ROLE OF NMDA IN THE VISUAL WORKING MEMORY OF THE MACAQUE MONKEY

by

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Abstract

Working memory refers to the ability to retain information for short periods of time to guide future behavior. This type of short-term memory has been shown to play an important role in mental disorders such as schizophrenia and therefore further investigations into the neural basis of this cognitive function may aid in the study of disease states where this cognitive function is defective.

A likely neural correlate of working memory has been identified in the persistent neural activity observed during the memory retention intervals of various behavioral tasks. Computational and cellular physiology has suggested that this persistent activity depends on NMDA receptor activation. Indeed, pharmacological studies on both human and animal subjects have reported a significant decrease in working memory task performance following the administration of NMDA-antagonists such as ketamine. However, the task and experimental design of these previous studies have not been ideal, and have therefore only shown equivocal evidence that NMDA-antagonists impair working memory, especially its capacity. Here we aimed to determine the effect of low-dose ketamine injection (0.25-mg/kg and 0.50-mg/kg IM) on the performance of macaque monkeys on a visual sequential comparison task, a task whose performance has minimal influence from other cognitive functions besides working memory.

All monkeys showed a detrimental effect of ketamine administration on visual working memory performance, either at higher ketamine doses or with high memory
loads. There was also an effect on performance in sessions without a memory component, indicating that the effect of ketamine was no limited to working memory maintenance.

Although the effect of ketamine on memory load varied per animal, this study provides solid evidence in support of the hypothesis that working memory maintenance is dependent on NMDA receptor integrity.
Contributions

Dr. Martin Paré was the principal investigator and supervisor for the studies described in this thesis. I composed this thesis in its entirety, with constructive criticism and editing provided by Dr. Martin Paré.

I performed all of the data collection and subsequent analysis. Kevin Johnston performed all the drug injections and the analysis programs were derived from programs used in the Shen et al. 2010 paper.
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Chapter 1 – Introduction

1.1 - Working Memory

Working memory refers to the ability to maintain relevant information online for a short period of time – usually just a few seconds – to guide future behavior. A common example of this memory system is remembering a phone number long enough to be able to dial it; once the number is dialed, this memory is no longer relevant and it is forgotten. This emphasis on relevance is one of the defining features that differentiates working memory from other types of short-term memory; such as semantic (memory of language usage) (Tulving, 1972) or procedural (memory for skills) (Squire et al., 1984) which can be stored or maintained in memory by pure repetition, not necessarily by relevance.

That is not to say that working memory and other forms of memory (including long-term memory) are utilized independently of each other. Working memory can be defined as the process for the retrieval and proper utilization of knowledge that has been acquired and stored in other memory systems (Goldman-Rakic, 1995). Working memory can therefore be thought of as the activation of information that is relevant to the task at hand, regardless if that information is acquired through sensory processes (e.g., reading that phone number from a piece of paper) or other memory stores (e.g., remembering your address long enough to write it down). As such, Alan Baddeley introduced it as the system that “supports human thought processes by providing an interface between perception, long-term memory and action” (Baddeley, 2003). In addition to relevance,
there are two other characteristics that differentiate working memory from other processes, especially long-term memory: it has a limited capacity and it is short-lived.

George Sperling first reported both of these characteristics in 1960. He was attempting to determine how much information is available to an observer when said information is displayed for only a brief amount of time. A majority of his experiments dealt with varying lengths of stimulus exposure time (in this case, arrays of letters) but in a subset of experiments, he examined the rate of decay of information by introducing a delay (retention interval) before the subjects reported which letters they observed. He noted that the performance in these sessions was different from the previous ones as it was dependent on the delay component: a longer retention interval resulted in a worse recall performance. He followed up by varying the number of items displayed and showed that this affected performance as well: the more items the observer had to remember, the worse their performance (Fig 1).

These results have been replicated since then in different tasks that involve retaining more abstract items such as randomly colored/shaded stimuli (Phillips, 1974; Pashler, 1988). These tasks have estimated the capacity limit of visual working memory to be roughly 3 to 4 items, with some subjects showing a capacity as high as 6, or as low as 1 (Purdy et al., 1980; Luck and Vogel, 1997; Vogel and Awh, 2008). An estimate for the maximum duration for which these items can be retained is unknown, as human studies have generally used retention intervals of 1 second.
Figure 1. Immediate-memory and available information with increasing retention interval durations. The parameter is the retention interval length in seconds. Heavy line indicates immediate-memory for the same materials. One subject (ROR) (Sperling, 1960)
These tasks have also been conducted in animal models such as non-human primates (NHP), which have shown similar characteristics in this respect to the human: they have demonstrated a capacity limit of at least 2 items (Petrides, 1991; Collins et al., 1998; Taffe et al., 2002; Heyselaar et al., 2011). The retention interval has also been tested for longer durations in these animals; studies have shown an ability to complete the task above chance level with retention intervals up to 10 seconds (Sobotka et al., 2005).

As working memory is theorized to support human thought processes, disruption of working memory would theoretically lead to symptoms of thought disorder. Such symptoms would be mainly manifested as an inability to maintain active representations of relevant information, which is an accurate description of some of the behavioral symptoms seen in schizophrenic patients (Park and Holzman, 1992; Goldman-Rakic, 1994; see for review Lee and Park, 2005; Forbes et al., 2009). Indeed, disruption of working memory in subjects, whether pharmacologically or via lesions, have presented with similar symptoms to those seen in schizophrenia (Freedman and Oscar-Berman, 1986). Other mental disorders have also shown deficits in working memory performance (e.g., ADHD; Martinussen et al., 2005). Therefore, further investigations into the neural basis of working memory may help develop the understanding of such disorders.

1.2 - Approaches to Studying Working Memory

As with any cognitive function, there are certain tasks that have been designed to measure working memory ability more accurately by minimizing interference from other
types of memory or cognitive functions. To ensure that the task successfully captures working memory performance and not any other cognitive function, the subject should show a drop in performance with increasing retention interval and memory load.

Working memory itself may be subdivided into different systems: verbal and visual (including spatial). The majority of the tasks used when investigating the neural basis of working memory have been spatial and/or visual working memory based and as such that subset of working memory will be focused on for this literature review.

1.2.1 - Delayed Response Tasks

Hunter originally devised the delayed response task in 1913 to attempt to differentiate the intelligence levels in different animals, including humans (Hunter, 1913). In the original task, the subjects faced two wells and observed as the experimenter placed a food item into one of the wells. Both wells were then covered with an opaque screen for a period of time (retention interval), and when the screen was lifted again, the subjects were required to retrieve the food item. A successful trial would be one where they retrieved the food item in one go.

Thanks to its simplicity, various animal models have also been trained in the delayed response task, namely macaque monkeys (e.g., Hikosaka and Wurtz, 1983; Funahashi et al., 1989) and rats (e.g., Smith et al., 2011). In the case of macaque monkeys, the main purpose of teaching them the task was to allow for subsequent neuronal recording to determine the underlying neural mechanisms of working memory. However, in order to accurately record from individual neurons, it is necessary for the
monkey to remain absolutely still and thus the task was adapted into its contemporary oculomotor form: the item to be remembered is a dot flashed on the screen, and the response is a saccade to the remembered location after a brief time interval (Fig. 2A).

Although relatively simple in appearance, there may be more than a single process reflected in the persistent activity observed in the delayed response task. Is this activity related to remembering the spatial location of the task or is it reflecting the planning of the response? (see Postle and D'Esposito, 1999)

Returning to the two characteristics of working memory mentioned in the previous section, the delayed response task is good for exploring the correlation between working memory performance and retention interval duration but it is difficult to test a response to more than one location, and hence this task is ill designed to investigate the capacity limits of working memory.

1.2.2 - Self-Ordered Tasks

The self-ordered task was originally devised in 1982 to assess frontal lobe dysfunction in human patients (Petrides and Milner, 1982). Such patients were shown a booklet on which the same items were displayed on every page, but their positions were different and randomly determined for each page. The subject was required to pick a novel item as they flip through the pages; the further they flip, the more items they must store in visual working memory (Fig. 2B). This task, like the delayed-response task, is also easy to teach and easy to administer. As such, it has been studied in macaque
Figure 2. Behavioural tasks used to test visual working memory A Delayed response task B Self-ordered task C Delayed match-to-sample D Sequential comparison task. All tasks depict a correctly executed trial. Dotted circles represent eye position; heavy squares represent manual selections.
monkeys in addition to humans (Collins et al., 1998; Petrides, 2000; Hasegawa et al., 2004).

