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Investigating the Primate Prefrontal Cortex Correlates of Cognitive Deficits In the Ketamine Model of Schizophrenia

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Graduate Program in Anatomy and Cell Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy
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**INVESTIGATING THE PRIMATE PREFRONTAL CORTEX CORRELATES OF
COGNITIVE DEFICITS IN THE KETAMINE MODEL OF SCHIZOPHRENIA**

(Thesis format: Integrated Article)

by

Kevin J Skoblenick

Graduate Program in Anatomy and Cell Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
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London, Ontario, Canada

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Abstract

The World Health Organization has classified schizophrenia as one of the five leading causes of disability worldwide. Afflicting almost 1% of the world's population, the disease's greatest impact stems from its reduction in patients' cognitive faculties. In order to better study these impairments, a pharmacological model has been developed using the NMDA antagonist, ketamine. This disease model successfully recreates the cognitive dysfunction of schizophrenia, allowing researchers to search for associated electrophysiological changes.

In this project I examined the behavioural and neurophysiological effects of ketamine on non-human primates performing the anti-saccade task. Success in this task requires a degree of cognitive control over behaviour and previous studies have described poor performance in both patients with schizophrenia and healthy controls administered ketamine. Our intracranial recordings are localized in the prefrontal cortex (PFC), a region associated with many of the cognitive functions impaired in schizophrenia.

The first study shows that neurons in the PFC exhibit selectivity for the task rule. This rule selectivity is lost after ketamine administration due to an indiscriminate increase in the neuronal firing rate. These changes were also associated with an increased error rate and longer reaction times. The second study shows that neurons in the PFC are also sensitive to the outcome of the trial, firing more for

either correct or erroneous responses. Once again, selectivity is lost following ketamine administration and the neurons show increased, nonspecific activity. Lastly, we recorded the local field potential of the PFC and found changes in the oscillatory patterns during the anti-saccade task. Prior to ketamine there was a significantly stronger beta-band activity after correct trials compared to error trials, but this selective activity was lost due to an overall decrease in the outcome selective oscillatory events.

These findings show that ketamine's effect on the PFC is one of selectivity reduction. Patients with schizophrenia have been shown to require increased PFC activity but only reach moderate performance levels in cognitive challenges. It is possible that their brains suffer the same changes highlighted in this research. Although the signals are still present in their PFC, they are being lost amongst the noise.

Keywords

Schizophrenia, ketamine, prefrontal cortex, working memory, local field potential, single neuron electrophysiology, anti-saccade, macaque, performance monitoring

Co-Authorship Statement

Kevin J. Skoblenick, Thilo Womelsdorf, and Stefan Everling.

As the primary author of the three experimental chapters, Kevin Skoblenick was responsible for designing the experiments, collecting the data, performing the data analysis, and writing the completed manuscripts. Stefan Everling supervised all projects and assisted in experiment design, data analysis, and manuscript revisions for all three experimental chapters. Thilo Womelsdorf assisted in the data analysis for chapter 4.

Epigraph

“Any man could, if he were so inclined, be the sculptor of his own brain.”
— Santiago Ramón y Cajal, *Advice for a Young Investigator* (1897)

Acknowledgments

First, I would like to thank Dr. Everling for taking me on as a PhD student, especially with my non-traditional start. With his enthusiasm for scientific discoveries and his encouraging attitude regardless of what came up in the project, he has renewed my passion for science. I hope to use the skills I have learned through his mentorship to instill the same kind of excitement for science in others.

Secondly, I would like to thank my family: Mom, Dad, Janice, Stephanie, Roger, and now Malcolm for supporting me over these many years of post-secondary education. It has been a long road to this point and their unwavering backing has been a comfort I know I could always rely on.

I would also like to thank my friends in the Everling lab over the years: Darren, Sahand, Jessica, Daniel, Michelle, Iman, Michael, Sabeeha, Susheel, Jason, Nikoo, Chao, Tyler, Alex and Andree. From methodological troubleshooting to origami folding, they have made the lab a great place to be the last few years. Kevin Johnston, for invaluable advice with a dose of sanity over the last few years and Thilo Womelsdorf, for helping me understand advanced neuroscience concepts.

Lastly, I'd like to thank my friends outside the lab, both in London and around the world. You've kept me grounded and kept life interesting to say the least.

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List of Abbreviations

ACC – Anterior cingulate cortex

AMPA - α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANOVA – Analysis of variance

BOLD – Blood oxygen level dependent signal

Ca²⁺ - Calcium ion

CT – Computerized tomography

dIPFC – Dorsolateral prefrontal cortex

EEG – Electroencephalograph

ERN – Error-related negativity

ERP – Event-related potential

fMRI – Functional magnetic resonance imaging

FP – Fixation point

GABA - Gamma-aminobutyric acid

GAD67 - Glutamate decarboxylase

Hz – Hertz

LFP – Local field potential

LPFC – Lateral prefrontal cortex

MAP – Multi-acquisition processor

MD – Medial dorsal

Mg²⁺ - Magnesium ion

MK-801 – Dizocilpine

MMN – Mismatch negativity

MRI – Magnetic resonance imaging

ms – Millisecond

NMDA – N-methyl d-aspartate

NMDAR – N-methyl d-aspartate receptor

PCP – Phencyclidine

PET – Positron emission tomography

PFC – Prefrontal cortex

Pv – Parvalbumin

ODR – Oculomotor delayed response

ROC – Receiver operating characteristic

rTMS – Repeated transcranial magnetic stimulation

SRT – Saccade reaction time

μ s – Microsecond

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Chapter 1

Introduction

1.1 - Schizophrenia

Schizophrenia is a debilitating mental disorder affecting nearly 1% of the world's population. A recent report by the World Health Organization ranked it among the 5 leading causes of disability worldwide (World Health Organization, 2008). In Canada alone this accounts for over 230,000 individuals (Goeree et al., 2005) and as a result produces a large strain on an already exhausted healthcare system. It has been estimated the morbidity of the disease accounts for 70% of the disease burden due to lost work (Goeree et al., 2005). Due to the symptomatic profile of the disease schizophrenia's effect on Canada's economy extends beyond the patient, negatively impacting the working life of the patients' family and loved ones as well. Patients with schizophrenia also have a much greater risk for suicide, estimated to be between 15 and 25 times higher than that observed in the general population (Allebeck, 1989; Caldwell and Gottesman, 1992). The future of treatments for schizophrenia is not encouraging either, with many pharmaceutical companies withdrawing from antipsychotic research because of poor returns (Hyman, 2012).

1.1.1 Etiology

There is currently no known etiology for the disease but a number of genetic and environmental factors have been proposed. A gene X environment hypothesis attempts to combine these findings by suggesting that genetic and epigenetic factors may pre-dispose an individual for schizophrenia but environmental factors add a second hit to these vulnerable persons and are the ultimate trigger for disease progression (Tsuang, 2000). Current studies have found that environmental risk factors include maternal infection during the second trimester of pregnancy, an urban upbringing, and even belonging to an immigrant group (Jablensky, 2000). Genetic susceptibility plays a strong role as well, with increasing genetic similarities increasing the risk of developing the disease; yet monozygotic twins only have a 50% concordance rate (Gottesman, 1991). The disease has a relatively later onset - in the late teens for males and early twenties for females - which confounds prospective studies but also influences a number of developmental models of the disease. Since this period in the brain's development consists of the final stages of neural pruning (Rakic et al., 1986; Rakic et al., 1994; Huttenlocher and Dabholkar, 1997; Insel, 2010), a growing body of evidence suggests that deficits in neural plasticity may contribute to the negative and cognitive symptoms of schizophrenia (Feinberg, 1982, 1990; Keshavan et al., 1994). The brain's ability to dynamically increase the strength of some pathways while decreasing (or pruning) others has already been well established to be critical in an organism's ability to learn and exhibit cognitive control over their actions (Segal, 2005). Thus, a disruption in the delicate balance

of this process could manifest as profound disabilities in a patient's cognitive faculties.

1.1.2 Symptoms

While schizophrenia is often associated with delusions and hallucinations (Stuart and Arboleda-Florez, 2001), these two symptoms are only a small part of a much larger spectrum of problems experienced by a patient with the disease. The problems are often broken down into three general categories of symptoms (Insel, 2010). Positive symptoms are those that produce traits or experiences not found in the non-schizophrenic population, such as auditory hallucinations and delusions of grandeur, paranoia, or persecution. Opposite to these are the negative symptoms, or traits not observed in the patient with schizophrenia that are present in the general population. Examples of this include anhedonia, social withdrawal, and emotional flattening. These two symptom categories follow different patterns of expression. While positive and negative symptoms often occur suddenly together in an acute event termed the 'first-episode', the negative symptoms only have a slow but constant worsening while positive symptoms have a fluctuating pattern of onset and offset.

Lastly, patients also experience deficits in their cognitive abilities including poor executive function, impaired memory, decreased concentration, and deficits in performance monitoring (Harvey, 1985; Heinrichs and Zakzanis, 1998;

Greenwood et al., 2005; Sergi et al., 2005). It has now been acknowledged that the cognitive symptoms constitute the core feature of schizophrenia and are most responsible for the disability associated with the disease (Elvevag and Goldberg, 2000).

