricity of $10^\circ$ from a white central fixation spot (CIE $x=0.323$, $y=0.325$, $L=9.9$ cd/m$^2$). The spatial arrangement of the stimuli remained identical across trials for each set size. For set size two, stimuli were on either side of the central fixation spot. For all other set sizes stimuli were presented at equal distances, with one stimulus always presented directly above the fixation spot. In each trial a set size was randomly assigned and the colors for the stimuli were chosen at random from a library of six highly discriminable colors: red (CIE $x=0.633$, $y=0.327$, $L=9.8$ cd/m$^2$), green (CIE $x=0.288$, $y=0.602$, $L=9.8$ cd/m$^2$), blue (CIE $x=0.155$, $y=0.063$, $L=9.9$ cd/m$^2$), magenta (CIE $x=0.345$, $y=0.168$, $L=9.9$ cd/m$^2$), yellow (CIE $x=0.432$, $y=0.485$, $L=9.9$ cd/m$^2$), or cyan (CIE $x=0.223$, $y=0.337$, $L=9.9$ cd/m$^2$). For each set size the colors of the stimuli could appear only once in a display. Luminance and chromaticity were measured using a Minolta CA100-Plus photometer, Ramsey New Jersey.

2.2 Behavioral Paradigm

Monkeys performed a visual sequential comparison task (VSCT; Figure 7). In this task, each trial started with the appearance of central fixation point (CIE $x=0.323$, $y=0.325$, $L=9.9$ cd/m$^2$).
Figure 7. Visual Sequential Comparison Task. A: Set size two (SS2). B: Set size three (SS3). C: Set size four (SS4). D: Set size five (SS5). Dotted circles represent eye position. White arrows indicate eye movement. All example set sizes displayed represent correctly executed trials.
L=9.9 cd/m²). The monkeys had to fixate at this point within 1000 ms of its appearance and maintain fixation continuously for 500-800 ms within a 2°x 2° window. This was followed by a memory array that was presented for 500 ms. The memory array was composed of a randomly assigned set of two to five colored squares and the duration of presenting this array was based on previous experiments (Heyselaar et al., 2011; Oemisch, 2012). Following memory array, there was a retention interval of 1000 ms, during which the stimuli were absent and the monkeys had to maintain fixation on the fixation spot on the screen. The duration of the retention interval was based on the retention interval used in human studies that used sequential-comparison tasks (Luck and Vogel, 1997; Vogel and Machizawa, 2004). Finally a test array that was identical to the memory except for a change in the colour of one of the squares of the memory array was presented. Simultaneously, the central fixation spot was dimmed (L=1.37 cd/m²) and the monkeys had to indicate which stimulus had changed by making a saccade to its location within 500 ms. A trial was marked ‘correct’ if the monkey made the saccade to the changed stimuli within 500 ms and this resulted in the delivery of a liquid reward. A trial was marked ‘incorrect’ and resulted in no reward if the monkey made a saccade to the unchanged stimulus or did not respond within 500 ms. Incorrect (saccade) responses and omissions, both were considered as errors. Incorrect trials were followed by a time out period of 3000 ms. For the completion trial, the monkey had to maintain fixation during the entire stretch of the trial unless when it is cued to make a saccade to the changed stimulus. If it aborted an ongoing trial by making a saccade away from the fixation window, prior to the presentation of the memory array, it was considered as a fixation break (Figure 8). If the monkey aborted the trial during
Figure 8. Illustration of the different variations of aborted trials. Red diagonal lines indicate abortion of the trial at that time point. In a fixation break, the monkey made a saccade away from the fixation window before the onset of the memory array. In an array break, the monkey made a saccade away from the fixation window during the memory array presentation. In a retention break, the monkey made a saccade away from the fixation window during the retention interval. In an omission, the monkey failed to make a response after test array presentation.
the presentation of memory array, it was considered as an array break. If it aborted an ongoing trial during the retention interval, it was considered as a retention break. Between every trial the monitor was illuminated by diffuse white light (1.5 cd/m2) in order to prevent dark adaptation.

2.3 Experimental Procedure

The animals used for this study had extensive experience with the VSCT, and had performed at least 10,000 trials in which they showed stable performance. Data regarding the performance of the animals (Monkey G and Monkey F) has been previously published (Heyselaar et al., 2011; Oemisch, 2012). While one of the monkeys (Monkey G) was exposed to different doses of D-serine in a pilot study conducted previously (1-1000 mg/kg), the other monkey (Monkey F) had no previous exposure to D-serine. In that pilot study, the effects of range of doses of D-serine on monkey’s performance in VSCT was tested. We had predicted that different doses would produce a dose-dependent change in the performance of the monkey. However, contrary to our predictions, the performance of the monkey remained within two standard deviations of the control experiment for all the doses tested. A drug washout period of eight weeks was given for the D-serine exposed monkey before starting our sub-chronic experiments.

The experimental set up and the time of the day the monkeys were tested was maintained according to how they had been trained previously and was different. While monkey G worked in the morning, monkey F worked in the afternoon. However, we made sure that the monkeys were tested at the same time every single day for all experimental sessions. To account for the different experimental set up that they were tested in and also
to establish the baseline performance for each of these monkeys, before the administration of drug, we ran them on pre-test experiments. In these experiments both the monkeys received 10ml orange juice as a vehicle 30 minutes prior to session start. The pre-test sessions were run until the monkeys showed a consistent task performance for all the four set sizes at least for 3 weeks before starting the test experiments. The monkeys usually took between 4-6 weeks to reach stable task performance and we took data from the last 3 weeks of stable task performance. The data collected for these 3 weeks are denoted as the ‘pre-test’ experiments.