Although this task is well suited to challenge the working memory capacity in this animal model, so far this has not been achieved, with the exception of a single study that tested up to eight items (Collins et al., 1998). The common version of the task used with animal models involves lever pressing or pointing at items. In the lever press, the animal must press the alternate lever to the first one they selected and thus the maximum number of items the animal retains is only one (Kubota and Niki, 1971b). With pointing at items (usually pots baited with treats), the maximum number of items used has been three (Petrides, 1991), and thus the maximum number of items to be remembered is two.

Although this task can be manipulated to explore both characteristics of working memory, the task itself is very open to strategy use, which influences the resulting performance. Even though the initial task changed the position of the items between each display, more recent studies using this task rarely do this. So the subject can employ a variety of strategies to aid them, such as moving in a clockwise/counter-clockwise direction. The existence of these strategies has been noted before in the study by Collins, in which the authors investigated the response sequences the monkeys used per trial (Collins et al., 1998). The authors reported that the animals did not move around in a random fashion and instead showed a preference to proximity items and moving in either a clockwise or counter-clockwise direction. These strategies, referred to as prospective strategies as it requires processing of a response before the retention interval, were shown
to significantly improve the performance in the task. Other tasks, such as the delayed non-match to sample task, could use retrospective strategies, where the response is prepared after the retention interval, and therefore requires memory to execute.

1.2.3 - Delayed Non-Match to Sample (DNMS)

The DNMS in its simplest form is a self-ordered task with only one repeat: the subject is initially shown one item; after a retention interval, the sample and a novel item are presented and the subject successfully completes the trial if they choose the novel item (Bachevalier and Mishkin, 1986). This task has mainly been used with macaque monkey models as they have a highly developed ability to recognize novel items, which allows them to perform at a high level in DNMS tasks (~80%) after as little as 100 trials (Mishkin and Delacour, 1975), compared to about 400 trials of the delayed match-to-sample task (see below) or the delayed response task which has reported training times of up to 2 months (Funahashi et al., 1993). Additionally, as there is only one novel item, the monkeys cannot use strategy to successfully complete the task, and thus the performance is a more accurate measure of working memory ability.

The task can also be altered into a delayed matching-to-sample task in which subjects match the sample stimulus at the end of the trial. This version has been used to measure the neural activity in the inferior temporal cortex as the monkey maintains a single item in mind while comparing it to non-matching items until the matching item appears. The task varies from displaying all the test samples simultaneously and the monkey points to the matching sample (Fuster and Jervey, 1981; Horel and Pytko, 1982)
to displaying the test items one by one in order to measure the neural response as more and more items are presented (Miller et al., 1991; 1993) (Fig. 2C).

Although useful for measuring neural activity and minimizing the use of strategy, this task can only afford the examination of the correlation between retention interval duration and performance of working memory and not investigations into the limited capacity characteristic, varying the memory load cannot be done practically.

1.2.4 - Visual Sequential Comparison Task

The visual sequential comparison task is more commonly used in human visual working memory experiments. It involves the presentation of a sample array of items, which are then removed for a retention interval and when presented as the test array, some, all or none of the items could have changed (Fig. 2D).

Initially, the task was designed as a simultaneous presentation of the sample and test arrays and the memory component was during the saccade made between the arrays (Egeth, 1966; Taylor, 1976). Even though saccade times are short due to the proximity of the two arrays, studies still reported a drop in performance as the number of items in the arrays (set size) was increased. The study of Taylor (1967) not only asked the subjects to report any difference (detection of novel stimuli) but also had an any-sameness condition (reporting non-novel stimuli). Even though the two tasks were conducted exactly the same, subjects still showed a significant increase in reaction time for the any-sameness condition compared to the any-difference condition suggesting that it was harder for subjects.
The contemporary version of the task involves the *sequential* presentation of the sample and test array, with a retention interval between array presentations. This version of the task has shown similar patterns of results compared to the simultaneous presentation version, including for the any-sameness and any-difference contrasts (Phillips, 1974; Pashler, 1988). However, as the arrays are presented in sequence, this allows manipulation of the retention interval correlation to performance, in addition to the limited capacity characteristic of working memory. This is the task that has been used to estimate the visual working memory capacity of humans, initially by using colored stimuli of varying number (set size) (Luck and Vogel, 1997). The capacity estimate of 3 to 4 items was later supported as an accurate estimate of visual working memory capacity by illustrating the same capacity limit when using items of varying complexities (Awh et al., 2007; but see also Alvarez and Cavanagh, 2004).

We recently showed that macaque monkeys are also able to perform this task with results comparable to the human (Heyselaar et al., 2011). Using a paradigm based on the Luck & Vogel (1997) model, the study showed that macaque monkeys had a capacity limit of at least 2 items, which is in the range of human visual working memory capacity. Additionally, the monkeys also showed a decrease in performance with increasing retention interval (Heyselaar et al., 2009; Oemisch et al., 2011), indicating that the monkeys were using working memory to complete the task.

The visual sequential comparison task can be viewed as an amalgamation of the previously discussed tasks; there is a delayed response variable, there are multiple items
and there is the emphasis on novel item selection. Additionally, employing a strategy to aid the memory component in this task is quite difficult and hence, performance in this task is a more accurate measure of an individual's working memory ability.

1.3 - Neural Basis of Working Memory

1.3.1 - Persistent Activity

In the “Approaches to Studying Working Memory” section, it was mentioned that some tasks had been developed or altered to allow for the simultaneous recording of neurons from animal models. Here the findings and where they have led us in understanding the neural basis of working memory will be further discussed.

A potential neural correlate of working memory was first recorded during delayed response tasks, with recordings from the dorsolateral prefrontal cortex (DLPFC) in macaque monkeys (Fuster and Alexander, 1971; Kubota and Niki, 1971b; Funahashi et al., 1989; 1990; 1991). It was reported that neurons exhibited a persistent activity that lasted for the entirety of the retention interval, when no external stimulus was present. This persistent activity is hypothesized to be the neural correlate of the memory for the spatial location as it bridges the temporal gap between the sample and response stages. Indeed, it was found that certain neurons were “tuned” for the spatial location of the item to be remembered; only neurons within the response field for the cued location would exhibit a persistent activity while neighboring neurons remained silent (Fig. 3).
Figure 3. Directional retention interval activity of a principal sulcus neuron during the oculomotor delayed response task. This neuron had strongly directional retention interval activity ($p < 0.001$), responding only when the cue had been presented at the bottom ($270^\circ$) location. It was suppressed during the retention interval when the cue was presented in the upper visual field.

(Funahashi et al. 1989)
Additional evidence only strengthens the claim for the explanation of this neural activity. Firstly, persistent activity was present during correctly completed trials only; during incorrect trials, the activity failed to persist throughout the entire delay period (Funahashi et al., 1989). Secondly, altering the DLPFC through either lesions or cooling caused the monkeys to become unable to do the task successfully (Funahashi et al., 1993; Chafee and Goldman-Rakic, 2000). And thirdly, persistent activity was shown not to be a representation of the location of the motor response, but a representation of the location of the stimulus cue (Takeda and Funahashi, 2002). An alteration of the task required the monkeys to make a saccade 90° clockwise from the cued location and yet the DLPFC neurons still showed a tuning for the location of the cue, not the location of the response.

This persistent activity has also been reported in other, non-spatial tasks, such as the delayed match to sample task that requires memory of an image instead of a location (Miller et al., 1996), further supporting that persistent activity could be the neural correlate of the item to be remembered, and not necessarily related to the planning of the response in these types of tasks. Persistent activity has also been observed in other brain areas that are known to be involved in working memory, such as the posterior parietal (Gnadt and Andersen, 1988; Chafee and Goldman-Rakic, 1998), and the inferior temporal cortex (Fuster and Jervey, 1981; Miller et al., 1993).