Cognitive symptoms appear slightly later and plateau for the majority of the patient's life, only to suddenly worsen after the age of 65 (**Figure 1**) (Harvey et al., 1999). Clinicians have found that specific tasks such as the Stroop task (Stroop, 1935) are very effective at establishing the severity of cognitive deficits common in patients with schizophrenia (Cohen et al., 1999). The task involves showing patients names of colors printed in a different color than the one described, for example the word 'red' may appear printed in blue ink. By asking the patients to either read the word or name the color of the ink, the examiner can test the patient's cognitive control over visual input and response. Schizophrenic patients are lacking in this dimension of cognitive control leading to increased error rates and can take much longer than control subjects when asked to state the color of the ink instead of reading the word (Perlstein et al., 1998; Henik and Salo, 2004). Additionally, patients are very often subject to increased impulsivity. Poor cognitive control over behavior and a strong 'stimulus-driven' behavioural profile have been suggested to be factors contributing to poor societal integration, substance abuse, and increased suicidality (Gut-Fayand et al., 2001; Enticott et al., 2008).

Progression of Symptoms

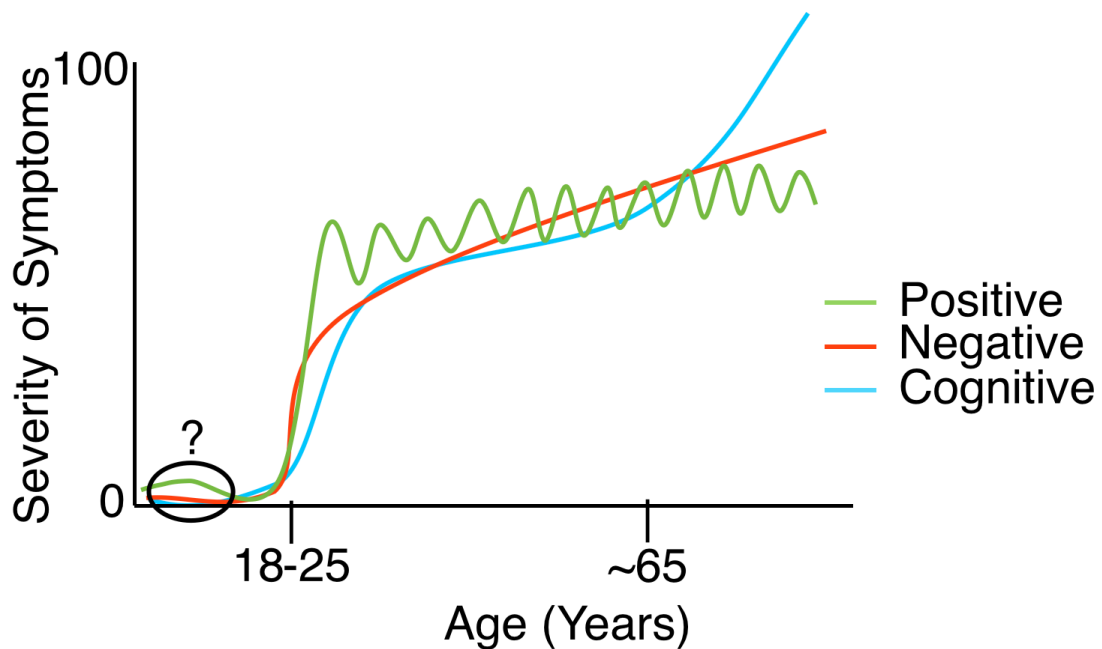


Figure 1.1: Schematic of schizophrenia symptom progression. It is unknown whether there is an event that occurs before the first episode but almost all cases begin with an acute episode of psychosis. The positive symptoms fluctuate throughout the course of the disease while the negative symptoms slowly progress over the entire course. Cognitive symptoms remain relatively stable after the first deterioration but often become much worse after 65. Data adapted from (Wiersma et al., 1998; Hafner et al., 1999; Harvey et al., 1999; Mancevski et al., 2007) .

1.1.3 Search for the Underlying Neuropathology of Schizophrenia

At the gross anatomical level, computerized tomography studies as early as the 1970s have found that patients with schizophrenia were more likely to display enlarged lateral ventricles (Johnstone et al., 1976; Weinberger et al., 1979a). Coinciding with this was a dilation of their interhemispheric and sylvan fissures (Weinberger et al., 1979b). Metabolic studies of schizophrenic patients' brains showed a decreased blood flow in the frontal cortex (Ingvar and Franzen, 1974) as well as a decrease in their frontal cortex glucose usage (Buchsbaum et al., 1982; Wolkin et al., 1985). With the increased availability of MRI, researchers soon discovered more subtle changes in the decreased brain tissue previously described. Studies confirmed the ventricular enlargement observed in CT studies but also noted a decrease in the grey:white matter ratio within the prefrontal cortex of patients with chronic schizophrenia (**Figure 1.2**) (Shenton et al., 2001). Additional MRI studies found correlations between the degree of change in prefrontal cortex volume and severity of negative and cognitive symptoms (Baare et al., 1999; Wible et al., 2001). Taking these results further with fMRI research, studies found that tasks with high cognitive demands like those requiring working memory, those requiring performance monitoring, and those involving inhibitory control over motor function showed abnormal activation of the prefrontal cortex (PFC) that was significantly different from controls (Manoach et al., 1999; Manoach et al., 2000; Carter et al., 2001; Rubia et al., 2001). Non-diagnosed

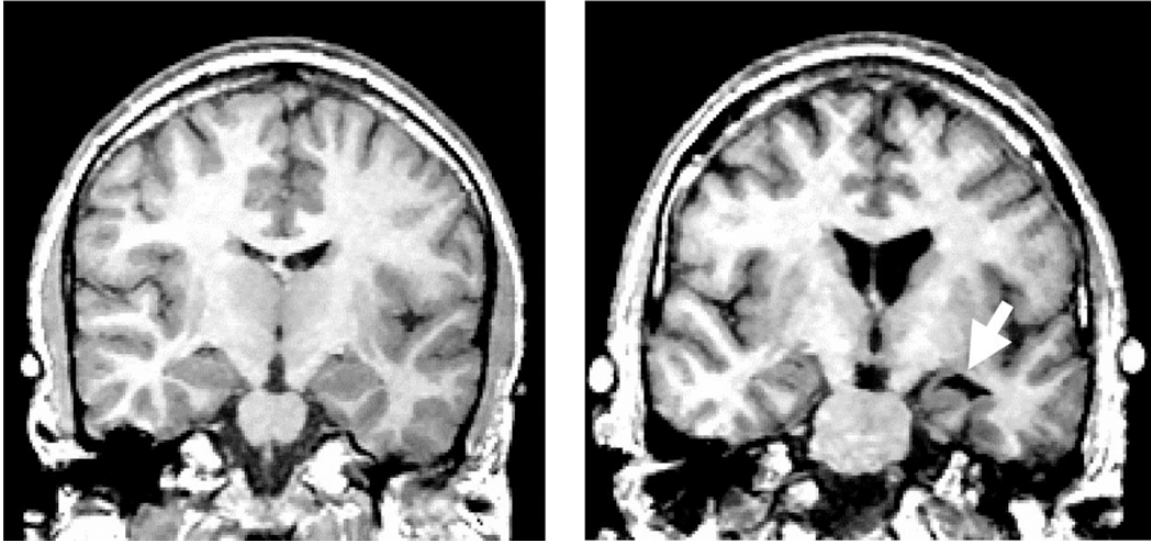


Figure 1.2: Coronal MRI sections of a normal control patient (left) and a patient with chronic schizophrenia (right). The arrow indicates an area of increased CSF in the sylvian fissure. Additionally, the increased lateral ventricle space in the patient with schizophrenia is visible. Reproduced with permission from: Shenton ME, Kikinis R, Jolesz FA, Pollak SD, LeMay M, Wible CG, Hokama H, Martin J, Metcalf D, Coleman M, McCarley RW (1992) Abnormalities of the Left Temporal Lobe and Thought Disorder in Schizophrenia — A Quantitative Magnetic Resonance Imaging Study. *The New England Journal of Medicine* 327:604-612. Copyright Massachusetts Medical Society.

siblings of schizophrenic patients also displayed these same changes in their PFC activity (Callicott et al., 2003).

Many changes in the brains of patients with schizophrenia have also been described at the cellular level. While pyramidal cells in the PFC remain largely unchanged between normal controls and patients with schizophrenia, the density of small interneurons is decreased in diseased brains (Benes et al., 1991). It was later discovered that this decrease was associated with parvalbumin (PV+) expression, an intracellular marker for a subset of interneurons (Hashimoto et al., 2003). These neurons have a high density of gamma aminobutyl acid (GABA) releasing terminals and thus are classified as inhibitory interneurons (McBain and Fisahn, 2001; Markram et al., 2004). Another intracellular marker associated with schizophrenia is the enzyme glutamate decarboxylase (GAD67), which is responsible for the synthesis of GABA (Erlander et al., 1991). Post-mortem studies have very consistently found that GAD67 mRNA is downregulated in the dorsolateral PFC (dlPFC) of patients with schizophrenia (Akbarian et al., 1995; Mirnics et al., 2000; Volk et al., 2000). This down regulation of GAD67 was found to be particular to PV+ interneurons and most notably that those interneurons with lower than normal PV+ expression were the same neurons that showed an absence of the expected GAD67 enzyme (Hashimoto et al., 2003). It is apparent that in the schizophrenic dlPFC the PV+ class of interneurons may be suffering from a functional impairment due to a decreased ability to synthesis GABA and

consequently are unable to properly inhibit post-synaptic pyramidal neurons (Lewis et al., 2012).

Aside from GABA transmission, the excitatory neurotransmitter glutamate has been implicated in schizophrenia as well (Olney and Farber, 1995). Glutamatergic receptors have been found to be downregulated in the PFC as well as the thalamus and hippocampus in schizophrenic patients (Konradi and Heckers, 2003). As the primary excitatory neurotransmitter system, this down-regulation has been regarded as a potential contributor to the 'hypofrontal' state associated with schizophrenia (Paz et al., 2008; Marek et al., 2010). Indeed, a blockade of the N-methyl D-aspartate (NMDA) type of glutamate receptors on PV+ interneurons with the antagonist ketamine induced a decrease in the GAD67 and PV expression of these cells (Kinney et al., 2006).