The test sessions were the same as pre-test experiments except that 100 mg/kg of D-serine was mixed in the vehicle. A fresh mixture was prepared daily before the start of every test session and administered orally 30 minutes prior to session start. A time frame of 30 minutes was chosen based on previous studies in humans (Tsai et al., 2008; Kantrowitz et al., 2010); no data exists in monkeys. Tsai et al. (2008) and Kantrowitz et al. (2010) studied the pharmacokinetics of administering D-serine in healthy humans and schizophrenic patients respectively. The first study tested a single high dose of 1000mg/kg of D-serine and the second tested chronic administration of 30mg/kg, 60 mg/kg and 120 mg/kg of D-serine. D-serine was detected in the serum within 30 minutes of administration (Tsai et al., 2008), and in both these studies peak levels occurred within 1-2 hours of administration. Kantrowitz et al. (2010), in addition to providing the pharmacokinetics of administering different doses of D-serine to schizophrenic patients also tested the effect of administering D-serine repeatedly for a time frame of 4 weeks. They compared the mean plasma levels of D-serine over 24 hours of acute and chronic treatment during week 1 and
week 4 respectively. They reported that the mean levels of D-serine observed during pharmacokinetic assessment on week 4 was greater than week 1 for doses 60mg/kg and 120 mg/kg. This finding forms the basis for us to choose a dosage of 100mg/kg for our monkeys. Similar findings have been observed in rodents (Dunlop and Neidle, 1997; Takahashi et al., 1997; Hashimoto and Chiba, 2004; Pernot et al., 2012; Rais et al., 2012). On 30mg/kg oral administration of D-serine in mice, the peak levels are attained within an hour of administration (Rais et al., 2012). Intraperitoneal injection of 1mg/kg D-serine in rats elevated serum levels of D-serine within 30 minutes of administration (Pernot et al., 2012). D-serine levels are elevated in the several brain regions approximately 3 hours (Pernot et al., 2012) to 6 hours (dose- 9 mmol/kg, Takahashi et al., 1997) after administration and remains elevated 24 hours post injection (Takahashi et al., 1997). In humans, Tsai et al. (2008) reported that D-serine has a mean elimination half-life of approximately 17 hours. Dunlop and Neidle (1997) observed that the decay rate of D-serine in mouse was 16.9 hours and in rats was 18.2 hours. Tsai and Lin (2010) in their meta-analysis documented that the administration of D-serine (dose-30 mg/kg) for 6 weeks was beneficial in reducing cognitive symptoms among various other symptoms in schizophrenic patients. Based on these findings we choose to test D-serine for 6 weeks in our monkeys and predicted that the D-serine produces its effect because of the accumulation of the drug daily in the system.

The drug was administered every day for a period of 6 weeks and the animals were tested 5 days a week. The animals received D-serine at the same time of the day even on weekends when they were not tested. To see if the effect of drug was present even after its
administration was stopped at the end of 6 weeks, we ran the monkeys on ‘post-test’ sessions. These sessions were similar to pre-test session where they received only vehicle and data from these sessions were collected for an additional 3 weeks.

2.4 Data Analysis

All the experimental data were analyzed using MATLAB (The Math Works, Natwick, MA) and Microsoft Excel. To assess the effects of D-serine on working memory performance, response accuracy and response latency were calculated from each session. Response accuracy is defined as the probability that the response was a saccade that landed on the changed stimulus. Response latency is defined as the time between the onset of the test array and the initiation of the saccade response. To verify that the changes in response accuracy and latency were not due to varying session lengths, only the first 600 trials of every session were evaluated. This amounted to approximately one hour of testing and also corresponded to the duration when D-serine levels in the serum are at its peak. Data across all pre-test experiments were compared using $\chi^2$ test ($p<0.05$). If there was no significant difference between individual pre-test experiments, they were pooled together. The pooled values of the pre-test experiments comprise of data from three weeks.

To test for a change in response accuracy as a result of accumulation of the drug, I did two types of comparison. First I pooled the values for the entire test and post-test experiments and compared it to the values of the pre-test experiments using $\chi^2$ test (Bonferroni corrected, $p<0.025$). I used this value of $p$ as I did two comparisons with the pre-test values, one with the test experiments and the other with post-test experiments. I expected to see an overall effect of D-serine in test and post-test experiments with this
comparison. Second I pooled the values of test and post-test experiments on a weekly basis and compared the data from each week to the pre-test values of the response accuracy using \( \chi^2 \) test (Bonferroni corrected, \( p<0.0055 \)). I choose this value of \( p \) as I did nine comparisons with the pre-test values, six comparisons for each week of the test session, and three comparisons for each week of the post-test sessions. I expected to see a time-dependent effect of D-serine for each week of test and post-test session with this analysis. To assess the changes in the response accuracy in each session only the trials labelled correct or incorrect were analyzed and trials that were aborted before the onset of the test array were excluded from the analysis. Omission errors, although rare, were treated as errors and included in the analysis. In addition, the percent changes from the average pre-test response accuracy for all set sizes across each week were calculated. Percent changes were assessed regarding their position within or outside of two standard deviations from the average pre-test response accuracy.