The above experiments have been conducted using animal models, exclusively the macaque monkey. However, persistent activity has also been observed in human studies using neuroimaging techniques, such as fMRI and event-related potentials (ERP).
FMRI studies have shown a sustained increase in BOLD activation in homologous brain regions, including the prefrontal (e.g., Postle and D'Esposito, 1999; Sakai et al., 2002), posterior parietal (e.g., Sereno et al., 2001), and inferior temporal cortices (e.g., Druzgal and D'Esposito, 2001a; 2001b). This increase in magnitude exhibited the same characteristics as the neural persistent activity recorded in the monkey brain; namely that there was a decrease in magnitude during incorrect trials (Sakai et al., 2002; Pessoa et al., 2002). The similarities between the human and macaque monkey data suggest that there is a common neural mechanism underlying working memory in these two species.

In some of these studies the tasks used also allowed for the manipulation of working memory load. FMRI data has shown an increase in the magnitude of the BOLD activation that was positively correlated with working memory load (Todd and Marois, 2004). In this task, subjects were run on a delayed match-to-sample task with an initial array of colored dots; after a 1.2 second retention interval, one of the dots was presented and the subject had to indicate whether that dot matched the one in the initial array in color and location. The authors varied the number of dots in the initial array from one to eight and measured activity in the intraparietal and intraoccipital sulci. They noted an increase in the magnitude of BOLD activation with increasing set size until the working memory capacity was reached; following this there was no more increase. These results further support that persistent activity might be the neural basis of working memory representation (Fig. 4A).
Figure 4. Changes in activation with increasing memory load

A fMRI Brain activation time course recorded in the bilater IPS/IOS. BOLD response function reached a plateau by set size 4 (t-test between 4 and 8, p < 0.05) (Todd & Marois, 2004)

B ERP difference waves at lateral occipital and posterior parietal electrode. Pairwise comparisons yielded significant differences in amplitude between three and four items (p < 0.001), but no difference between three and four items (p > 0.20) (Vogel & Machizawa, 2004).
As the persistent activity is closely linked to the timing of the task, further analysis into this phenomenon was done using ERP, which has a better temporal resolution and is a more direct measure of neural activity compared to fMRI. The studies reported a large, broadly distributed negative slow wave during the retention interval of working memory tasks (Klaver et al., 1999) that increased with increasing memory load (Vogel and Machizawa, 2004; McCollough et al., 2007) and was decreased or non-existent during incorrect trials (McCollough et al., 2007) (Fig. 4B). Unfortunately, the effect of memory load on persistent activity has not yet been investigated in monkey models.

1.3.2 - Basis of Persistent Activity

As persistent activity plays an instrumental role in working memory, there have been many different neurocomputational models that attempt to explain the characteristics seen in experimental sessions. Two of the more prominent ones will be reviewed below.

Persistent activity may be maintained in a neural network through recurrent excitation within cell assemblies (Amit, 1995; see for review Durstewitz et al., 2000; Wang, 2001) (Fig. 5). In this model, the group of neurons within a cell assembly could collectively encode one stimulus and as such are connected together. When this stimulus is presented, the feed-forward connections within this assembly will slowly boost the activity to an upper suprathreshold level that will allow the activity to persist even after
Figure 5. Structure of the persistent activity network model. Two patterns (‘cell assemblies’, green and yellow boxes) coding for two different objects are embedded in the symmetric synaptic weight matrix. Neurons within the same cell assembly are connected reciprocally by high synaptic weights \( w^+ \), whereas neurons not belonging to the same assembly are connected by low synaptic weights \( w^- \). Single neurons might participate in more than one cell assembly (green/yellow box; this has also been observed experimentally). In addition to these local recurrent excitatory connections, there is a global feedback inhibition \( \text{IN} \) driven by input from the excitatory neurons that allows only one pattern to stay active at a time, and an external afferent input \( \text{I}_{\text{aff}} \) to each unit in the network. (Durstewitz et al. 2002)
removal of the stimulus. Although simple, simulations with this model have shown activity patterns similar to those seen *in vivo* (Amit, 1995).

Another prevailing model bases persistent activity on cellular bi-stability, which does not require any pre-existing connections. Bi-stability refers to a neuron's ability to have two different stable states, one resting state and one continuously spiking ‘up’ state (Durstewitz et al., 2000). This up state could be supported by voltage/Ca\(^{2+}\)-gated membrane currents as well as in the presence of sufficient synaptic drive.

The data obtained from these models does not conclusively show whether either of these models would be able to maintain the robust persistent activities recorded *in vivo*. It was initially postulated that the persistent activity was supported mainly by activity through the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor. However, this receptor current exhibits a fast decay, such that the time course would be much too short to support any recurrent activity (~5ms). The \(N\)-methyl \(D\)-aspartate (NMDA) receptor current, however, has a much slower dynamic that could help stabilize the working memory persistent activity (~100ms; Wang, 2001). In fact, if the system was modeled with AMPA receptors, the feedback excitation would need to be very finely tuned to allow a stable persistent activity for any period of time, whereas if the same system was modeled with NMDA receptors, the tolerance for the feedback excitation is much higher, making it biologically more feasible (Seung, 1996). The slow NMDA receptor dynamics will also help control the firing rate in any recurrent network/cell model.
NMDA receptors are ion channels that are activated by excitatory amino acids, namely glutamate and aspartate. In the human brain, NMDA receptors are located predominately in the cerebral and hippocampal cortices (Kornhuber et al., 1989), where it is known to play a crucial role in the induction of long-term potentiation, a requirement of long-term memory consolidation (Collingridge et al., 1983). A key property of NMDA is its voltage-dependent activation; there is a magnesium blockade that needs to be removed by depolarization before the receptor itself can be activated. In terms of working memory retention, this could aid the selective retention of relevant stimuli: during stimulus presentation, the cells that are activated by the stimulus will be more depolarized than the rest of the network, differentially removing the magnesium block from those NMDA receptor channels resulting in an enhanced synaptic reverberation and persistent activity within this group of neurons (Wang, 2001).

Seamans and colleagues (2003) conducted a study with prelimbic cortical neurons from male rats to determine the synaptic basis of persistent activity. They divided persistent activity into three main components; an initial excitatory post-synaptic current (EPSC) with a large amplitude response, a second slower response that was maintained throughout depolarization and small asynchronous synaptic events that rode along the second wave (Seamans et al., 2003). The initial EPSC was completely eliminated by either NMDA or non-NMDA antagonists, suggesting that a mix of NMDA and non-NMDA currents initiates persistent activity. However the second, slower response was
only removed with the addition of NMDA-antagonists, suggesting that it is the maintenance of persistent activity that is dependent on NMDA receptor current.

1.3.3 - NMDA Antagonist Studies

There have been several pharmacological studies, both human and animal, that have provided evidence that NMDA receptors play a role in the maintenance of persistent activity during the retention interval.

In humans, the study of the effect of NMDA-antagonists on working memory has been conducted mainly using verbal working memory tasks. In these tasks, the subjects are required to either just remember the words/digits (maintenance) or to rearrange them into an instructed order (manipulation). There have been numerous studies done with healthy volunteers and they have shown a deficit in working memory performance after the administration of NMDA-antagonists (Ghoneim et al., 1985; Javitt and Zukin, 1991; Krystal et al., 1994; Lahti et al., 1995; Ahn et al., 2003; Honey et al., 2003; Morgan et al., 2004). Additionally, administration of NMDA-antagonists have been used to model schizophrenic symptoms in healthy subjects, as they induce both positive and negative symptoms (Krystal et al., 1994; Adler et al., 1998; Newcomer et al., 1999).

The role of NMDA in visual working memory tasks has mainly been studied in animal models such as the macaque monkey. Dudkin and colleagues conducted a series of experiments with macaque monkeys executing delayed visual discrimination tasks. The subject was presented with two colored squares, a yellow and a blue, and was required to remember the position of the yellow square in order to make a saccade to it
after a retention interval (Dudkin et al., 1996; 1997; 2001). During this task, the authors recorded from neurons in the PFC and visual cortex either without any pharmacological manipulations, or after the administration of NMDA-antagonists (APV) or agonists (NMDA glutamate). The results showed that NMDA-antagonists caused a decrease in activity in both brain regions (Dudkin et al., 2001), whereas the NMDA glutamate increased the synchronization of the neural firing during the retention interval (Dudkin et al., 1995; 1996). All of these results were also supported with behavioral changes in performance; NMDA-agonists caused a significant increase in performance whereas NMDA-antagonists caused a significant decrease in performance.