Dopamine was the first neurotransmitter implicated in the pathology of the disease, due to the therapeutic effects of dopamine receptor antagonists (for review see Seeman, 1987). While these findings were consistent with the positive symptoms of schizophrenia, the models of the disease and therapies targeting the dopaminergic system were less successful at describing the negative and cognitive dimensions (Angrist et al., 1980; Lipska and Weinberger, 2000; Neill et al., 2010). A post-mortem study found that dopamine was decreased in the dIPFC of patients with schizophrenia (Abi-Dargham and Moore, 2003). Possibly

to compensate for this decreased dopamine, another study found that an increased dopamine D1 receptor availability in this area was associated with poor performance during a working memory task in patients with schizophrenia (Abi-Dargham et al., 2002). Interestingly, the previously mentioned PV+ interneurons are the only type of interneuron within the PFC to receive dopaminergic synaptic inputs (Sesack et al., 1998).

Taken together, the functional disabilities and neuroanatomical changes point to a strong role of the prefrontal cortex in schizophrenia symptomology.

1.2 Prefrontal Cortex

Initially described by Brodmann in the early 20th century, the PFC was believed to be unique to primates (Brodmann, 1905; Brodmann, 1909). Years later it was discovered that analogous regions existed in non-primate mammals such as rabbits, sheep, cats, and rats as defined by the projections from the medial dorsal (MD) nuclei of the thalamus terminating in these areas of the frontal cortex (Rose and Woolsey, 1948; Akert, 1964; Divac et al., 1978a). Localized subdivisions of the PFC were also identified based on their anatomical connectivity and cytoarchitecture (Brodmann, 1905; Walker, 1940; Petrides and Pandya, 1999; Petrides, 2005). Newer techniques using functional magnetic resonance imaging (fMRI) have gone further and identified functional

connectivity in the human and non-human primate PFC (Hampshire and Owen, 2006; Hampshire et al., 2012; Hutchison and Everling, 2013).

When classifying these regions based on their cytoarchitecture, researchers often look at the distribution of cell types in the various layers of cortex. The granulated cortex is the histological attribute that defines the difference between the primate dlPFC and other PFC subregions (Brodmann, 1909). This name stems from the granulated appearance of neurons within layer IV of the lateral PFC, also named the internal granulated layer (Preuss, 1995). Particularly in the dlPFC (Brodmann areas 9/46), layer IV in these regions is much denser and better developed when compared to other bordering regions (**Figure 1.3**) (Petrides and Pandya, 1999). The density and increased development of layer IV in these regions is in part due to the MD thalamic projections terminating in this area (Tobias, 1975) as well as the highest concentration of P_v+ neurons (Hashimoto et al., 2003). Structural connectivity experiments involving the injection of tracer dyes showed that area 9/46, or the dorsolateral and ventrolateral prefrontal cortex had extensive inputs from multimodal areas such as the superior temporal sulcus, rostral superior temporal gyrus, and the anterior & posterior cingulate cortex (Petrides and Pandya, 1999). Their outputs projected onto both temporal multimodal areas as well as paralimbic areas like the cingulate and retrosplenial cortex (Petrides and Pandya, 1999). Initial studies on the cytoarchitecture in the PFC examined and defined

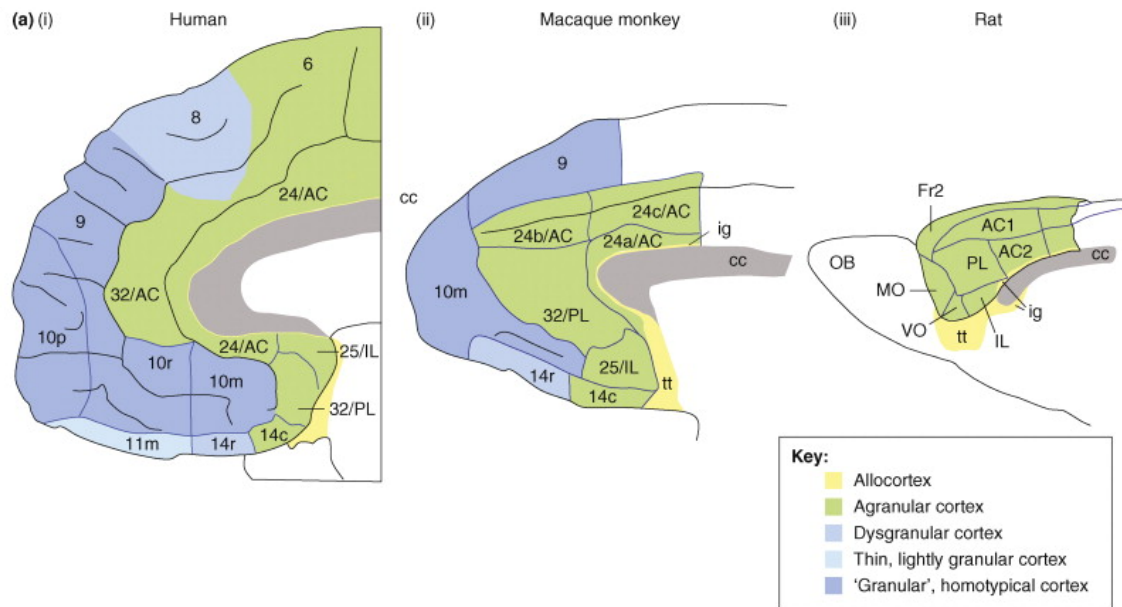


Figure 1.3: Sagittal illustrations of the frontal lobe of humans, macaque monkeys, and rats. Homologous areas are mapped between species by their indicated colours. The granulated prefrontal cortex has no corollary area in the rodent brain but agranular regions are still present. Reprinted with permission from: Wise SP. (2008) Forward frontal fields: phylogeny and fundamental function. *Trends in Neuroscience*. 31(12):599–608.

these areas separately in the human and non-human primate PFC (Brodmann, 1905; Brodmann, 1909; Walker, 1940). More recent research efforts have now collated this data with additional experiments, resulting in area definitions that are applicable across both species (Petrides and Pandya, 1999).

Differences still remain between the PFC of the two most common animal models used for schizophrenia: rodents and non-human primates (Brown and Bowman, 2002). Whereas projections from the MD thalamus terminate in the medial, orbital, and dorsolateral surfaces of the primate PFC, the rodent PFC only contains medial and orbital regions (Ongur and Price, 2000). This difference in PFC subregions is critically important when studying the cognitive functions impaired in schizophrenia, as higher order brain functions like working memory (Goldman-Rakic, 1987; Kimberg and Farah, 1993), attention control (MacDonald et al., 2000), and decision making (Heekeren et al., 2008a) have all been attributed to the dorsolateral aspect of the primate PFC (Miller and Cohen, 2001). The rodent may have developed analogous areas or methods of performing these cognitive tasks, but the two homologous regions (medial and orbital) of PFC do not substitute for the primate dIPFC (Kolb, 1990). Some aspects of cognitive deficits in schizophrenia have been associated with the anterior cingulate cortex (ACC), a medial region of the PFC (Devinsky et al., 1995; Dolan et al., 1995; Carter et al., 2001). Similarities exist between the ACC and the medial PFC of rodents, meaning experiments that investigate cognitive

dysfunction associated with this area have valid homologues in rodent models of the disease (Preuss, 1995).

The described interconnectedness of the PFC allows it to use highly processed sensory information to generate a top-down, executive control over behaviour (Miller and Cohen, 2001). Two components of this executive function are particularly impaired in schizophrenia: working memory, which maintains, integrates, and manipulates sensory information over short periods of time (Goldman-Rakic, 1994); and performance monitoring, which gives feedback on the outcome of an action in order to better guide actions in the future (Carter et al., 2001).

1.2.1 Working Memory

Neural computational models of cognitive control often require a set of neurons to act as a temporary storage space, keeping immediately relevant information online for the integration of past and present stimuli, previous outcome experiences, and internally generated goals (Dehaene and Changeux, 1991; Durstewitz et al., 2000). This is achieved through the use of working memory. Often describe as the scratchpad of the brain, working memory temporarily retains information to guide future behaviours (Baddeley, 1986). Appreciating working memory is an important aspect to understanding the overall function of the PFC as its effects on the brain are extensive (Goldman-Rakic,

1987). Studies in both humans (Goldman-Rakic, 1987; Fuster, 1997; Knight and Stuss, 2002) and non-human primates (Funahashi and Kubota, 1994; Petrides, 1994; Funahashi and Takeda, 2002) have verified the importance of the dlPFC in working memory function.

Working memory function can be demonstrated in a number of modalities. Tasks like the oculomotor delayed response (ODR) have shown that the dlPFC has sustained activity during the retention interval, an epoch in the working memory task that engages spatial working memory (Curtis and D'Esposito, 2003). The ODR task requires a subject to fixate on a central fixation point, after which a suddenly appearing visual stimulus will be briefly displayed in one of 8 cardinal locations surrounding the fixation point. After the stimulus has disappeared from the subject's visual field, they must wait until they receive a cue instructing them to look towards the blank screen location where the visual stimulus previously appeared. While they wait for this cue, their spatial working memory is maintaining the location of where in the visual field the stimulus appeared, so that they may generate a saccade to the remembered location when instructed. The sustained activity in the PFC during this retention interval couples the initial visual stimulus with the later behavioural response. By training non-human primates on the task, experimenters were able to obtain single neuron recordings in the dlPFC to show increased activity in the retention interval (Funahashi et al., 1989; Sawaguchi and Goldman-Rakic, 1994). Single neuron

recordings in the primate also showed that neurons in the dlPFC were selectively tuned to specific locations in the visual field (Funahashi et al., 1989, 1990). These data provided a neurophysiological representation of spatial working memory in the primate dlPFC.