Similar to the analysis of response accuracy, the changes in the response latency as an effect of D-serine was calculated on an overall basis and on a weekly basis. The response latency in correct trials and error-trials were different. As a consequence, a difference in overall (all trials) response latency could have simply be due to a difference in response accuracy (i.e., difference in the proportion of correct and error trials). So, I choose to include response latency values for correct trials only. This gave a better idea if the response latency shortened on correct trials, as expected, following D-serine administration. To evaluate if D-serine produced an overall effect on test and post-test sessions, the response latency data pooled for the entire test and post-test experiments were
compared to the pre-test experiments using t-test (Bonferroni corrected, p<0.025). I choose this value of p as I did two comparisons with the pre-test values, one with the test experiments and the other with post-test experiments. For evaluating the time-dependent effect of D-serine administration on test and post-test experiments, I pooled the response latency value for each week of the test and post-test sessions and compared it to the response latency values from the pre-test experiments using t-test (Bonferroni corrected p<0.0055). I used this value of p as I did nine comparisons with the pre-test values, six comparisons for each week of the test session, and three comparisons for each week of the post-test sessions. Differences between the latencies across different pre-test experiments were assessed using one-way ANOVA (p<0.05). To assess the monkey’s motivation, the proportion of all aborted trials, including fixation, array and retention breaks in the pre-test, test and post-test experiments were compared using $\chi^2$ test (Bonferroni corrected, p<0.025).
Chapter 3

Results

3.1 Pre-Test Performance

The animals have been trained on the VSCT and performance data from them has been collected for our previous studies (Heyselaar et al., 2011; Oemisch, 2012). In this study, the test and post-test experiments were compared to the pre-test experiments and the difference in performance between them was taken to reflect the effect of D-serine on the monkey’s task performance. Also, the performance data obtained by each monkey during the pre-test sessions were compared to their performance data collected from previous studies (Heyselaar et al., 2011; Oemisch, 2012). For the sake of comparison we took data after the monkey had reached a stable performance, after the initial training and this comprised of monkey G having performed the VSCT for 69,802 trials in 96 sessions and monkey F for 40,171 trials in 60 sessions. In both monkeys, the data from the pre-test sessions remained within two standard deviations of the average response accuracy of previous data. This comparison enabled us to determine that the monkeys had reached a steady performance before entering this study and whatever changes occurred after administering the drug was solely due to the effect of the drug and not due to learning processes.

Proportion correct across three weeks of pre-test experiments were pooled and plotted as a function of set size for each animal in Figure 9. Response accuracy for Monkey G averaged 0.85, 0.59, 0.49 and 0.37 for set sizes two to five, respectively. Response accuracy for Monkey F averaged 0.87, 0.71, 0.59 and 0.49. These proportions
Figure 9. Response accuracy in pre-test sessions. The black line represents response accuracy across three weeks of pre-test experiments pooled and plotted as a function of set size for each animal (mean±s.d). Dashed lines indicate chance performance.
exceeded chance performance at all set sizes for both the monkeys (z-test, p<0.0001) and they significantly decreased as a function of set size (ANOVA, p<0.0001). These results compared well with the performance levels that were previously observed and that have been reported previously for monkey G and monkey F (Oemisch, 2012).

The average response latency for correct and error trials together was 151±25 ms (mean± s.d) for monkey G and 199 ± 36 ms for monkey F across all pre-test sessions. Response latency for correct and incorrect responses across all the pre-test experiments were assessed using two way ANOVA, with set size (two to five) and trial outcome (correct or error) as factors. In monkey G, only main effects of set size was significant (F (3, 8783) =881.26, p*<0.0001) and the interaction between outcome and set size was significant (F (3, 8783) =10.37, p*<0.0001). The main effects of outcome was not significant (F (1, 8783) =117.21, p= 0.5987; mean latency for error and correct trials for all set sizes were 145 ms vs.156 ms respectively). Post-hoc comparisons revealed that the monkey took significantly more time on error trials compared to correct trials for set size two (t (8783)= 4.21, p*<0.001). This was not significant for set size three (t (8783) = 0, p=1). For set size four (t (8783) =3.45, p*<0.001) and five (t (8783) =3.08, p*<0.001), it took significantly less time on error trials compared to correct trials (Student-Newman-Keuls test, p<0.05). In monkey F, main effects of both set size (F (3, 7451) =118.78, p<*0.0001) and trial outcomes was significant (F (1, 7451) = 388.88, p*<0.0001; mean latency for error and correct trials for all set sizes were 210 ms vs.193 ms respectively), but there was no significant interaction (F (3, 7451) =1.07, p=0.3595). Response latency on error trials was significantly longer than on correct trials for all set sizes (for all, p*<0.0001; mean latency:
The latency of the animals’ error responses should be equal to or greater than that of correct responses since both outcomes result from the same deliberative process. The increased response latency on error trials indicates that errors made by monkeys were diligent guesses, and not random responses.

3.2 Effect of D-Serine on Response Accuracy

Response accuracy declined as a function of set size across all the test and post-test sessions in each monkey (ANOVA, p<0.0001). According to my hypothesis administration of D-serine should first produce a gradual increase in response accuracy because of the accumulation of the D-serine in the system. Following this, when the administration is stopped, the increased performance should gradually drop back to the levels observed during the pre-test experiments.