Recently, the main NMDA-antagonist used in these pharmacological studies has been ketamine. Ketamine is much less potent than other noncompetitive NMDA-antagonists such as phencyclidine and MK-801 and thus induces much less behavioral side-affects unlike the phencyclidine-induced psychosis or exacerbation in psychotic symptoms in patients (Krystal et al., 1994). High doses of phencyclidine in rats has been shown to produce pathological neuronal changes in the brain, but due to ketamine’s decreased potency and the use of low doses, these side-affects are uncommon (Olney et al., 1989; see for review Mouri et al., 2007). At high, fully anesthetic doses, ketamine has been found to bind to μ- and σ-opioid receptors (Øye et al., 1991), in addition to dopamine and serotonin receptors (see for review Wolff and Winstock, 2006), which could cause other behavioral side-affects. However, in the studies discussed below the doses of ketamine administered are below anesthetic effects, as all the subjects are awake...
and behaving. At these doses, ketamine’s effect on other receptors beside NMDA is minimal.

In 2002, Taffe and colleagues conducted a study investigating the effects of ketamine on cognitive processes. They ran macaque monkeys on a battery of cognitive tasks, including (but not limited to) response time, motor movement and visual working memory to determine the effect sub-anesthetic doses of ketamine (0.3-, 1.0- and 1.78-mg/kg ketamine, intramuscular) had on the monkey’s performance in these tasks. They used self-ordered and delayed match-to-sample tasks to assess visual working memory performance. Within these tasks, the authors were able to manipulate memory load as well as the retention interval duration. The data showed a decrease in performance at the highest ketamine dose for the delayed match to sample task, and a decrease in performance at the higher memory load for the self-ordered task (Taffe et al., 2002) (Fig. 6). The effect of ketamine on delayed match-to-sample task performance has also been reported a previous study, although the subject was only required to remember one item, and thus the effect of sub-anesthetic doses of ketamine (2- and 4-mg/kg) on memory load was not assessed in this study (Buccafusco and Terry, 2009). Studies using this task, but with other NMDA-antagonists, have also reported similar results (Buffalo et al., 1994; Frederick et al., 1995).

A detrimental effect of sub-anesthetic doses of ketamine (approximately 1-mg/kg) on delayed response task performance has also been reported, where monkeys are required to indicate a baited well out of two possible wells after varying retention
Figure 6. Effect of ketamine on delayed matched to sample performance. The mean (± S.E.M.; N = 6) proportion of correct choices was reduced in a dose- and delay-dependent manner consistent with the interpretation that ketamine increased the rate of forgetting. (Taffe et al. 2002)
intervals. The results showed a decreased performance compared to control with a further decreasing performance at increased retention intervals (Roberts et al., 2010a; Roberts et al., 2010b; 2010c; Castner et al., 2010; Castner et al., 2011).

All of these studies support the hypothesis that NMDA receptors play an important role in working memory, most likely by stabilizing persistent activity so that it lasts throughout the retention interval.

1.4 - Scope of the Study

Numerous computational and cellular studies have shown evidence in support of the hypothesis that NMDA receptors play an important role in the ability of an individual to maintain mnemonic information. However, the effect of NMDA-antagonists on working memory task performance has not been studied adequately, as the tasks and experimental design used allowed for the influence of other cognitive functions besides working memory. Therefore, behaviorally, the effect of NMDA-antagonists on working memory is still not accurately known. Additionally, these studies have not adequately investigated a key working memory characteristic: its limited capacity.

As neuroimaging studies have shown an increase in persistent activity with increasing memory load, what effect would ketamine have on memory loads below and above working memory capacity? If NMDA does modulate persistent activity, then antagonizing the receptors should show a greater behavioral effect with increasing memory loads.
This study aims to determine the effect of acute, low-dose ketamine (<1-mg/kg) on the working memory performance of monkeys performing a visual sequential comparison task. This task has been shown to provide accurate measures of working memory in addition to allowing easy manipulation of memory load. Animals were given low-dose ketamine and run on the task immediately after administration, as ketamine shows a behavioral effect as fast as 4 minutes post injection (Bergman, 1999). This contrasts previously studies that have waited up to 45 minutes after drug administration, at which point an accurate interpretation of the effect of dose is questionable (Buccafusco and Terry, 2009).

Additionally, animals will be run on low and high memory load arrays to determine the effect of ketamine on the maintenance of working memory above and below capacity.
Chapter 2 – Materials and Methods

2.1 - Subjects and Apparatus

Data were collected from three female rhesus monkeys (*Macaca mulatta*, 5.0 – 6.0 kg, 10-11 years old) cared for under experimental protocols approved by the Queen's University Animal Care Committee and in accordance with the Canadian Council on Animal Care guidelines. Animals were prepared for experiments by undergoing surgery, in which a head restraint and subconjunctival search 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 post-surgery recovery period, after which they were trained with operant conditioning and positive reinforcement to perform fixation and saccade tasks for a liquid reward until satiation.

Behavioural paradigms, visual displays, and data acquisition were controlled using the QNX Real-Time Experimentation Software (REX) system (Hays et al., 1982). The 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 (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 the magnetic search coil technique (Robinson, 1963). Field coils were used to generate opposing vertical and horizontal magnetic fields around the animal. The
voltage recorded was proportional to the horizontal and vertical angular eye position generated from the scleral eye coil sampled at 1kHz and accurate to 0.1°. For monkey B, eye movements were recorded using an infrared eye-camera system (Eyelink II, SR Research) running on a Dell Dimension 8300 computer, with a sampling rate of 500 Hz.

Each stimulus array was defined as a set of two or four colored squares positioned at an eccentricity of 10° from a central fixation spot. For each set size, the spatial configuration of the stimuli remained identical across trials. For set size two, stimuli were on the right and left side of the fixation spot. For set size four, stimuli were on the right, left, directly above and below the fixation spot (Fig. 7). Stimuli were identical in size (1.2°x1.2°), and closely matched in luminance (10 cd/m²). Each trial was randomly assigned a set size of two or four stimuli, the colors of which were drawn from a library of 6 predetermined highly discriminable colors; red (CIE x = 0.635, y = 0.327), green (CIE x = 0.289, y = 0.600), blue (CIE x = 0.155, y = 0.064), magenta (CIE x = 0.321, y = 0.156), yellow (CIE x = 0.469, y = 0.458) or cyan (CIE x = 0.219, y = 0.319). The same color could not appear at more than one location in the array. The luminance and chromaticity of each stimulus was measured using a Minolta photometer (CRT Color Analyzer, CA-100 Plus).
Figure 7. Depiction of a correctly performed trial in the visual sequential comparison task. Dotted circle and arrow represent eye position and saccade response.
2.2 - Visual Sequential Comparison Task

Each experimental trial was performed in complete darkness, except for the relevant visual stimuli. Between trials the screen was illuminated with diffuse white light (1.5 cd/m$^2$) for an interval of 1000-1500 ms to prevent dark adaptation.

Each trial (Fig. 7) began with the appearance of a fixation spot (0.5º, CIE $x = 0.30$, $y = 0.289$, $L = 10.2$ cd/m$^2$) in the centre of the display monitor. The animal was required to look at the central fixation spot within 1000 ms of its appearance. After a successful continuous fixation for 500-800 ms, a memory array composed of randomly determined set of two or four stimuli was presented during which the animal was required to maintain fixation on the central fixation spot. After a presentation time of 500 ms, the stimuli (except for the central fixation spot) were removed for 1000 ms (hereafter referred to as retention interval). The array was then presented again (test array), with one stimulus being randomly assigned a different color and the central fixation spot dimmed (L=1.37 cd/m$^2$). Within 1 second following the display of the test array, the monkey was required to initiate a single saccade within a computer-defined window to the stimulus that had changed (hereafter changed stimulus). If the task was correctly performed the trial was labeled ‘correct detection’ and the animal was given the maximal liquid reward. If the target stimulus was not found, the trial was labeled as ‘incorrect’ and no reward was given. Monkeys’ behaviors were monitored to ensure they did not make saccades to non-stimulus locations, as these responses would not be due to errors in memory and
hence will not be included in the analysis. Fortunately, none of the monkeys exhibited such behavior.