By incorporating electroencephalography (EEG) methods, researchers also identified that certain frequencies of neuronal oscillations are sensitive to the retention interval epoch of the trial during which verbal or spatial working memory is required. These frequencies may help synchronize the PFC with other regions of the brain also required for the task (Sarnthein et al., 1998). Higher frequencies were negatively correlated with the difficulty as well, which led researchers to conclude that there is a quantifiable working memory load that exists within the PFC and it can be measured through EEG (Gevins et al., 1997).

Aside from the spatial location of a visual stimulus, working memory can also maintain features of an object, as demonstrated by the delayed match-to-sample task (Hasegawa et al., 1998). In this task, the subject is briefly shown an object, after which they must remember the identity of the object over a delay period while continuing to hold a hand lever. During this delay period of this task the subject's dlPFC was shown to exhibit sustained neuronal activity analogous to the activity observed in the ODR task. Following the brief delay period, the subject is shown another object and they are required to release the lever if the

object is the same as the cue object previously shown. The sustained activity recorded in the dlPFC during the delay period was found to be dependent on what object was being stored, a feature of working memory similar to the selectivity observed in spatial working memory (Miller et al., 1996; Johnston and Everling, 2006b).

Not limited to only storing simple properties of visual stimuli, the working memory system is capable of operating at a higher order level through the storage of abstract rules (Wallis et al., 2001). This rule-based working memory is then employed by the subject to guide an imminent behavioural response to a stimulus (Bunge, 2004). In these tasks it is not the stimuli that is being stored, rather an internally developed concept associated with a visual cue that is being maintained in working memory (Miller and Cohen, 2001). Neurons within the dlPFC have been recorded that display a selectively increased activity for specific rules during the delay period (Wallis et al., 2001; Everling and DeSouza, 2005; Johnston and Everling, 2006b; Johnston et al., 2009) and these neurons project directly to the superior colliculus, an area responsible for the generation of the oculomotor response (Everling et al., 1999; Johnston and Everling, 2006a).

Proper dlPFC function is required to use these learned rules to guide behaviour, as is evident by the increased error rate in the face of an incongruent or stimuli-response challenge, such as the previously described Stroop Task

(Perret, 1974; Alexander et al., 2007) or the anti-saccade task (Hallett, 1978; Guitton et al., 1985) following PFC damage. Although other brain regions are also involved in this working memory activity, the PFC is the only region in which impairment causes severe deficits in task performance (Kane and Engle, 2002). Functional MRI scans showed the strongest BOLD signals in the lateral PFC during working memory tasks (Owen, 1997). Theoretical models first predicted that because of its ubiquitous nature, working memory impairment might have a widespread impact on general cognitive function (Kimberg and Farah, 1993). This diverse cognitive disability stemming from impaired working memory was later confirmed to occur in humans (Silver et al., 2003; Alexander et al., 2007; Koerts et al., 2009). Similar cognitive disabilities may also arise due to deficits in error monitoring, another executive function associated with the PFC.

1.2.2 Error monitoring and performance feedback

The purpose of performance monitoring is to gather information regarding the outcome of a trial in the context of the behavior used to reach this outcome. Both correct and error outcomes have been shown to elicit specific neuronal responses, where neurons may be selective for only one of the two. This outcome specific activity has been localized to the ACC, a region bordering the PFC (Carter and van Veen, 2007). Different regions of the ACC have been shown to exhibit different activation patterns for correct or erroneous outcomes (Holroyd et al., 2004; Margulies et al., 2007). The ACC is connected via

dopamine projections to the ventral tegmental area, which allows correct conditions to produce an intrinsic sensation of reward and network reinforcement (Divac et al., 1978b; Watanabe, 1990; Leon and Shadlen, 1999). Reciprocal connections to the lateral PFC (Bates and Goldman-Rakic, 1993; Petrides and Pandya, 1999) combine this performance information with associated behaviors and goals stored in working memory (Watanabe, 1992; Ridderinkhof et al., 2004). Together, this network acts to make sense out of action-outcome sets.

The first demonstration of the outcome sensitivity of neurons in the ACC was recorded using intracranial electrodes in non-human primate ACC. The experiment employed a task that required the animal to exert cognitive control over their response to a cue, delaying the trained behavioural reaction in order to gain a reward (Niki and Watanabe, 1979). In these experiments, it was determined that neurons showed sensitivity not only to either a correct or error outcome, but that the stimulus tied to the outcome could also influence the ACC activity. An additional study confirmed these results and found that if the animal performed a correct trial but no reward was given, it was the error neurons that increased their activity in the performance-monitoring epoch (Amiez et al., 2005). Neurons within the rodent ACC were also found to assemble into coherent states, where a network of neurons would organize in discernable patterns dependent on the cognitive demands of the task and falter during tasks with error

responses (Lapish et al., 2008). This sensitivity of the ACC to erroneous responses had previously been demonstrated in humans through EEG studies.

One of the soundest and most reproducible EEG findings in the human PFC is the error related negativity (ERN) response. First described in simple reaction time tasks (Falkenstein et al., 1991), the ERN is a brief negative deflection of the event-related potential as recorded by EEG over the ACC (Yeung et al., 2004). The ERN regularly occurs 100ms after the subject has made an erroneous response to a stimulus and has been described in humans and non-human primates alike (Falkenstein et al., 2000; Godlove et al., 2011; Phillips and Everling, 2014). The robust nature of the ERN is due in part to its occurrence in the ACC independent of both the modality of stimuli presentation and behavioural response required (Holroyd et al., 1998). The ERN can be triggered in two types of errors. The first type occurs when the subject makes a behavioural response that the ACC recognizes to be erroneous and attempt to remedy by quickly switching to the correct response. This type of intrinsic error correction can often go unnoticed by the subject and the behavioural correction occurs too fast to be consciously registered (Murphy et al., 2012). The second ERN trigger occurs when the subject performs a task correctly and is expecting a reward but receives none (Holroyd et al., 2003; Yasuda et al., 2004). It has been hypothesized that this second type of ERN helps the PFC to continually adjust to

which behaviours will not be rewarded and suppresses those unrewarded events appropriately (Walsh and Anderson, 2012).

The localization of performance monitoring to the PFC has implications in humans with abnormal PFC function, such as patients with schizophrenia or those that have sustained PFC damage. Without the ability to properly detect correct responses from errors, the entire network of behavioural modification and optimization begins to suffer. Consequently, in both of these situations experiments have shown that patients have trouble adapting their behavior in the face of incorrect responses and error outcomes. Error monitoring deficits are particularly evident in patients with schizophrenia, as EEG studies have found a decrease in the amplitude of the ERN (Kopp and Rist, 1999), as well as a decreased BOLD signal during the performance-monitoring epoch of a trial (Carter et al., 2001). Commonly patients with schizophrenia report a feeling that their thoughts and behaviours are being externally controlled (O'Grady, 1990). This unique symptom has been attributed to a disturbance in their ability to recognize and monitor internally generated actions (Frith, 1987; Frith and Done, 1989). The decreased awareness of behavioral outcomes leads to a subsequent decrease in the general cognitive awareness of the patient's sense of self. Interestingly, many of these symptoms associated with schizophrenia have been elicited with compounds that antagonize the NMDA glutamate receptor.

1.3 Glutamate

Glutamate is the primary excitatory neurotransmitter of the brain, ubiquitous across the central nervous system and necessary for the transfer of action potentials across the synaptic cleft (Fain, 1999). It acts primarily on three classes of post-synaptic ionotropic receptors. The α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are responsible for the fast response to presynaptic glutamate release and initiate the excitatory post-synaptic potential (EPSP) at the post-synaptic density (Meldrum, 2000). The kainite receptor family is poorly understood but it appears to have a role in the regulation of the AMPA and NMDA receptor responses in the synapse (Lerma and Marques, 2013). Lastly is the NMDA receptor, which has been the most extensively studied and has been implicated in a number of neurological disease models (Dingledine et al., 1999).

1.3.1 NMDA Receptor

The NMDA receptor was named after a selective agonist for this subtype of glutamate receptor. It is a voltage gated Ca^{2+} channel that has a Mg^{2+} ion resting inside the channel pore during its inactive state, This Mg^{2+} ion acts as a plug to prevent the inward flow of Ca^{2+} . Upon depolarization the Mg^{2+} ion is released and the receptor goes through a conformational change to open the ion channel. The open pore allows an influx of Ca^{2+} which helps to release additional intracellular stores of Ca^{2+} and subsequently cause depolarization. This

ultimately leads to the generation of an action potential (**Figure 1.4**). NMDA activation is also required for the changes in the post-synaptic receptor profile that produce long-term potentiation and neuronal plasticity (Malenka and Nicoll, 1999). The most common class of antagonist for the NMDA receptor acts by binding inside the channel pore near the Mg^{2+} binding site (Kroemer et al., 1998). This secluded location for the antagonist binding site requires the activation of the receptor before the compound can bind and exert its effect (Anis et al., 1983). The pharmacodynamic profile induced by this antagonist binding pattern shows higher binding rates with higher values of agonist and is called uncompetitive antagonism. Uncompetitive antagonists for the NMDA receptor such as ketamine, phencyclidine (PCP), and dizocilpine (MK-801), have all been shown to have very potent effects on the activity of neurons at the cellular level and were initially used in high doses to produce an anesthetic effect. However, at subanesthetic doses, these drugs can produce startling behavioural changes (Lipton, 1993, 2004). Of all of the NMDA uncompetitive antagonists, ketamine has been studied most extensively because of the similarities between its behavioural effect profile and the behavioural phenotypes observed in patients with schizophrenia.