To investigate this, I did two kinds of analysis. First, I compared the pooled data of all the test and post-test experiments to the pre-test experiments to observe if there was an overall effect. Contrary to my predictions the overall changes in response accuracy in the test and post-test sessions, for both the monkeys remained within two standard deviations of the pre-test response accuracy. For monkey G, response accuracy for test experiments were not significantly different from the pre-test experiments for all the set-sizes (p>0.05 for all set-sizes; χ² test, Bonferroni corrected p>0.025). However response accuracy for set size 5 in the post-test experiments were significantly reduced (p*=0.023 for SS5; p=0.029 for SS2, p>0.05 for SS3 & SS4; Figure 10). For monkey F, response accuracy was found to be significant only for set size 3 in test experiments (p*=0.018 for
Figure 10. Response accuracy in all the sessions. The left, center and right panel represent the response accuracy for each session of the pre-test, test and post-test experiments for all four set sizes respectively (set sizes 2-5 represented by blue, red, green and purple respectively). The mean (±C.I) response accuracy are represented at zero on the y-axis. The shaded portion extends the mean±c.i of the average values. Tick marks represent the response accuracy for each session (pre-test n=15 for Monkey G and n=13 for Monkey F, where ‘n’ represents number of sessions; test session n=30 for Monkey G and n=29 for monkey F; post-test session n=15 sessions for Monkey G and n=14 sessions for Monkey F). For monkey G response accuracy in post-test sessions for SS5 was significantly different from pre-test experiments (p*=0.022, χ² test, Bonferroni corrected p<0.025). For monkey F response accuracy in test experiments for SS3 was significantly different from pre-test experiments (p*=0.018, χ² test, Bonferroni corrected p<0.025).
SS3, p>0.05 for all other set sizes; Figure 10). This significant change was due to improved performance for this set size. Only set size 3 in post-test experiments was different from pre-test experiments, however this failed to reach statistical significance (p=0.0265 for SS3, p>0.05 for all other set sizes).

In my second analyses, I pooled data from the test and post-test sessions on a weekly basis, each week containing either four or five sessions and compared this to the pre-test data. This comparison enabled me to observe if the response accuracy varied from one week to the next. Contrary to my hypothesis, the percent change in response accuracy in the test experiments did not show a gradual improvement for both the monkeys for all the set-sizes tested. In fact, the changes were either due to an increase or decrease in task performance. In addition, the changes that were observed remained within two standard deviations of the pre-test response accuracy (Figure 11). For monkey G the percent change in response accuracy was significantly different for only set size 5 for three out of the six weeks of test experiments (SS5: p*<0.001, p*=0.0049, p*=0.0045, p>0.01 for all other set sizes across each week; χ² test, Bonferroni corrected p<0.0055). For monkey F, it was significant only for set size 3 for the one week of the test session (SS3: p*=0.0052; p=0.0056 for T6; p>0.05 for all other set sizes across each week). There were no changes observed in the weeks during which post-test experiments were conducted in both the monkeys (for all p>0.1). A correlation analysis was also carried out to see the relationship between the percent change in response accuracy of each test session on a daily basis. In both the monkeys, percent changes in response accuracy
Figure 11. Percent change in response accuracy with respect to pretest experiments. Data for test and post-test sessions were pooled on a weekly basis. Bins represent weekly percent change in test and post-test sessions with respect to pre-test experiments (T1-T6: test weeks, 1 to 6; PT1-PT3: post-test weeks 1 to 3; n=5 sessions for all the weeks for monkey G; n=5 for all the weeks for monkey F except for T1 and PT1 for which n=4). Asterisk represents the week for which response accuracy is significantly different from pre-test experiments ($\chi^2$ test, Bonferroni corrected p<0.0055). Dashed lines represent percent change corresponding to two standard deviations of the pre-test response accuracy. Percent change in response accuracy for the test and post-test sessions for each week, remained within two standard deviations of the pre-test response accuracy for both the monkeys.
<table>
<thead>
<tr>
<th>Set size</th>
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<td>Monkey G</td>
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<tr>
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<td>Monkey F</td>
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<tr>
<td>SS5</td>
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<td>0.047115</td>
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**Table 1**: Correlational analyses showing the relationship between the percent change in response accuracy in test sessions over time.
showed low correlation with respect to time (\( r \leq 0.41 \) in cases where there was positive correlation; see table 1).

In conclusion, the changes observed in both the monkeys were marginal and inconsistent either across weeks or across set sizes.

3.3 Effect of D-Serine on Response Latency

Another parameter indicative of the monkey’s performance in the sequential comparison task is response latency. As response latency increases following inactivation of NMDAR, I expected that the activation of NMDAR via D-serine would decrease response latency. That is, the monkeys will take lesser time to initiate a saccade during test experiments when compared to pre-test experiments. Second, I predicted that once the drug administration is stopped, the response latency of the monkeys will gradually return to their normal response latency.

To quantify this I examined the response latencies for correct trials only, for all the experiments. First I compared the response latencies for pooled data from all the test and post-test experiments to the pre-test experiments. This comparison allowed me to see if there was an overall effect of the drug on response latency. In contrast to my hypothesis, both monkeys took more time to initiate a saccade on test and post-test experiments when compared to pre-test experiments (Figure 12; t-test, Bonferroni corrected \( p<0.025 \)). For Monkey G response latency for correct trials during pre-test, test and post-test were 156 ms, 158 ms (\( t (15138) =6.335, p^*<0.001 \)) and 157 ms respectively (\( t (10289) =3.63, p^*<0.001 \)). The test and post-test sessions were only marginally different from the pre-test sessions and the significance was due to a large number of
Figure 12. Percent change in response latency for correct trials with respect to pre-test experiments (mean±c.i.). The left panels show the percent change in response latencies for the entire test and post-test experiments. The right panel shows the change in response latencies obtained for each week of the test (T) or post-test (PT) sessions (T1-T6: test week 1 to 6; PT1-PT3: post-test weeks 1 to 3; n=5 sessions for all weeks for monkey G; n=5 for all weeks for monkey F except for T1 and PT1 for which n=4). Asterisk represents data significantly different from pre-test experiments. For Monkey G, the average response latency during pre-test sessions was 156 ms and for Monkey F it was 193 ms. Dashed lines represent percent change corresponding to two standard deviations of the pre-test response latency. Percent change in response latency for the test and post-test sessions for each week, remained within two standard deviations of the pre-test response latency for both the monkeys.
trials included for the analysis. Monkey F showed an average pre-test response latency of 193 ms for correct trials. The average response latency for correct trials in test sessions was 199 ms (t (16275) =10.52, p*<0.001; t-test, Bonferroni corrected p<0.025; Figure 12) and post-test was 197 ms (t (10714) =6.73, p*<0.001).