The monitor screen was illuminated with diffuse white light (1.5 cd/m$^2$) during the inter-trial interval (1000 ms following correct trials, 3000 ms following incorrect trials) to prevent dark adaptation.

2.3 - Experimental Procedures

Before experiments were conducted and data collected, all animals were trained on an identical paradigm with set sizes two to five (see for details Heyselaar et al., 2011) until a stable performance was reached (monkey B: 57 sessions, monkey G: 109 sessions, monkey F: 30 sessions). Based on those results, the current study was run with set size two and four to represent low and high mnemonic loads respectively.

Before data were collected for this study, animals performed at least 10 training sessions in this reduced version of the visual sequential comparison task. Additional sessions with no retention interval were collected to control for the effect of ketamine on processes other than memory maintenance, as these sessions would only require minimal use of visual working memory. For these trials, the monkey was required to make a response immediately after the 500 ms presentation time, cued by the dimming of the fixation spot (L=1.37 cd/m$^2$).

Each treatment session was composed of an initial block of 100 trials, during which motivation could be assessed, followed by a treatment block consisting of a
minimum of 600 trials (the equivalent of > 60 minutes). Between the initial block and the
treatment block, animals randomly received an intramuscular (quadriceps for monkey B
and G, forearm for monkey F) injection of ketamine (Ketaset; 0.25- or 0.5-mg/kg diluted
to 0.3ml with saline), 0.3ml of saline solution (0.9% sodium chloride) as a vehicle
control, or no injection. The same individual administered all injections. Although
ketamine is a dissociative anesthetic, doses ≤ 1.0-mg/kg have been reported not to cause
debilitating effects on behavior while reliably affecting saccade latency and metrics
(Shen et al., 2010).

Results from treatment sessions were contrasted with results from control
sessions, which were all collected the day before each treatment session. Data were
collected from three treatment sessions per treatment per monkey (33,324 trials). For the
data collected without a retention interval, two treatment sessions per treatment per
monkey was collected (23,916 trials). Before this study, each monkey had received a
varying total amount of ketamine for anesthetic and study purposes: monkey B, 4.7ml;
monkey G, 21.3ml; and monkey F, 10.2ml.

2.4 - Data Analysis

To assess the effect of ketamine treatment on working memory performance,
response accuracy and latency were calculated for each dose and control sessions.
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. These measures were pooled within each session type, treatment doses and control. Response accuracy was consistent across each animal’s control sessions; pair-wise $\chi^2$ tests on the ensemble of trials in these sessions revealed no difference that was statistically significant. Pair-wise Kolmogorov-Smirnov tests were run to assess the variability in response latency between each animal’s control sessions. Differences in response latency were obtained between some sessions in all three monkeys (p<0.05), but these differences were generally minimal. The median response latency ranged within approximately 10% (monkey B: 178 – 192 ms; monkey G: 158 – 168 ms; monkey F: 174 – 203 ms), which may reflect the natural day-to-day variability in this measure that is also expected in treatment sessions independently of the treatment effects.

The effect of ketamine on response latency and accuracy was quantified by calculating the average response latency and the proportion of correct trials respectively in a 30-trial moving window for both control and ketamine sessions. Each measure was then plotted against the mean time (after injection) of the 30-trial window. To quantify all behavioral effects of ketamine injections, we determined the interval during which response latency in the treatment sessions was significantly longer (rank sum test, p<0.05) by at least 15% from the control sessions. This was done to maximize the number of trials included in the interval, to ensure there was sufficient statistical power.

The statistical significance of the differences in response accuracy between treatment and control sessions was determined using $\chi^2$ tests for each window (p<0.05).
A treatment session was deemed to show a significant effect on response accuracy if at least 15 consecutive $\chi^2$ tests were significant.
Chapter 3 - Results

The behavioral effects of low-dose ketamine injections were rapid but time-limited. Figure 8A illustrates the time course of the effect on response latency for monkey B at all three doses of ketamine. In this animal, following the 0.25-mg/kg ketamine injection, response latency became significantly longer than control at 1.5 minutes post injection (p<0.05, rank-sum test) reaching a peak of 35.9% increase from control (255 vs 186 ms) within 6 minutes. This was followed with a more gradual decay lasting up to 17.5 minutes post injection. Response latency following the 0.50-mg/kg ketamine injection showed a more pronounced effect with a 72.5% increase from control (324.1 vs 186 ms) occurring 8 minutes post injection, and lasting 31 minutes. No significant effect on response latency was observed following 0-mg/kg ketamine (saline) injections.

To quantify the behavioral effects of low-dose ketamine injections, the interval during which response latency in the treatment sessions was significantly longer (rank sum test, p<0.05) by at least 15% from the control sessions was determined. For monkey B, this time interval extended from 1.5 minutes until 17.5 minutes after the injection of 0.25-mg/kg ketamine (Fig. 8A). The effect was much longer lasting following the 0.50-mg/kg ketamine injection. This pattern was also seen in the other monkeys, with longer time intervals at the higher dose (Table 1). Therefore, to standardize the analysis interval during which the behavioral effects of low-dose ketamine injections were assessed, all
Table 1. Time window of significant increase in response latency for sessions with a retention interval. Time in minutes.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Dose (mg/kg)</th>
<th>Start</th>
<th>End</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.5265</td>
<td>17.507</td>
<td>15.9805</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.1921</td>
<td>31.004</td>
<td>28.8119</td>
</tr>
<tr>
<td>G</td>
<td>0.25</td>
<td>2.882</td>
<td>22.06</td>
<td>19.178</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.6561</td>
<td>24.232</td>
<td>22.5759</td>
</tr>
<tr>
<td>F</td>
<td>0.25</td>
<td>4.7355</td>
<td>9.0143</td>
<td>4.2788</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.5365</td>
<td>30.657</td>
<td>29.1205</td>
</tr>
</tbody>
</table>
Figure 8. Representative effects of ketamine on visual sequential comparison task performance (monkey B). A Mean response latency for each 30 trial running window post ketamine injection. The grey shaded area indicates the epoch during which there was a significant increase in response latency from control for the 0.25-mg/kg ketamine session (p<0.05, rank-sum test). This is the window used for all further analysis. Black line represents the control data; dotted line represents 2 standard deviations above and below the average control value B Mean response accuracy for each 30 trial running window post ketamine injection. Grey shaded area is an overlay of the grey shaded area from part A showing that the drop in performance corresponds with the significant increase in response latency. Black horizontal line represents average proportion correct for the entire control session; dotted lines represent 95% CI on either side of the control value. C Proportion of omission errors (out of total errors) executed per 2 min intervals following ketamine injection. Grey shaded area is an overlay of the grey shaded area from part A showing that the increase in number of omissions corresponds with a change in behavior shown in parts A and B.
further analyses were conducted within the interval identified in each animal’s 0.25-mg/kg treatment injections. In monkey G, this interval lasted from 2.9 minutes to 22.1 minutes post ketamine injection, whereas in monkey F it lasted from 4.7 minutes to 9 minutes (Table 1). Within this analysis interval, mean response latency increased significantly following ketamine injections and in a dose-dependent manner across animals (p<0.0001, ANOVA) (Fig. 9A). Figure 9B shows the same data as percent change.

Figure 8B illustrates the effect of low-dose ketamine injections on response accuracy for monkey B on the task array of set size 4. Following each ketamine injection, the proportion of correct responses dropped quickly and remained lower than control for about 20 minutes, with a more pronounced drop at the higher ketamine dose. Within the predetermined analysis interval, the decrease in the proportion of correct responses at set size 4 was significant for both the 0.25-mg/kg and 0.50-mg/kg ketamine injections (p<0.05, \( \chi^2 \) test)(Fig. 10A). This monkey also showed a non-significant decrease in response accuracy for set size 2 for the 0.25-mg/kg ketamine injection (p=0.4280), but no effect for set size 2 at the 0.50-mg/kg ketamine injection. Figure 10 summarizes the results for all monkeys. Monkey G showed a decrease in performance following the 0.50-mg/kg ketamine injection at both set sizes, but no significant changes in response accuracy following the 0.25-mg/kg ketamine injection was observed (p>0.5228). Monkey F showed a decrease in accuracy at 0.50-mg/kg ketamine injection but this did not reach statistical significance (p>0.2317). On closer investigation of the individual sessions, one
Figure 9. Changes in response latency following ketamine injections for the sessions with a retention interval. **A** Average response latency (± SE) for each animal and for each treatment within the analysis interval. Heavy bars represent a significant difference from control (no injection) sessions (p < 0.05, rank-sum test).