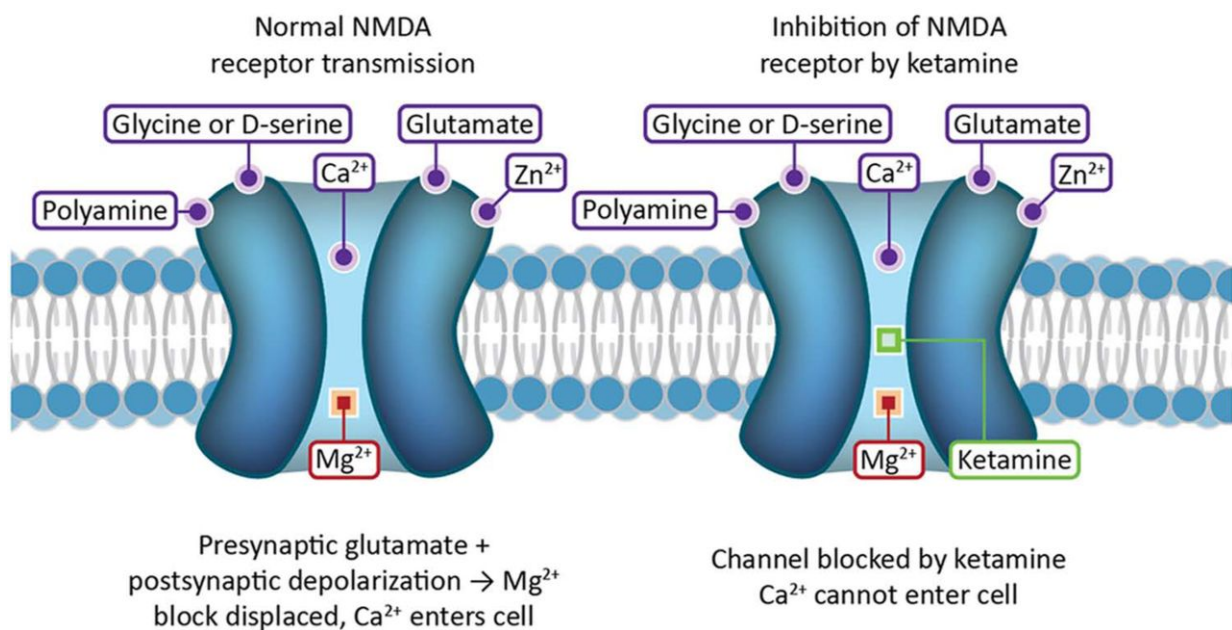


Figure 1.4: The NMDA receptor and the ketamine binding site. This ionotropic receptor requires two steps for activation. First, an agonist must bind to an extracellular site to induce a conformational change and second, the membrane surrounding the receptor must become depolarized to free the Mg^{2+} block in the channel. This allows Ca^{2+} to enter the cell. Reprinted with permission from: Frohlich J and Van Horn JD (2014). Reviewing the ketamine model for schizophrenia. *Journal of Psychopharmacology* 28(4) 287-402.

1.3.2 Ketamine

First created in 1962 as a less neurotoxic alternative to PCP (Rowland, 2005), ketamine was intended to be used as a dissociative anesthetic that would produce fewer side effects such as hallucinations and delirium that were common with PCP (Corssen and Domino, 1966). Since its initial discovery, it has been found to exert analgesic and amnesic effects as well, both of which are complementary to its intended purpose as a surgical anesthetic. More recent studies have suggested it may even have clinical use as a treatment for intractable major depressive disorder (Berman et al., 2000) and post-traumatic stress disorder (Feder et al., 2014). Hallucinations are a known side-effect of the drug, however they are rare in children under the age of 16 years old (Reich and Silvey, 1989), which is similar to the rarity of schizophrenia presenting with a first episode of psychosis before late adolescence (Hafner et al., 1993). After the administration of ketamine *in vitro*, a washout period is sometimes observed that can produce spontaneous action potentials in the absence of EPSP input, possibly due to a neurophysiological response to the drug that increases the NMDA receptor current for sodium, leaving the neurons more easily excitable (Benoit et al., 1986).

Despite ketamine's antagonism of an excitatory receptor, it has been well documented that it - and other NMDA antagonists - have a net positive effect on the general excitatory activity of the brain. It has been suggested that this is due

to an increase in compensatory glutamate release (Rowland, 2005; Kim et al., 2011; Stone et al., 2012). Due to the increased glutamate release following NMDA receptor antagonism, this family of drugs is prone to inducing excitotoxicity in the brain (Horvath et al., 1997; Wozniak et al., 1998; Farber and Olney, 2003). Human studies have shown that the behavioural changes associated with this drug can be attenuated by blocking the ketamine-induced glutamate release, particularly the increased activity in the ACC (Deakin et al., 2008). Additionally, there is a strong correlation between the amount of glutamate released in response to ketamine and the severity of the ketamine-induced psychotic symptoms (Stone et al., 2012).

The NMDA receptor and the functional effects of its antagonism are of particular importance when discussing schizophrenia. Post-mortem studies of the brains of schizophrenic patients have demonstrated decreased levels of proteins required for functional NMDA receptors (Karlsgodt et al., 2011). Polymorphisms of the NMDA receptor subunits have also been found in patients with schizophrenia (Li and He, 2007). The link between NMDA receptor function and schizophrenia is further strengthened by studies that found specific NMDA hypofunction via both post mortem mRNA analysis and positron emission tomography (PET) scans in the brains of schizophrenics (Law and Deakin, 2001; Pilowsky et al., 2006). The PET study also found that the level of behavioural abnormalities induced by blocking the NMDA receptor with ketamine was

correlated with the amount of dopamine released in the lateral PFC and the ACC. This hyper-responsive dopaminergic state in the frontal cortex is often observed in rodent models of schizophrenia employing NMDA receptor antagonists such as PCP. These animals show an increased sensitivity to the dopamine-releasing properties of amphetamine in their frontal cortex (Javitt et al., 2004). Similarly, human schizophrenia patients also have a heightened response to amphetamine at both the cellular and functional level (Laruelle et al., 1996; Breier et al., 1997).

1.4 Pharmacological Models of Schizophrenia

These concepts highlight the two strongest theories behind the neurophysiology of schizophrenia; the dopamine hypothesis and the more recent glutamate hypothesis. Taking cues from the observation that dopamine receptor antagonists were successful at treating the positive symptoms of schizophrenia (Seeman and Lee, 1975), the dopamine hypothesis posits that the behavioural abnormalities observed in the disease stem from an excessive amount of dopamine signaling in the striatum (Seeman, 1987) and decreased dopamine signaling in the prefrontal cortex (Abi-Dargham and Moore, 2003). Unfortunately, these anti-dopaminergic drugs were unsuccessful at ameliorating the most debilitating negative and cognitive symptoms of the disease (Lieberman and Stroup, 2011).

The more recent hypothesis involving glutamate dysregulation stemmed from the description of a schizophrenia-like phenotype in human patients administered PCP (Erard et al., 1980). This concept did not gain traction until the pharmacological properties of PCP were better understood (Javitt and Zukin, 1991) and the shortcomings of the dopamine theory became apparent. Similar to its synthesis history, ketamine became preferred over PCP as a model of schizophrenia because of its decreased neurotoxicity. The acute administration of either of these NMDA antagonists induced a behavioural profile very similar to schizophrenia in normal patients and triggered a psychotic episode in patients already diagnosed with schizophrenia (Krystal et al., 1994). These behaviours include very specific types of memory disturbances and thought disorders that are fairly unique to schizophrenia (Adler et al., 1999a). Dopamine dysregulation is likely still involved in the etiology of schizophrenia but it is probably occurring as a downstream repercussion of changes in the NMDA receptor activity (Roberts et al., 2010). For example, chronic users of NMDA antagonists have shown alterations in their dopaminergic signaling similar to patients with schizophrenia (Jentsch and Roth, 1999).

1.4.1 Parallels between Schizophrenia and the NMDA Antagonism Model

While NMDA antagonists can induce a net increase in neuronal activity (Homayoun and Moghaddam, 2007), chronic use of either PCP (Cochran et al., 2003) or ketamine (Deakin et al., 2008) have been shown to cause a regional

hypoactivity in the prefrontal cortex. Once again, we see a parallel in the human schizophrenia population with patients often displaying a hypofrontal pattern of activation (Liemburg et al., 2012). Additionally, chronic ketamine use decreases prefrontal grey matter in a similar fashion to the decreased grey matter found in MRI scans of schizophrenia patients (Liao et al., 2011).

Similar to the ERN, the mismatch negativity (MMN) is a phenomenon observed in EEG studies on patients with schizophrenia. The MMN response is a brief negative potential elicited after a different stimulus appears to a subject's sensory field after they have been presented with a repeating and unchanging stimuli. For example, a patient may be continually presented with series of blue circles but the 10th circle in the series is coloured red instead. This red circle is an oddball stimulus and will elicit a MMN. This effect holds true with auditory stimuli as well. In patients with schizophrenia, the MMN event is blunted both in amplitude and duration. The ketamine model of schizophrenia causes an identical change in the MMN response in normal subjects treated with ketamine (Oranje et al., 2000; Umbricht et al., 2000; Kreitschmann-Andermahr et al., 2001; Heekeren et al., 2008b; Schmidt et al., 2012). Non-human primates treated with ketamine (Gil-da-Costa et al., 2013) and other NMDA antagonists also display a similar change in their MMN response (Javitt et al., 1994; Javitt et al., 1996).