In my second analyses, I compared the response latencies from test and post-test sessions to the pre-test experiments on a weekly basis. This enabled me to observe if there were any gradual changes in response latencies for correct trials. The percent change in response latencies for each week of the test and post-test experiments for both the monkeys remained within two standard deviations corresponding to their pre-test response latencies (Figure 12). The significant changes that were observed were all positive in nature for both the monkeys. For monkey G, response latency was significant for 4 weeks of test experiments (p*<0.001; p*=0.0027 for T5; p>0.01 for the 2 weeks that were not significant) and 2 weeks of post-test experiments (p*<0.001; p>0.1 for PT3; Figure 12; t-test, Bonferroni corrected p<0.0055). For monkey F, response latency was significant for all the six weeks of test experiments, and all the three weeks of post sessions (p*<0.001 for all; Figure 12; t-test, Bonferroni corrected p<0.0055). The correlation of percent change in response latency overtime was low for both the monkeys (Monkey G: r (28) = -0.25, p=0.17; Monkey F: r (27) =0.15, p=0.40).

Contrary to my predictions, response time observed in both the monkeys did not shorten. The increased response latencies did not have a definite pattern and were inconsistent both within and between the monkeys. Overall, the changes in response
latency following sub-chronic administration of D-serine for 6 weeks produced minute changes in the response latency of test and post-test sessions.

3.4 Effect of D-Serine on Motivation

To examine if the lack of effects of D-serine on monkeys performance was not due to altered motivation, the proportion of trials that were not completed (i.e., aborted) on test and post-test experiments were compared to that of pre-test experiments (see Methods and Figure 13). In monkey G the overall proportion of aborted trials for test and post-test experiments being 2.2% and 2.1% respectively, were not different from pretest experiments (approx. 2.3%; \( p>0.1; \chi^2 \) test, Bonferroni corrected \( p<0.025 \); Figure 13).

For monkey F, the overall proportion of aborted trials for pre-test, test and post-test experiments were 4.3%, 5.7% and 6.9% respectively. The proportion of aborted trials for the test and post-test experiments were significantly different from those of the pre-test experiments (\( p^*<0.01 \) for both; \( \chi^2 \) test, Bonferroni corrected \( p<0.025 \); Figure 13). For test sessions, the increase was mainly because of the increased proportion of array and retention breaks that were significantly different from that of the pre-test values (\( p^*<0.01 \); \( \chi^2 \) test, Bonferroni corrected \( p<0.025 \)), each contributing approximately 2.5% of the total trials (Figure 13). In post-test experiments, the significance was due to an increased proportion of all the three types of breaks that were significantly different from that of the pre-test values (\( p^*<0.020; \chi^2 \) test, Bonferroni corrected \( p<0.025 \); Figure 13). A closer look at the data from each session revealed that the increase in breaks seen for test and post-test experiments did not have a specific pattern. The significant change was
Figure 13. Effect of D-serine on trial abortions. The figure represents the proportion of breaks out of the total number of trials of the pre-test, test and post-test experiments. Error bars represent seem of the proportion of breaks. Asterisk represents the proportion of breaks that was significantly different from the pre-test experiments ($\chi^2$ test, Bonferroni corrected $p < 0.025$).
contributed due an increase in breaks on certain days when the monkey was not motivated to work.

The purpose of examining a measure of motivation was to verify if the lack of effect of D-serine on monkeys' task performance was not due to altered motivation. Monkey F showed an increased proportion of aborted trials on test and post-test sessions which suggest that D-serine reduced the motivation of this monkey. This may have contributed to the lack of effect of D-serine on the response accuracy and response latency in the WM task. However, as the proportion of aborted trials were not altered in monkey G and so was motivation, this suggests that the lack of effect of D-serine on performance was not likely due to reduced motivation in both the monkeys.
Chapter 4

Discussion

Working memory is dependent on the maintenance of persistent activity within specific neuronal networks mediated by the NMDAR. Our lab has shown blockade of NMDAR using the antagonist ketamine produces dose and memory load-dependent working memory deficits along with an increase in response latency in monkeys performing a WM task (Heijselaar, 2011). Schizophrenia has been hypothesized to rest on the NMDAR hypofunction. Previous studies have shown that some symptoms of this disorder are alleviated using agents that target NMDAR co-agonist sites, presumably by increasing NMDAR activation. Based on these studies, I hypothesized that administering the NMDAR co-agonist D-serine would increase the activation of NMDAR and improve the performance of monkeys on a working memory task. More specifically, I predicted that daily administration of D-serine for six weeks would gradually improve the WM task performance of monkeys. Contrary to my hypothesis, changes in response accuracy were marginal and not memory load-dependent. Interestingly, the monkeys’ response latency increased in the WM task, similar to what was observed following antagonist administration. Lastly, following D-serine administration, the proportion of aborted trials increased for one of the monkeys, suggesting that D-serine reduced the motivation for this monkey. Being the first study to test the effects of D-serine administration on WM performance in monkeys, my results suggest that D-serine might not produce enough activation of NMDARs that can result in improving the performance of monkeys in WM task.
4.1 Modulation of NMDA Receptors by D-Serine