**B** Percent change in response latency for each animal and for each treatment compared with corresponding control data (monkey B, 187.8 ms; monkey G, 167.9 ms; monkey F, 197.4 ms) within the analysis interval. Heavy bars represent a significant difference from 0 (p < 0.05, rank-sum test).
Figure 10. Changes in response accuracy following ketamine injections for the sessions with a retention interval. 
A Average proportion correct (± 95% CI) for each animal, for each treatment and for each set size within the analysis interval. Heavy bars represent a significant difference from control (p<0.05, χ2 test) B Percent change in response accuracy for each animal, for each treatment and for each set size within the analysis interval relative to control accuracy (monkey B: set size 2 = 0.85; set size 4 = 0.69, monkey G: set size 2 = 0.82; set size 4 = 0.54, monkey F: set size 2 = 0.84; set size 4 = 0.54). Heavy bars represent a significant difference from 0 (p<0.05, χ2 test)
A 0.50-mg/kg ketamine session did show a significant decrease in the proportion of correct responses for set size two. In summary, ketamine appears to have a detrimental effect on response accuracy for two out of the three monkeys.

The calculated response accuracy of the monkeys is influenced by two different incorrect responses: 1) detection errors, i.e. making a saccade to an unchanged stimulus, and 2) omission errors, i.e., the failure to make any saccade during the presentation of the test array. Omission errors occurred almost exclusively following ketamine injections and therefore may reflect a failure in the comparison process (Fig 8C). As another measure of working memory performance, the proportion of omissions (out of the total incorrect responses) within the predetermined analysis interval for each ketamine injection and control sessions was tallied, and compared using Fisher’s Exact test. The number of trials that contributed to this analysis is shown in Table 2.

The proportion of omission errors is shown in Figure 11. For monkey B the distribution of these omission errors were significantly higher during the predetermined analysis interval for both set sizes following the 0.50-mg/kg and 0.25-mg/kg ketamine injection (p<0.05, Fisher’s Exact test) when compared to control. Monkey G and monkey F made significantly more omission errors during the predetermined analysis interval for both set sizes following the 0.50-mg/kg ketamine injection (p<0.05, χ² test), but no effect following the 0.25-mg/kg ketamine injection (monkey G: p=0.9709, χ² and Fisher’s Exact test; monkey F showed 0 omission errors for both ketamine and control sessions following 0.25-mg/kg ketamine injection). This analysis shows a significant
Table 2. The total number of errors exhibited per set size for each ketamine dose for sessions with a retention interval.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Dose (mg/kg)</th>
<th>Set Size</th>
<th># Errors</th>
<th># Omissions</th>
</tr>
</thead>
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<tr>
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<td>15</td>
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</tr>
<tr>
<td></td>
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<td></td>
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</tr>
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<td>2</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>73</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No Injection</td>
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<td>2</td>
</tr>
<tr>
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<td></td>
<td>4</td>
<td>58</td>
<td>4</td>
</tr>
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</table>
Figure 11. Proportion of error trials (± 95% CI) that were caused by omissions for each animal, for each treatment and for each set size, in the retention interval sessions, within the analysis interval. Heavy bars represent a significant difference from control (p<0.05, Fisher’s Exact test).
increase in the proportion of omission errors, compared to error detection, following ketamine injection.

Another measure of working memory performance can be taken as the number of breaks from fixation the animal makes during the retention interval (retention breaks). An increase in these breaks after ketamine injection could indicate that ketamine is affecting the monkeys’ ability to either encode the initial stimulus properly, or an inability to maintain it until the end of the retention interval. To analyze the number of retention breaks, pairwise comparisons were run between the proportion of fixation breaks (out of the total number of trials) within the analysis interval in ketamine and control sessions. Fixations breaks were present in the control sessions, although at a low proportion (monkey B: 17%; monkey G: 2%; monkey F: 2%). There was a significant increase in their occurrence following ketamine injection for two monkeys (monkey B: 26% p<0.0001; monkey G: 5% p<0.05; monkey F: 0%, Fisher’s Exact Test).

The animals’ motivation could also affect their performance in the task. One way to measure their motivation is to determine the number of trials they aborted as a function of time; an aborted trial refers to the event when the monkey breaks fixation from the central fixation spot during the memory array presentation. When these happen during the initial presentation of the stimulus array, they are referred to as memory breaks and an increase in these could indicate a decrease in motivation to initiate the trial. These were analyzed the same was as the retention breaks mentioned above. There were very few fixation breaks for all three monkeys in the control session (monkey B: 1.7%; monkey G:
0.8%; monkey F: 0%) and only monkey B showed a significant, yet moderate, increase following ketamine injection (monkey B: 5% p<0.05; monkey G: 1.5% p=0.24; monkey F: 0 Fisher’s Exact test), suggesting no effect of ketamine injection on monkey G and F’s motivation. Even for monkey B, the proportion of aborted trials is quite small, and thus the animal’s motivation should not be detrimentally affected.

If ketamine did impair the monkeys’ ability to maintain a memory representation, it can be hypothesized that there may be an increase in the amount of guessing. As discussed in Heyselaar et al. (2011), if the subject employed the diligent guessing strategy, then response times for the error trials should be equal or greater to the correct trials. The difference between the latency of correct and incorrect responses during the time course for ketamine was tested for each animal with a three-way ANOVA, with dose (0.50-mg/kg, 0.25-mg/kg, 0-mg/kg and control), set size (2 and 4), and trial type (correct vs. error) as factors. There was no effect of ketamine dose on the difference in response latency between correct and incorrect trials. All monkeys did show an effect of set size on response latency (p<0.001), except for monkey B who showed no effect at set size 2. Post hoc analysis showed that in the cases of a significant effect, the response latency of incorrect trials was significantly longer than of correct trials (p<0.05; Student-Newman-Keuls test). This suggests that while under the influence of ketamine, the subjects did not engage in diligent guessing, something that could be hypothesized if their memory maintenance is impaired.
3.1 – Non - (memory) maintenance processes

The working memory processes required for the visual sequential comparison task can be divided into three stages: sensory integration (memory consolidation), memory maintenance, and a response in the form of a motor command. A decline in response accuracy could be caused by a failure at any one of these stages. To control for ketamine affecting two non-maintenance stages (sensory integration and motor response), we ran a subset of sessions without a retention interval, as it requires minimal memory maintenance for the task to be completed successfully.

Figure 12 shows the effect of ketamine on response latency for all three monkeys. The pattern of effect on response latency is the same as it was for the retention interval sessions: ketamine causes a significant increase in the response latency that is more pronounced and longer at the higher doses (p<0.05, rank sum test). The sessions without a retention interval showed a shorter time epoch of ketamine effect across all monkeys, and as such the analysis window was re-determined for the sessions without a retention interval, although still using the 0.25-mg/kg ketamine injection sessions (Table 3). Due to the shortness of the task, even though the analysis interval is shorter, the number of trials completed is roughly equivalent to the sessions with a retention interval, hence two repeats was adequate enough to have the same statistical power between the two experiment types.