The links between the disease and disease model extend down to the cellular level as well. Interneurons that express the protein Pv express the protein at lower level in patients with schizophrenia (Hashimoto et al., 2003). These Pv+ interneurons have a very strong glutamatergic inputs synapsing onto them in the PFC (Melchitzky and Lewis, 2003), the region most associated with schizophrenia. This is especially interesting for the ketamine model of schizophrenia, as it has been shown that Pv+ interneurons are strongly affected by PCP, ketamine, and other NMDA antagonists in their Pv expression and ability to inhibit PFC neurons (Cochran et al., 2003; Behrens et al., 2007). With NMDA receptors driving the excitation of GABA-releasing inhibitory interneurons, proper NMDA receptor function is critical for the inhibition and synchronization of pyramidal cells in the PFC (Behrens et al., 2007; Homayoun and Moghaddam, 2007). This decreased inhibitory control has been linked to the manifestation of behavioural changes caused by ketamine through the use of GABA agonists. The administration of a GABA agonist shortly after the ketamine administration resulted in an amelioration of the normal behavioural changes expected with ketamine (Castner et al., 2010). In summary, it is the NMDA receptor hypofunction that is causing a disinhibition of the PFC network via decreased excitatory input to the Pv+ GABAergic interneurons. These changes are cumulative as well, with chronic administration of NMDA receptor antagonists permanently altering the post-synaptic environment of these cells (Cochran et al., 2003; Amitai et al., 2012).

1.5 Brain Oscillations in Schizophrenia

Interneurons are also responsible for the synchronization of neuronal firing in the cortex (Lytton and Sejnowski, 1991; Lewis et al., 2005) and thus poorly functioning interneurons will alter the generation of functionally relevant oscillatory activity, a necessity for interregional communication in the brain (Schnitzler and Gross, 2005). Schizophrenia has been proposed to be a disorder of connectivity (Friston, 1998) with early studies showed fewer functional interactions between different regions of the brain (Volkow et al., 1988). More advanced techniques confirmed these findings of global reductions in the functional connectivity of the schizophrenic brain (Calhoun et al., 2009). Anatomically, the connections have been shown to be smaller via decreased axonal and cortical thickness (Bassett et al., 2008; Wang et al., 2011; Zalesky et al., 2011), while an fMRI analysis of patients with schizophrenia has also shown that at resting state the brain exhibited fewer hubs of connectivity and a decreased efficacy of those remaining connections (Lynall et al., 2010).

Researchers have delineated different ranges of oscillatory frequencies that facilitate these types of long distance communications and neural synchrony. The most thoroughly investigated range in schizophrenia research is the gamma band, which is most often defined as frequencies between 30-90 Hz (Lee et al., 2003). A number of cognitive functions have been associated with gamma band

oscillations – short-term memory (Tallon-Baudry et al., 1998), working memory (Haenschel et al., 2009), attention (Tiitinen et al., 1993) and social cognition (Williams et al., 2009). Patients with schizophrenia demonstrate an increase in the amplitude of gamma band activity in their PFC during steady-state responses to stimuli (Plourde et al., 1997), an effect also observed in ketamine-challenged healthy human subjects (Hong et al., 2010) and animals (Pinault, 2008). The increase in gamma band activity did not confer improved performance though, as patients challenged with a working memory task exhibited an increase in the gamma band activity but an overall decrease in performance (Basar-Eroglu et al., 2007). Some studies show an increase in the functional connectivity of brain regions at rest after the administration of ketamine but these increases in connectivity do not correspond with improved outcomes (Driesen et al., 2013). Tests with repeated transcranial magnet stimulation (rTMS) have found that the application of an rTMS field decreased the abnormally high gamma band activity in patients with schizophrenia during a working memory task (Barr et al., 2011) and this decrease corresponded with an improved cognitive performance (Farzan et al., 2012). Further, other studies have found correlations between gamma band activity and the positive symptoms expressed by schizophrenic patients (Baldeweg et al., 1998; Kehrer et al., 2008; Spencer, 2008). The remaining ranges have shown some degree of abnormal function in schizophrenia as well but their contributions to the schizophrenia phenotype has yet to be fully discovered (Roopun et al., 2008; Uhlhaas and Singer, 2011, 2012, 2013).

1.6 Eye movement deficits in schizophrenia

Previous studies have established that patients with schizophrenia display abnormalities in a number of dimensions when eye movements are examined. Two types of canonical movements can be demonstrated in this patient group; a disturbance in the voluntary control of saccades and dysfunctions when attempting to perform smooth pursuit movements (Diefendorf and Dodge, 1908; Kojima et al., 1990). Smooth pursuit movements were reported to be deficient in patients with schizophrenia by Holzman et al. who demonstrated that eye-tracking patterns in this population were disturbed when attempting to follow the rhythmic movements of a pendulum (Holzman et al., 1974). Further, this study and others also showed that non-schizophrenic first-degree relatives of these patients also showed similar but less pronounced deficits, supporting the genetic component of schizophrenia's etiology (Hutton and Kennard, 1998; Adler et al., 1999b).

In contrast to smooth pursuit eye movements, a saccade is the rapid, reorientation of the fovea onto a new visual location (Munoz and Everling, 2004). While smooth pursuit eye movements attempt to keep an object on the fovea, saccades exist to bring a new item or visual field area onto the fovea. Saccadic eye movements can be prepotent, such as looking towards a suddenly appearing visual stimulus, or they may be influenced by internal goals such as looking towards a memorized location (Mort et al., 2003). Cognitive control is required for

both of these saccade types and this control has been known to be deficient in schizophrenia (Fukushima et al., 1988; Broerse et al., 2001). A lack of control over the prepotent saccades would negatively impact an organism's ability to focus on salient stimuli and block out irrelevant but novel visual stimuli that act as distractors (Braff, 1993). In the second type, an animal's cognitive system is responsible for integrating their internal cues and memories in order to generate a target location for the saccade (Broerse et al., 2001; Munoz and Everling, 2004). Both types of saccade can easily be described in the successful operation of a car. Novel but irrelevant stimuli are constantly presented to a driver's visual field while traveling along the road. If the driver were unable to filter these out, their gaze and attention would not be focused on the road ahead. This unfocused gaze would eventually cause an undesirable outcome, thus illustrating the importance of an inhibitory control over prepotent saccades. Alternatively, if the driver wishes to turn they must look towards the direction they want to travel in and scan for hazards. This requires the integration of internal goals and previous memories about visual areas of importance. By purposefully generating a saccade towards these internally selected saccade locations, the driver is illustrating the importance of cognitively guided saccades. Both types of cognitive control over saccades can easily be tested in the laboratory setting with the anti-saccade task (Hallett, 1978; Everling and Fischer, 1998; Munoz and Everling, 2004).

1.7 Anti-saccade task

Originally developed in 1978 by PE Hallett (Hallett, 1978), the anti-saccade task can probe multiple components of cognition known to be deficient in schizophrenia. The subject of the experiment is trained to recognize that a visual rule cue in the form of a red or green coloured circle will indicate whether they are to make a saccade towards a suddenly appearing novel stimulus (pro-saccade) or to a location that is diametrically opposite to this novel stimulus (anti-saccade). Each trial consists of displaying the rule cue to the subject then showing a novel stimulus either to the left or right of this cue. A successful pro-saccade trial simply requires the subject to make a saccade towards the novel stimulus. In contrast to this, if the rule cue indicates an anti-saccade trial the successful completion of the trial would have the subject looking away from the novel stimulus after its appearance (**Figure 1.5**). To complete the anti-saccade trial correctly requires a number of cognition-controlled steps. First, the subject must suppress the pre-potent or stimulus-driven response of automatically looking towards the novel stimulus. Next, they must generate the voluntary anti-saccade location in their visual space. This location has no visual indicators so it must be calculated in relation to the angle and distance of the novel stimulus from the central fixation point. Lastly, the subject must execute this saccade to the internally generated saccade target location.