Most studies have tested the role of D-serine in humans and rodents. In patients with schizophrenia, D-serine was shown to have beneficial effects of reducing the cognitive symptoms of the disorder (Tsai and Lin, 2010). In rodent models of schizophrenia, D-serine administration has been shown to reduce schizophrenia-like behaviour induced by antagonists of NMDAR (e.g., Andersen et al., 2004; Lipina et al., 2005). Andersen et al. (2004) studied the effects of administering high doses of D-serine to mice which had undergone a sub-chronic treatment with PCP. Here, mice that received sub-chronic treatment of PCP showed impaired performance in different versions of Morris water maze task which tests their learning and spatial working memory. They noticed that following D-serine administration, the impairment was reversed, as reflected by a better performance at the task. Similarly, several other studies using rodent models of schizophrenia, produced by alterations to genes involved in D-serine pathway have shown the reversal of impairment following D-serine administration (For review see Labrie et al., 2011). The type of genes altered included serine receptor racemase gene (SRR) involved in the synthesis of D-serine (e.g., Labrie et al., 2009c), Grin1 gene responsible for maintaining the structure of the co-agonist sites (e.g., Kew et al., 2000) and DAO gene responsible for regulating the levels of DAO (e.g., Labrie et al., 2009b). Genetic alterations produced several deficits including impairment in spatial working memory, long-term memory and decreased spatial recognition. However, to date, very few studies have tested the effects of D-serine on WM in particular. One study conducted in mice suggested that the group
receiving a daily oral administration of D-serine performed better than the control group on a delayed alternation task (Bado et al., 2010). In this task, mice had to remember to alternate between two arms of a maze on subsequent trials. Improved task performance was taken to reflect an improved WM, which was attributed to the effects of D-serine administration. In contrast to this, the results from another study conducted in rats (Stouffer et al., 2004) and ours suggest that D-serine might not be beneficial in improving the WM task performance. Stouffer et al. (2004) tested the effects of D-serine administration on rats performing a delayed match-to-place version of the Morris water maze task. In this task, rats were initially placed on a sinking platform and were required to swim to a hidden platform in order to escape. The time taken to do so was termed 'escape latency'. The change in escape latencies during three consecutive trials was taken as the measurement of spatial WM. It was reported that there was no difference in escape latencies between the D-serine and the saline treated group, indicating that D-serine did not influence WM. The discrepancy in results from Bado et al., Stouffer et al. and my study suggest that the effects of D-serine are species specific. However it is important to note that results from Bado et al. do not provide conclusive evidence that D-serine improved WM in mice; improvement in performance of mice that received D-serine was significantly different from the saline group only on the last day of testing. Thus, the difference in results might not indicate the species-specific effects of D-serine. Further, the delayed alternation task used in that study may not provide an accurate measure of WM as a constant retention interval cannot be maintained between trials. In addition, that task does not allow the manipulation memory load. Several other studies in rodents have shown that prolonged administration of D-serine
reverses the cognitive impairments induced by NMDAR antagonists (e.g., Andersen et al., 2004; Lipina et al., 2005), suggesting that D-serine has influence on cognitive domains besides working memory.

Another possible explanation for the lack of effect of D-serine on WM performance could be that a disease model was not used here. Hypofunction of NMDAR may underlie the pathophysiology of schizophrenia. In support of this, a meta-analysis conducted in schizophrenia patients suggested that D-serine is beneficial in improving the cognitive symptoms of the disorder (Tsai and Lin, 2010). As our monkeys were healthy monkeys that do not show any hypofunction of NMDAR, it may be expected that sub-chronic administration of D-serine would not have ameliorating effects. One way to test this would be to model the disease condition by reducing the activation of NMDAR. Indeed, a previous study conducted in our lab demonstrated that blockade of NMDAR activation by NMDAR antagonists impairs WM (Heijselaar, 2011). Another study conducted by Roberts et al. (2010) tested the effects of GlyT1-I administration on monkeys WM. In this study, GlyT1-I pre-treatment was beneficial when followed with a ketamine injection but not when administered alone. This suggests that the effects of using agents targeting the NMDAR co-agonist sites might only be beneficial when there is an underlying impairment that is a result of hypofunction of NMDARs.

The next explanation could be that an impaired circuitry might be required in order for D-serine to have an effect. This suggestion is based on previous fMRI studies in which schizophrenia patients performing WM tasks show altered connectivity between PFC and PPC (e.g., Kang et al., 2011) that have been shown to be important for the proper WM
function in monkey (Chafee and Goldman-Rakic, 1998, 2000). In addition to altered connectivity between the PFC and PPC, Kang et al. (2011) reported that schizophrenia patients show a disrupted connectivity between PFC and posterior visual association cortical areas which could contribute to the deficits in WM task performance. They suggested that altered PFC and visual networks might also be involved in guiding a subject's performance in WM. As our monkeys have intact connectivity between these brains regions, their performance may not have been affected by D-serine administration. A future experiment involving localized injections of ketamine or cortical cooling of one brain region followed by an administration of D-serine may provide more evidence whether D-serine is beneficial in improving WM.