Ketamine still produced an effect on response accuracy (Fig 13). Monkey B showed a significant decrease in response accuracy for set size 4 following both doses of
Table 3. Time window of significant increase in response latency for sessions without a retention interval. Time in minutes.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Dose (mg/kg)</th>
<th>Start</th>
<th>End</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
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<td>13.206</td>
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</tr>
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Figure 12. Changes in response latency following ketamine injections for the sessions without a retention interval. A Average response latency (± SE) for each animal and for each treatment within the analysis window. Heavy bars represent a significant difference from control (p<0.05, rank-sum test). B Percent change in response latency for each animal and for each treatment compared with corresponding control data (monkey B, 211.4 ms; monkey G, 187.8 ms; monkey F, 207.1 ms) within the analysis window. Heavy bars represent a significant difference from 0 (p<0.05, rank-sum test).
Figure 13. Changes in response accuracy following ketamine injections for the sessions without a retention interval. A Average proportion correct (± 95% CI) for each animal, for each treatment and for each set size within the analysis window. Heavy bars represent a significant difference from control (p<0.05, χ² test) B Percent change in response accuracy for each animal, for each treatment and for each set size within the analysis window relative to control (monkey B: set size 2 = 0.98; set size 4 = 0.96, monkey G: set size 2 = 0.96, set size 4 = 0.85, monkey F: set size 2 = 0.98; set size 4 = 0.94). Heavy bars represent a significant difference from 0 (p<0.05, χ² test)
ketamine injection (p<0.05, $\chi^2$ test), whereas monkey G showed a significant effect on set size 2 following the 0.50-mg/kg ketamine injection only (p<0.05, $\chi^2$ test). Monkey F showed a significant decrease in response accuracy for both set sizes following the 0.50-mg/kg ketamine injection (p<0.05, $\chi^2$ test). However, on closer inspection of the individual sessions at each dose for each monkey, it was discovered that the effects are inconsistent between repeats; this was not observed for the sessions with a retention interval were all the individual sessions showed an effect on response latency and accuracy. For monkey B and G, one session showed a significant decrease and the other did not. Monkey F’s results were consistent across all repeats. Despite this inconsistency, there was still a response latency effect for all repeats, suggesting that the lack of effect on performance is not due to insufficient ketamine administration, or tolerance. Instead, these results suggest an inconsistent effect of ketamine on non-maintenance processes, resulting in a detriment in performance.

Ketamine could be affecting sensory integration and/or the ability for the monkey to make a motor response. The ability to elicit a motor response can be quantified by the number of omissions the monkey made during this subset of sessions (Fig 14). There are still significantly more omissions made during the analysis interval in the sessions without a retention interval, suggesting that the ability to make a motor response may have been effected (Table 5). Monkey B showed a significant increase in omissions for the set size 4 arrays for both ketamine injections, whereas monkey G showed a significant increase in omissions for both ketamine injections and for both set sizes.
Figure 14. Proportion of error trials (± 95% CI) that were caused by omissions for each animal, for each treatment and for each set size in the sessions without a retention interval, within the analysis interval. Heavy bars represent a significant difference from control (p<0.05, Fisher's Exact test).
Table 4. The total number of errors exhibited per set size for each ketamine dose for sessions without a retention interval.

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Dose (mg/kg)</th>
<th>Set Size</th>
<th># Errors</th>
<th># Omissions</th>
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<tr>
<td>No Injection</td>
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<td>8</td>
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(p<0.05, Fisher’s Exact test). Monkey F also showed a significant increase in omissions, even though this monkey failed to show any for the sessions with a retention interval. This monkey showed it following the 0.50-mg/kg ketamine injections. These results suggest that ketamine also affects non-maintenance processes in addition to memory maintenance.
Chapter 4 - Discussion

Monkeys were administered low-dose ketamine injections while completing a visual working memory task with varying memory loads. All three monkeys showed a significant increase in response latency shortly after the ketamine injection that lasted for an average of 20 minutes. During this time period, the monkeys showed a decrease in response accuracy as well as an increase in omission errors and retention breaks, indicating that NMDA receptor integrity is plays an important role in the maintenance of working memory.

4.1 – Results

4.1.1 – Effects on Response Latency

Ketamine caused a significant dose-dependent increase in the response latency of all three monkeys, suggesting that the process of neural activity integration underlying the accumulation of sensory evidence was slowed down. This increase in eye movement response latency following ketamine injection has been reported elsewhere (Shen et al., 2010; see also Condy et al., 2005). The effect was short-lived, which is consistent with the known pharmacokinetics of ketamine (Bergman, 1999). As this effect is present in all the monkeys it indicates that ketamine is administered in a high enough dose to cause a behavioural effect. If any of the monkeys had developed a tolerance (Pouget et al., 2010) due to their previous exposures to the drug, this would have presented as a lack of (or lesser) effect on response latency, which we did not find.
4.1.2 – Effects on Response Accuracy

All three monkeys showed a detrimental effect of ketamine on response accuracy, although each monkey showed a different pattern. Monkey B showed a memory load dependent response, with the higher memory load causing a significant decrease in response accuracy, although with a slightly higher magnitude of effect at the higher ketamine dose. Monkey G showed a dose dependent response to ketamine, with increasing doses of ketamine causing a significant decrease in response accuracy regardless of memory load. Monkey F also showed a dose-dependent drop in working memory response accuracy, although this did not reach significance. As the analysis interval for monkey F was much shorter compared to the other two monkeys, there may not have been enough trials to gain sufficient statistical power to detect significance.

As previous studies have shown an increase in the magnitude of persistent activity with high memory loads until the capacity limit is reached, it was hypothesized that antagonizing the NMDA receptors should show a greater behavioural effect with increasing memory loads; a hypothesis that is difficult to support from this data alone.

Monkey B and G have been previously run in a study that looked at the effect of increasing memory load in the visual sequential comparison task on performance (Heyselaar et al., 2011) and therefore a working memory capacity estimate is known for these two monkeys (monkey B: k=2.4; monkey G: k=1.8; capacity (k) = p(correct) x set size, (Pashler, 1988; Cowan, 2001)). As such, it is likely that set size 2 – although
included to represent a memory load below capacity, may be above the capacity limit of monkey G, but still below the capacity limit for monkey B.

Therefore, monkey B may still have more resources available to allow for the encoding of more stimuli past set size 2. This may require more NMDA-receptor activation, and thereby causes a differential effect between the two memory loads used in this study. Monkey G, however, may not have any more resources available due to her low estimated working memory capacity, and thus a further increase in stimuli to encode will not cause an increase in NMDA-receptor activation. This would be represented behaviorally by a lack of a differential change with increasing memory load, which is consistent with what was observed in this study. This account may also apply to monkey F, who showed a capacity estimate (k=1.9) similar to monkey G.

### 4.1.3 – Omission Errors and Retention Breaks

All three monkeys made significantly more omission errors following ketamine injection compared to control. If this was due to a failure to maintain the stimulus in memory, this could also be reflected in the response latency of the error detection (the other type of incorrect trials). If the subject was unable to maintain the stimulus in memory, then during the response stage, they could make a random response to any stimulus with the chance of getting a reward. These responses do not need to be preceded with a comparison process between the stimulus and test arrays and as such should be significantly faster than the correct responses. However, the response latency analysis of
incorrect versus correct trials showed that there was no significant difference. Hence, they do not make random responses; instead, maintaining fixation could be the response expressed when they were unable to maintain the stimulus array over the retention interval.

This hypothesis can be explained by looking at the previous training these monkeys have had. Monkey B and G have been previously trained on no-change trials - trials where none of the stimuli had changed color – and they were trained to respond to a no-change by maintaining fixation (Heyselaar et al., 2011). Therefore, perhaps the increase in omission errors is an indicator that the monkeys do not remember and hence respond by maintaining fixation. What is interesting, however, is that monkey F, who has not had this training, also showed an increase, albeit smaller, in the number of omissions following ketamine injection. This suggests that perhaps ketamine has an effect on non-maintenance processes that could result in the monkeys being unable to make a response.

Additionally, the increase in retention breaks may be another indicator of a failure in memory maintenance. If the memory maintenance decayed completely before the end of the retention interval, a phenomenon that has been measured neurally (Funahashi et al., 1989; Zhang and Luck, 2009), the monkeys may have responded by aborting the trial. An increase in the retention breaks under the influence of ketamine suggests that the memory trace is more vulnerable to sudden decay.
4.1.4 – Effects on processes other than memory maintenance

To control for any effect of ketamine on non-maintenance processes, the monkeys were run on sessions without a retention interval. In theory, these sessions should show minimal decline in response accuracy as the maintenance component plays a smaller role in these sessions. However, all three monkeys did show a significant decline in response accuracy, indicating ketamine was affecting other processes that are necessary to successfully complete the task.