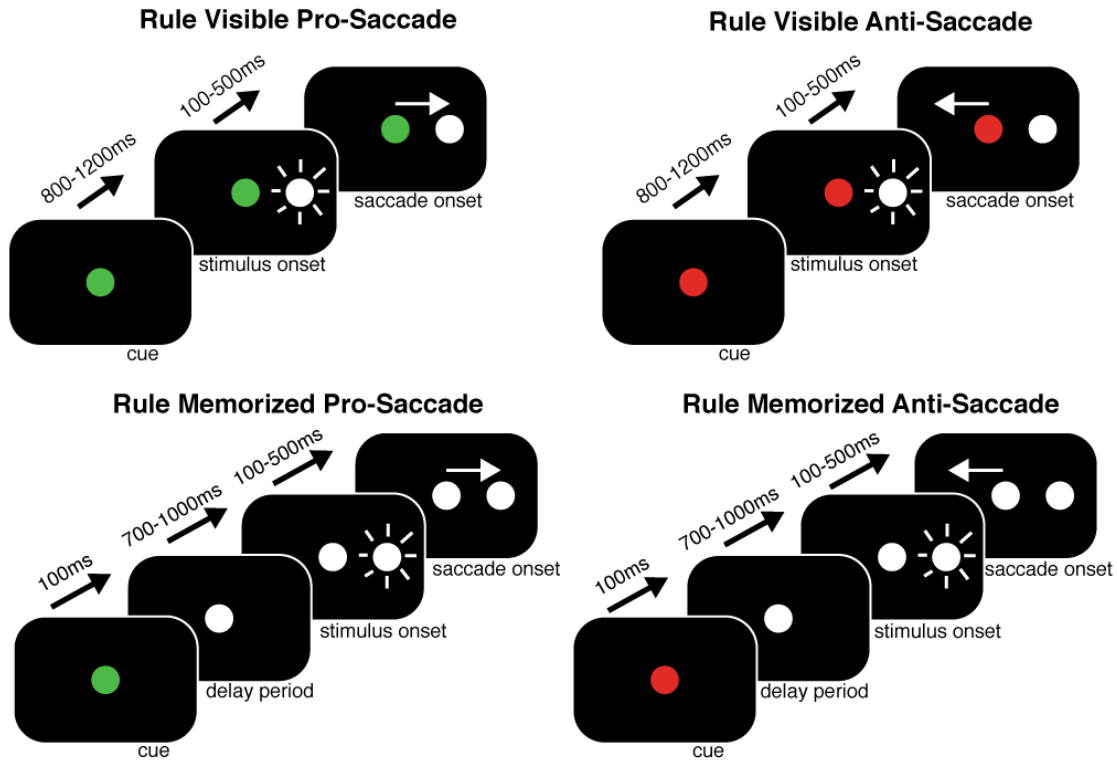


Figure 1.5: The anti-saccade task. The task can be administered in both rule visible (top row) and rule memorized (bottom row) conditions. In pro-saccade trials, the subject learns that a coloured cue represents the pro-saccade rule and indicates that they should saccade towards the novel stimulus appearing shortly after. The anti-saccade rule is indicated by a different colour and a successful trial involves looking diametrically opposite of the novel visual stimulus. Rule memorized versions introduce a delay period during which the subject must maintain the pro- or anti-saccade rule in their working memory in order to correctly respond to the stimulus when it appears.

1.8 Objectives

1.8.1 Examine the effect of ketamine on anti-saccade performance and rule-based working memory in the PFC

A previous study has shown that the ketamine model of schizophrenia is capable of inducing a behavioural phenotype reminiscent of patients with schizophrenia when challenged with the anti-saccade task (Condy et al., 2005). Meanwhile, our laboratory has previously described neurons within the PFC that are sensitive to the rule for the current trial in the anti-saccade task (Everling and DeSouza, 2005; Johnston et al., 2009). Since schizophrenia is most commonly associated with changes in the PFC (Barch et al., 2001) I will look for changes to these rule-specific neurons in the PFC and analyze the role their activity plays in the behavioural deficits associated with the ketamine model of schizophrenia. The goal of this objective is to identify how changes in the PFC activity during working memory manifest as changes in the subject's behavioural performance on the anti-saccade task.

1.8.2 Test for ketamine-induced changes to error monitoring feedback behaviours and their neuronal representations

Disconnection between brain regions has been hypothesized to have a strong impact on the cognitive symptoms of schizophrenia. One of the regions that shares a strong reciprocal connection with the PFC is the anterior cingulate cortex (ACC), a brain region associated with performance monitoring (Gehring and Knight, 2000). Studies in human patients with schizophrenia have noted their

decreased ability to recognize errors in behaviour when they occur (Carter et al., 2001). Poor performance monitoring activity in the ACC may ultimately be represented in the schizophrenia-associated PFC where it is integrated with other signals to produce a behavioural response. Therefore, I will look for these changes to performance monitoring in the anti-saccade task following the administration of a subanesthetic dose of ketamine. This data will be combined with single unit recordings in the PFC before and after ketamine administration to determine if the ketamine model of schizophrenia produces changes that coincide with those observed in patients with schizophrenia.

1.8.3 Examine PFC neuronal oscillatory activity for changes caused by ketamine and their behavioural correlates

Oscillatory neuronal activity has been described as the physiological phenomenon capable of synchronizing distant regions of the brain and facilitating interregional communication at a scale greater than single unit activity (Buzsaki and Draguhn, 2004). Specific frequency ranges of this oscillatory activity have been shown to be abnormal in the schizophrenic patient (Uhlhaas and Singer, 2010), but the EEG methodology used to record it is limited in its spatial resolution. Since the PFC is a hub for multiple sources of information, I will therefore look at a more accurate representation of oscillatory activity within the PFC by analyzing local oscillatory activity within the PFC both before and after the administration of ketamine to non-human primates while they perform the

anti-saccade task. By using intracranial recordings I will be able to identify changes in this oscillatory activity at a very precise level. Results from this study will help to relate neuronal oscillatory abnormalities observed externally in the schizophrenia patient with changes in local field potentials induced by an NMDA receptor antagonist.

1.9 Summary

It is clear that schizophrenia is a complex mental disorder with a number of underlying neuronal dysfunctions that all contribute to the final observed behavioural symptoms. Glutamate, and its signaling through the NMDA receptor, plays a central role in the disease pathology. Previous studies have shown that the NMDA receptor antagonist ketamine produces an animal model with a striking number of resemblances to schizophrenia at both a behavioural and neurological level. I intend to improve our understanding of how schizophrenia manifests itself in the dlPFC, a region unique to primates. My project will couple electrophysiological activity recordings with behavioural performance analysis during a task that requires cognitive control over saccade responses. I will use these techniques to investigate how ketamine affects both dimensions simultaneously and the interaction between neuronal and behavioural changes. By improving our understanding of the ketamine model of schizophrenia I hope to ultimately provide us with a better understanding of the disease itself so that better therapies may be pursued.

1.10 References

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Chapter 2

NMDA antagonist ketamine reduces task-selectivity in macaque dorsolateral prefrontal neurons and impairs performance of randomly interleaved pro- and anti-saccades

A version of Chapter 2 is published as K Skoblenick and S Everling, (2012) NMDA antagonist ketamine reduces task-selectivity in macaque dorsolateral prefrontal neurons and impairs performance of randomly interleaved pro- and anti-saccades. *The Journal of Neuroscience*.

2.1 Introduction

The use of N-methyl-D-aspartate (NMDA) receptor antagonists to induce a preclinical animal model of schizophrenia has been gaining traction over recent years. Acute doses of ketamine, a non-competitive NMDA antagonist, have been shown to induce short-lived behavioural profiles that include the positive, negative, and cognitive symptoms of schizophrenia in humans (Krystal et al., 1994; Lahti et al., 1995; Adler et al., 1999; Newcomer et al., 1999; Taffe et al., 2002). Further, a subanaesthetic dose of ketamine can often trigger a psychotic episode in patients already suffering from the disease (Malhotra et al., 1997; Lahti et al., 2001).

The ketamine-induced preclinical model of schizophrenia generates robust cognitive impairments as demonstrated by tasks probing working memory and

the suppression of prepotent responses to stimuli (Tsai et al., 1995; Olney et al., 1999; Javitt, 2009). Reduced cognitive function is considered to be the most debilitating aspect of schizophrenia as the severity of these symptoms directly relates to the patient's quality of life and current pharmaceutical interventions provide minimal improvement (Elvevag and Goldberg, 2000; Goldman-Rakic et al., 2004; Goeree et al., 2005; van Os and Kapur, 2009). Non-human primates also show similar cognitive deficits following systemic subanaesthetic doses of ketamine as patients with schizophrenia in a number of behavioural tasks (Condy et al., 2005; Stoet and Snyder, 2006). An example is the anti-saccade paradigm, which requires the inhibition of a prepotent pro-saccade towards a flashed peripheral stimulus in favour of the generation of a saccade away from the stimulus towards the opposite direction (Everling and Fischer, 1998; Munoz and Everling, 2004). Following ketamine injections, non-human primates exhibit increased reaction times and error rates on anti-saccade trials (Condy et al., 2005). These impairments seem to mimic the deficits observed in patients with schizophrenia (Fukushima et al., 1988; McDowell et al., 2002) and patients with prefrontal cortex (PFC) lesions (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991).

The behavioural profile of cognitive deficits following subanesthetic doses of ketamine has been well documented, however, the neural mechanisms in the primate PFC responsible for these changes are still unknown. Although rodent

studies have reported an increase in frontal cortex neural activity following acute ketamine administration (Jackson et al., 2004; Homayoun and Moghaddam, 2007), rodents lack a granular PFC which is characteristic for lateral, ventral, medial, and frontopolar prefrontal areas in primates (Povinelli and Preuss, 1995; Preuss, 2000; Wise, 2008). To directly investigate the effects of ketamine on task-selective neural activity in the primate lateral PFC, we recorded single neuron activity in macaque monkeys before and after the administration of subanesthetic doses of ketamine during the performance of randomly interleaved pro- anti-saccade trials.

2.2 Materials and Methods

All experiments were performed in accordance with the Canadian Council of Animal Care policy on the use of laboratory animals and all procedures were approved by the Animal Use subcommittee of the University of Western Ontario Council on Animal Care (**Appendix A**). Two male rhesus monkeys (*Macaca mulatta*) weighing 7 kg (Monkey O) and 10 kg (Monkey W) participated in the study. Both animals had a plastic head restraint and plastic recording chambers implanted above their lateral PFC as previously described by our laboratory (Johnston and Everling, 2006b). Animals received analgesics and antibiotics postoperatively and were closely monitored by a university veterinarian. Following implantation surgery, we obtained anatomical MR images of both monkeys using an actively shielded 7 Tesla 68-cm horizontal bore scanner

(Varian, Yarnton, UK) and a Siemens AC84 gradient subsystem (Erlangen, Germany). Plastic grids filled with betadine were inserted into the recording chambers during the scan which allowed for precise electrode targeting and subsequent reconstruction of recording locations.