Kantrowitz et al. (2010) conducted the first study that explicitly reports the effects of D-serine on WM in humans. They found that in schizophrenia patients, D-serine did not improve WM. In this study, they tested the effects of a high dose of D-serine on different cognitive domains including WM. The cognitive symptoms were assessed using MATRICS, which includes tests for the verbal and non-verbal aspects of WM. They reported that the patients showed improvement in most of the cognitive symptoms other than WM. This result strengthens our finding in suggesting that D-serine fails to affect WM in particular, even in the case of schizophrenia patients who might have an altered circuitry.

Further, most clinical studies that report beneficial effects of D-serine involved the assessment of schizophrenia patients using different psychometric scales. These involve an interviewer rating the symptom severity in patients. The ratings provided by each
interviewer may vary and might not be accurate in identifying the improvements, if produced by a drug.

4.2 Modulation of NMDA Receptors by targeting its co-agonist sites

Our aim was to increase NMDAR activation by targeting its co-agonist site. Though D-serine has not been tested in monkeys, other agents, particularly GlyT1-I has been previously tested (Roberts et al., 2010). In this study, they showed that when GlyT1-I alone was administered, it had no impact on the WM task performance of monkeys, which was consistent with my findings. However, as mentioned before, when pre-treated with the NMDA antagonist ketamine, GlyT1-I was beneficial in improving WM of monkeys. The mechanism of activation of NMDARs by the two co-agonists is different. That is, in our study D-serine was exogenously supplied whereas, GlyT1-I work by binding to the glycine transporter to increase the extracellular levels of glycine, which in turn activates the NMDAR. Increasing the levels of co-agonists by exogenous application would not have the same effect as increasing their levels endogenously. Also, GlyT1-I was administered sub-cutaneously while oral administrations of D-serine were used in my study. Further, the basis of using ketamine in studies that test the effects of co-agonist administration is to increase the stimulation of the glycine site of the NMDARs indirectly. Ketamine causes the blockade of only some of the NMDAR sites. Thus, when the extra-cellular co-agonist levels are increased, the activities of remnant NMDARs are enhanced.

The idea behind using co-agonist, D-serine for increasing the activation of the NMDARs was solely based on the assumption that the co-agonists sites are not saturated (Chen et al., 2003; Fossat et al., 2011). However based on our study and the study by
Roberts et al. (2010) it might be possible that in monkeys, these sites are either completely saturated or very few unsaturated sites exist.

### 4.3 Effect of D-Serine on Response Latency

Besides response accuracy, the response latency of monkeys was also assessed. As previous studies have reported an increase in response latency following the administration of the NMDA antagonist ketamine (e.g., Shen et al., 2010; Heijselaar, 2011), I predicted that D-serine would decrease response latency. I expected that an increased activation of NMDARs by D-serine would lead to better processing which would be reflected with shorter response latency. I found that the response latency of both monkeys increased, although to a very small extent. At present, my study being the only one to report the effects of D-serine on response latency, further studies are required to validate these findings.

### 4.4 Limitations

**Degradation of D-serine**

In my study, D-serine was administered orally. D-serine is normally degraded by its catabolic enzyme D-amino acid oxidase (DAO). A limitation of this study is that D-serine may have been degraded even before it could reach its target site. Rais et al. (2012) studied the pharmacokinetics of oral administration of D-serine in mice with or without DAO activity (DAO knock-out mice). Here, they compared the plasma levels of D-serine in normal mice after they were administered with D-serine alone or in combination with DAO-inhibitor. Further, they compared these to the plasma levels of DAO-knockout mice administered with D-serine. They observed that the terminal half-life of D-serine in normal
mice (with or without DAO inhibitor) was approximately 1.2 hours and 1.5 hours respectively. In knockouts, the D-serine levels remained elevated even after 4 hours of administration. This suggests that DAO tightly regulates the activity of D-serine and is responsible its rapid clearance when supplied orally. One possible way to overcome this is by microinjection of D-serine to specific brain regions involved in WM or the systemic administration of DAO inhibitors along with D-serine.

D-serine blood levels

In my study, I chose to orally administer D-serine in monkeys as this is the most common route of administration in humans. Based on a pilot study and the study conducted in schizophrenia patients by Kantrowitz et al. (2010), I chose a dose of 100mg/kg/day for monkeys. A blood serum analysis of monkeys during the pre-test, test and post-test would have confirmed if the D-serine administered was absorbed by the system. Although we have collected the blood sample of a monkey towards the end of test and post-test experiments, it was beyond the scope of this thesis to perform blood serum analysis. Kantrowitz et al. performed a blood serum analysis 24 hours after the first day and last day of the D-serine administration in their study, reporting that following chronic administration of D-serine (60mg/kg and 120mg/kg for 4 weeks); the mean serum levels had increased in a dose-dependent manner on the fourth week compared to the first week. Similar to this study, I would expect the serum levels of D-serine to have increased in monkeys due to accumulation of drug across 6 weeks of administration. While conducting the experiments, I ensured that both monkeys received the entire mixture of D-serine and orange juice. Both monkeys had no problems in taking the drug. As D-serine did not
produce a beneficial effect in this study, a blood analysis would also reveal if the levels of D-serine were comparable to the amount that was ingested or if it was degraded by its catabolic enzyme DAO.