A ketamine effect on a task performance without a retention interval is not uncommon. It has been observed in a previous study: ketamine administration (>2.0-mg/kg) caused a significant decline in performance in a delayed match-to-sample task without a retention interval (Buccafusco and Terry, 2009). The study explains this phenomenon as ketamine affecting other cognitive processes, such as attention, due to the high ketamine dose used (Krystal et al., 1994; Paule et al., 1998). Even though the ketamine doses used in my study are not as high, any deficit in attention would present itself as an increase in fixation breaks, as the monkey cannot focus attention long enough to complete a task without being distracted. None of our monkeys showed a sizeable increase in fixation breaks, indicating that a deficit of attention does not account for the behavioural changes seen. However, ketamine could still be affecting other processes.

NMDA receptors are widely distributed throughout the brain, primarily in the hippocampus and the outer layers of the cerebral cortex (Maragos et al., 1988; Young et al., 1990). As ketamine was administered systemically, it is reasonable to argue that
certain non-memory maintenance processes necessary for successful completion of the task could also have been hindered. In fact, drifts in eye position following ketamine injection were observed indicating that ketamine was affecting neurons in the oculomotor system (Mettens et al., 1990; see also Seung et al., 2000). Two of the main processes that could be affected are the monkey’s ability to discriminate color, and/or the ability to plan/execute a motor response. Lesion studies have shown that color discrimination is regulated in the lateral geniculate nucleus (LGN) (Schiller et al., 1990), and that the LGN can be antagonized by ketamine administration (Kwon et al., 1992). Studies have shown that macaque monkeys are unable to complete simple color discrimination tasks after the injection of NMDA-antagonists such as MK-801 (Buffalo et al., 1994), and thus it is reasonable to assume that this effect could also be present in our study. If the monkeys were unable to discriminate color, it would be harder to determine which of the colors had changed in the test array, and thus their performance would have shown a detriment not due to a failure in memory maintenance, but because of a failure in perception.

NMDA-antagonists have also been shown to decrease reflex and locomotion in rats (Carter, 1994), due to their distribution in the basal ganglia (Maragos et al., 1988) where they regulate long-term potentiation. A neurophysiological study in macaque monkeys showed that there are movement related neurons in the motor cortex that were suppressed by NMDA-antagonist APV (Shima and Tanji, 1998). Additionally, ketamine has also been shown to affect eye movements. Studies in the rat have shown that the bursts of activity in the superior colliculus result from NMDA-mediated reverberation.
These high-frequency bursts are a critical determinant of activating the down-stream saccade generator circuits (Schiller and Koerner, 1971; Wurtz and Goldberg, 1972). Therefore, there is a possibility that ketamine could have antagonized these neurons, affecting the monkeys’ ability to make a response following ketamine injection.

4.2 – Literature Review

4.2.1 – NMDA Basis of Working Memory

The effect of ketamine on the visual working memory task performance with increasing memory load has been studied previously (Taffe et al., 2002). However, the task used in this study was not a direct measure of working memory, as there were a variety of strategies that were not controlled for that could have influenced the resulting performance. There have been other studies that used the delayed response task, here again the experimental designs of these studies (drug administration) led to equivocal evidence in support of an NMDA-antagonist effect on working memory maintenance (Castner and Goldman-Rakic, 2004; Roberts et al., 2010a; 2010b; 2010c; Castner et al., 2011). The current study used a task that provides a more accurate measure of working memory task performance, and thus the results shown here provide stronger evidence in support of the hypothesis that NMDA-antagonists affect working memory maintenance.

However, previous studies have also shown that the ketamine effect on working memory performance can be reversed by administering AMPA-, D1-, and GABA-
agonists, as well as glycine transport inhibitors and nicotinic acetylcholine receptor stimulation. Therefore, is NMDA really necessary for working memory maintenance? All of these agonists enhance NMDA receptor activity; as ketamine is only a partial agonist, some NMDA receptors will still be available and these agonists help enhance that signal (see for review Castner and Williams, 2007). For example, NMDA has binding sites for both glutamate and glycine; a glycine transport inhibitor would only increase the concentration of glycine in the synaptic cleft, increasing binding with NMDA receptors. With respect to dopamine, however, it has been shown that the two receptor types are intricately connected. For example, Seamans and colleagues (2001) have shown that D1-receptor stimulation selectively enhances the NMDA-mediated components of the EPSC in layer V pyramidal cells. Therefore, the reversal of the working memory deficit is possible as the drugs used enhance the NMDA receptors that are not blocked by antagonists. This provides more evidence that working memory maintenance is dependent on NMDA-receptor function.

4.2.2 – Maintenance vs. Manipulation

Reviews on human studies have suggested that NMDA-antagonists may affect the manipulation of working memory, not the maintenance (Fletcher and Honey, 2006). The difference between these two being that the subject must not only retain the information, but must also change it in some way. A prime example of this would be mental math, where you are given a set of numbers and instructions that you must remember and follow. But what really is the difference between manipulation and maintenance in terms
of neural circuits and mechanisms? Neuroimaging studies have shown an increase in activation in the DLPFC during both maintenance and manipulation tasks (see for review D'Esposito et al., 2000), and although currently there are no models for the neural mechanisms underlying only manipulation, it can be assumed that sustained activity is still necessary, for which NMDA receptors are still a likely candidate due to its slow kinetics. Therefore, even though human verbal working memory studies have shown no effect on maintenance performance following ketamine injection (Ghoneim et al., 1985; Honey et al., 2003), this may be due to the tasks used in the studies as verbal working memory tasks can employ many strategies to aid in the completion of the task. Indeed, certain human spatial working memory tasks have shown a deficit in maintenance performance following ketamine injection (Newcomer et al., 1999), in addition to all the monkey studies mentioned previously (e.g., Buffalo et al., 1994; Frederick et al., 1995; Dudkin et al., 2001; Buccafusco and Terry, 2009; Roberts et al., 2010a; Castner et al., 2010; Roberts et al., 2010b; 2010c).

4.3 – Concluding Remarks

4.3.1 – Limitations and Future Directions

Although this study shows that blockage of NMDA receptors with ketamine has a significant effect on working memory maintenance, it is difficult to conclude the effect ketamine has on response accuracy with increasing working memory load as each monkey presented with a different pattern of working memory deficit. One hypothesis is
that the working memory process was not challenged enough (or already over challenged) to produce an observable trend. Therefore, administering a higher dose of ketamine may help to challenge the system further, or at least to produce a third ketamine dose to compare to the two recorded here. Studies have shown that increasing doses of ketamine, although still sub-anaesthetic, start to cause greater deficits in behavior and thus any response accuracy deficit may not be due to a deficit in working memory processes. However, doses under 1.0-mg/kg ketamine have shown not to cause debilitating effects (Shen et al., 2010) and thus a 0.75-mg/kg ketamine dose should still show an effect on working memory without affecting other systems such as attention.

Additionally, local NMDA-antagonist injections into the brain areas known to contain neurons with persistent activity will help minimize the influence of other cognitive functions. This has been done previously using delayed response tasks and the study reported a significant deficit in working memory performance (Dudkin et al., 2001), complementing our results here. However, this has not yet been done with increasing working memory loads.

The effect of these injections can also be used to support the influence of other cognitive functions by determining the effect of local NMDA-antagonist application on the visual cortex and oculomotor centers. Previous studies have shown an increased deficit in performance with NMDA-antagonist application on the visual cortex (Dudkin et al., 2001) although no studies have yet looked into the effect of NMDA-antagonist
application in the frontal eye fields or superior colliculus on working memory performance.

A further limitation is the lack of effect shown by monkey F. This may be due to the small analysis interval obtained for this monkey, and hence there was not sufficient statistical power to be able to detect a significance. An additional repeat could provide extra trials that will help boost the power of the test if that is really the case, as this monkey did not show any effects of ketamine insensitivity, such as a decreased effect on response latency, that may have otherwise accounted for the lack of an effect.

4.3.2 - Conclusion

Overall, ketamine was observed to cause a deficit in working memory performance, providing evidence in support of the dependence of working memory maintenance on NMDA receptor integrity. Although the effect on memory load varied per subject, possibly due to individual differences in working memory capacity, this study provides solid evidence of the effect of NMDA-antagonists on working memory performance, as the task and experimental design used minimized the influence of other cognitive processes on performance.
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