Effect of age and gender

The monkeys used in this study were adult monkeys which have received several years of training in the VSCT. One argument for not seeing any beneficial effects of the drug could possibly be due to the use of highly trained monkeys whose performance might be saturated. However, this was not the case. If the monkeys had already reached their optimal performance, then they would have shown the same accuracy for all set-sizes. However, this was not the case. The performance for both the monkeys scaled according to set-sizes for all the experiments. So, if not for obvious effects of the drug on WM performance at lower set sizes, the prediction of observing a pronounced effects at higher set sizes is justified. That is, though we are using highly trained monkeys, there is still scope of improving its performance. The negative findings of this study does not imply that the task performance of the monkeys cannot be improved.

In this study, the tests were performed on female macaque monkeys which have cycling levels of estrogen. In humans its been shown that elevated levels of estrogen during the reproductive age of females, is associated with better performance in tasks that tested their verbal and spatial WM (Segal, 2012). Similarly, following the administration of estrogen along with hormone replacement therapy resulted in a better performance in WM tasks in post-menopause females (Duff and Hampson, 2000). Based on this we could say that the performance of the monkeys might vary depending on what phase of its estrogen
cycle it was. However, the pre-test experiments performed controlled for all these changes. Also the response accuracy of the monkeys (see figure 10) did not have a specific pattern on a particular day for each set size. As in, if estrogen cycling influenced the performance of the monkeys on a particular day, then it would have been reflected by either a decreased or increased performance on all four set sizes on that day compared to others. But this was not the case. The performance of the monkeys remained within two standard deviations of the pre-test performance in all sessions, suggesting that the estrogen cycle had limited influence on their working memory ability.

D-serine on motivation

In order to assess if there were changes in motivation following D-serine administration, we assessed the number of trials aborted. As only one monkey was found to abort more trials following D-serine administration, our results do not provide a conclusive evidence if it altered the motivation of the monkeys. Motivational deficits are one of the negative symptoms of schizophrenia. In schizophrenia patients, previous studies have shown that following D-serine administration, the negative symptoms are reduced (e.g., Kantrowitz et al., 2010; Tsai and Lin, 2010). However none of these studies exclusively report if the motivational deficits are ameliorated by D-serine. In our study, the reduced motivation for this monkey was not consistent. That is, the monkey aborted more trials on certain days of testing that might not have been dependent on D-serine administration. In addition to this, other factors such as thirst, size of the reward could have also influenced the performance of a monkey on a daily basis.
4.5 Future Directions and Conclusion

Future studies can clarify certain questions that are beyond the scope of this thesis and extend our understanding of using NMDAR co-agonists to enhance the activation of these receptors. Analysis of blood levels of D-serine would be the next immediate step after this study. It would help us understand how D-serine is absorbed by monkeys and if it is comparable to other species such as humans and rodents. In addition, as I speculated that the failure of D-serine to improve WM in monkeys might have been due to its degradation by its catabolic enzyme DAO, a follow up study that would test the role of DAO inhibitors along with D-serine would be helpful.

Additionally, studies have to be conducted to verify if combinations of different treatment options can enhance the performance of monkeys in WM task. These include sub-chronic treatment with ketamine along with acute and sub-chronic treatment of D-serine for the same dose as in our study or higher doses. Using a sub-chronic administration of ketamine might help in achieving a primate model of schizophrenia. Although previous studies have used chronic doses of PCP (e.g., Linn et al., 2007) and ketamine in monkeys (e.g., Yu et al., 2011), none of them have tested their effects on WM.

In schizophrenia patients, studies testing the effects of increasing the activation of NMDARs (via co-agonists) explicitly on WM have to be carried out. This could be achieved by using the sequential comparison task similar to ours. Further, imaging studies have to be carried out in patients before and after drug administration, while they are performing the task. This would give a better understanding as to which areas of the brain are specifically altered following the administration of the drug. This technique is called
pharmaco-fMRI (PhMRI) and has been used to see the effects of different drugs in humans (For review see Jenkins, 2012) and in rodents (e.g., Gozzi et al., 2008). Utilizing fMRI in order to obtain high level spatial and temporal images, PhMRI isolates the effects of drug action in different areas of the brain. It enables the visualization of the time window for which the drug is active in different brain regions and the order in which these regions are activated during that time window. This technique may be used for the development of personalized treatment options to patients with schizophrenia, by testing them on different doses of many drugs and choosing the best suitable option for them. Also, this technique may be used to help understand the mechanism of the action of the drug. In fact, Gozzi et al. (2008) used PhMRI to study the effects of PCP in rats pre-treated with D-serine. In this study, they observed that following acute doses of PCP, there was an activation of the cortico-limbo-thalamic circuits. These regions were completely inhibited when PCP administered rats were pre-treated with D-serine. Utilizing imaging techniques, this is one of the first studies that enabled the visualization of the action of D-serine in different brain regions. As D-serine reversed the effects induced by NMDA antagonist, this study supports the hypothesis that targeting the co-agonist site might be a suitable treatment option. As an extension to Gozzi et al. (2008) and our study, further studies should use PhMRI, to see the effects of D-serine administration in the absence of NMDAR antagonists. Conducting these in different healthy species including rats, monkeys and humans would provide evidence if the effects of D-serine are species specific. Testing subjects, particularly humans on working memory task similar to ours would provide further understanding of the effects of D-serine on WM.
Conclusions

The findings from this study indicated that the sub-chronic administration of D-serine does not improve the working memory task performance of monkeys. Being the first study to test the effects of D-serine administration on monkeys WM, my findings were contradictory to my hypothesis. A gradual, memory load-dependent increase in response accuracy and a gradual decrease in response latency that was predicted by the end of six weeks did not occur. In conclusion, this study indicated that the prolonged activation of NMDARs via D-serine might not influence the working memory ability per se.
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