2.2.1 Behavioural Task

Both animals were trained on rule-visible and rule-memorized variants of the pro/anti-saccade task (**Figure 2.1**). The rule for the current trial was presented following an initial 100 msec fixation period by changing the central white fixation spot to either green or red, with each color indicating whether the animal had to perform a pro-saccade (green fixation cue) or anti-saccade (red fixation cue) in response to the ensuing peripheral stimulus. On rule-visible trials, a peripheral stimulus appeared 8° to either the left or right following a 1000-1200 msec fixation period on the rule cue. To challenge the animal's working memory, the rule-memorized trials presented the rule cue for only 200 msec, after which the color of the cue returned to white for 800-1000 msec. Following this delay period, the peripheral stimulus was presented in a manner identical to the rule-visible trials. To obtain a juice reward, the animal was required to look to the correct target location within a 8° circular window. On pro-saccade trials, this required a saccade towards the peripheral stimulus, whereas on anti-saccade trials, the correct target location was diametrically opposite of the peripheral stimulus. Every block performed consisted of a pseudo-randomized ordering of

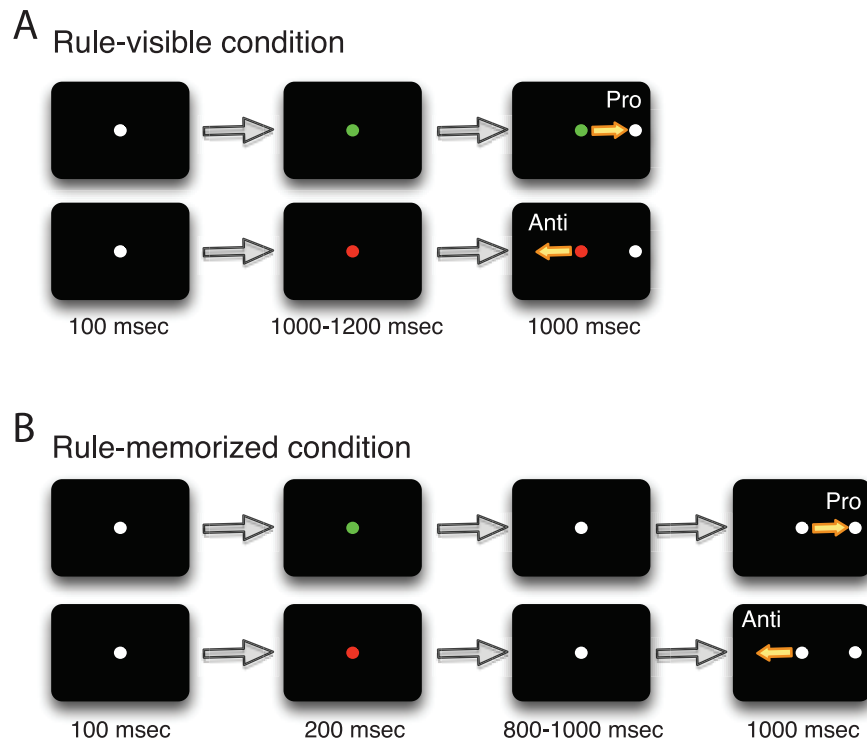


Figure 2.1: Experimental paradigm. *A*, Rule-visible task. Each trial began with an FP signaling, by its color, a prosaccade or antisaccade trial. A stimulus then appeared either 8° to either the left or right. *B*, Rule-memorized task. Same as *A*, but the color of the FP changed to white 800-1000 ms before stimulus presentation. This required the monkey to memorize the task rule.

the eight trial variants: pro-saccade left and right; anti-saccade left and right; with each of these four conditions appearing as either rule-visible or rule-memorized. Error trials were not repeated. The animals' eye positions were recorded and digitized at 120Hz using an ISCAN infra-red pupillary tracking system (ISCAN Inc., Woburn, MA).

2.2.2 Multi-electrode recording

Each behavioural testing session was accompanied by electrophysiological recordings from the left or right lateral PFC in monkey O and the left lateral PFC in monkey W. Initial recordings were performed with twenty screw mini-microdrives (Miller laboratory, MIT, Boston, MA), each equipped with two tungsten electrodes (FHC Inst., Bowdoinham, ME). Neural activity was combined with performance data and eye position data into a single recording file using a multi-acquisition processor (MAP) system (Plexon Inc., Dallas, TX). Recorded neurons were sorted off-line using 2-D and 3-D principal component analysis. In later recording sessions, we used a custom-designed semi-chronic screw microdrive recording system which contained 32-recording electrodes and 2 reference electrodes (Neuronitek, London, Ontario). Initial insertion of the semi-chronic drive consisted of lowering individual electrodes through both a thin silicone membrane and the animal's dura until stable units were isolated on a maximal number of channels. Each subsequent recording day only required reconnecting the 32-channel headstage (Plexon Inc., Dallas, TX) and minimal

depth adjustments to isolate new units to record for that day's experimental session. The microdrive system remained implanted for two weeks before it was removed for cleaning and maintenance.

2.2.3 Drug administration

Each experimental session began with a 15-minute period of baseline activity during which the animal performed approximately 200-250 trials in a pseudo-randomized order. Both animals were trained extensively at the task and performed at a <20% error rate during these pre-injection periods. After 15 minutes of trials, the experiment was briefly paused and the animal received a single intramuscular injection of ketamine (0.4mg/kg Monkey O; 0.8mg/kg Monkey W) diluted in sterile saline to 0.4 ml into their right triceps brachii muscle. The injection process only interrupted the animal's experimental session for less than 15 seconds after which the monkeys would continue the behavioural task. We previously performed titration experiments with each monkey to determine animal-specific concentrations of ketamine (between 0.2mg/kg and 1.0mg/kg) for the experiment, as these values have previously been found to elicit cognitive deficits with minimal anaesthetic effects (Condy et al., 2005). Monkey W had more exposure to ketamine prior to this study for surgeries and routine veterinary procedures and developed a greater tolerance to the drug (Pouget et al., 2010). The behavioural effects of ketamine became apparent after approximately 5 minutes (50-75 trials). Control experiments involving the intramuscular injection

of saline only were also performed. No changes in behavior (error rates and reaction times) or single neuron activity between the pre-injection and post-injection periods were observed (data not shown here). Experimental sessions were separated by at least 4 days to ensure the ketamine had a sufficient wash-out period and thus avoid cumulative dosing effects.

2.2.4 Data Analysis

Behavioural data was calculated with the pre-injection periods defined as 15 minutes prior to injection and the post-injection period defined as 5 minutes after injection until 25 minutes after injection, as this was the time period that displayed the strongest behavioural effects of the drug. Directional errors were trials in which the animal made a saccade in the opposite direction of a correct response (anti-saccade on a pro-saccade trial and vice versa). No response trials were those in which the animal maintained fixation during the response period, while fixation errors consisted of trials in which the animal did not maintain fixation until the stimulus onset. Significance was calculated between pre-injection and post-injection values for each category using paired t-tests. Later, time-course behavioural data was calculated by combining all experimental sessions for both monkeys and sorting the data into 5 minute bins for error rates and saccadic reaction times. A one-way analysis of variance (ANOVA) followed by a post-hoc Dunnett's test was used to probe each trial type for significant differences between the animal's pre-injection performance and their performance at each

binned time-point. For the analysis of neural data, we included correct and directional error trials. The calculation of indexed values for the change in each neurons discharge frequency included neural activity from the entire trial period defined as 1000 msec preceding stimulus presentation until 500 msec after stimulus presentation. Neurons were subjected to further analysis to highlight changes in task-selectivity following ketamine administration. Task-selectivity was calculated as $(\bar{X}_{\text{preferred}} - \bar{X}_{\text{non-preferred}}) / (\bar{X}_{\text{preferred}} + \bar{X}_{\text{non-preferred}})$ using the mean discharge rate from each neuron during a pre-stimulus epoch and a stimulus/response epoch. Preference was defined as either task preference (pro-saccade versus anti-saccade) or direction preference (contralateral stimulus versus ipsilateral stimulus). Whichever comparison exhibited a greater selectivity was used for the pre-injection versus post-injection scatter plot defined by epoch and rule visible or rule-memorized conditions. Statistical significance for each neuron was tested using a Mann-Whitney U-test for changes in task selectivity caused by the ketamine administration.

To define cells as either narrow-spiking (putative interneurons) or broad-spiking neurons (putative pyramidal neurons), we calculated mean trough to peak times for each neuron's extracellular waveform and constructed a histogram of the resulting values as previously described (Johnston et al., 2009). In accordance with this previous study, which used the same recording system and the same type of microelectrodes, we defined neurons with waveform duration

shorter than 270 μ sec as narrow-spiking neurons and any neurons with waveform durations longer than 270 μ sec this as broad-spiking neurons.

Finally, a sliding receiver operating characteristic (ROC) curve was computed using the neurons that were shown to exhibit selectivity in either of the two epochs so that any differences in the significance of task-selectivity across the entire trial period both pre- and post-injection would be apparent. The ROC value was calculated using a sliding 100 msec window beginning 1000 msec before peripheral stimulus onset, advancing in 1 msec increments, and extending 500 msec beyond stimulus presentation. Significance cutoff values for the 5th and 95th percentile were calculated using a bootstrap method (Koval et al., 2011).

2.3 Results

2.3.1 Behavioural deficits

Both animals displayed acute increases in response errors, fixation errors, and no response trials when attempting to perform both pro- and anti-saccade trials following ketamine administration (**Figure 2.2A**). The effect lasted approximately 20 minutes in monkey O and 35 minutes in monkey W, with performance returning to pre-injection error rates afterwards in monkey O. Error rates decreased in monkey W over time after the initial ketamine effect, but never returned to pre-injection levels in the timespan of each experimental session. The

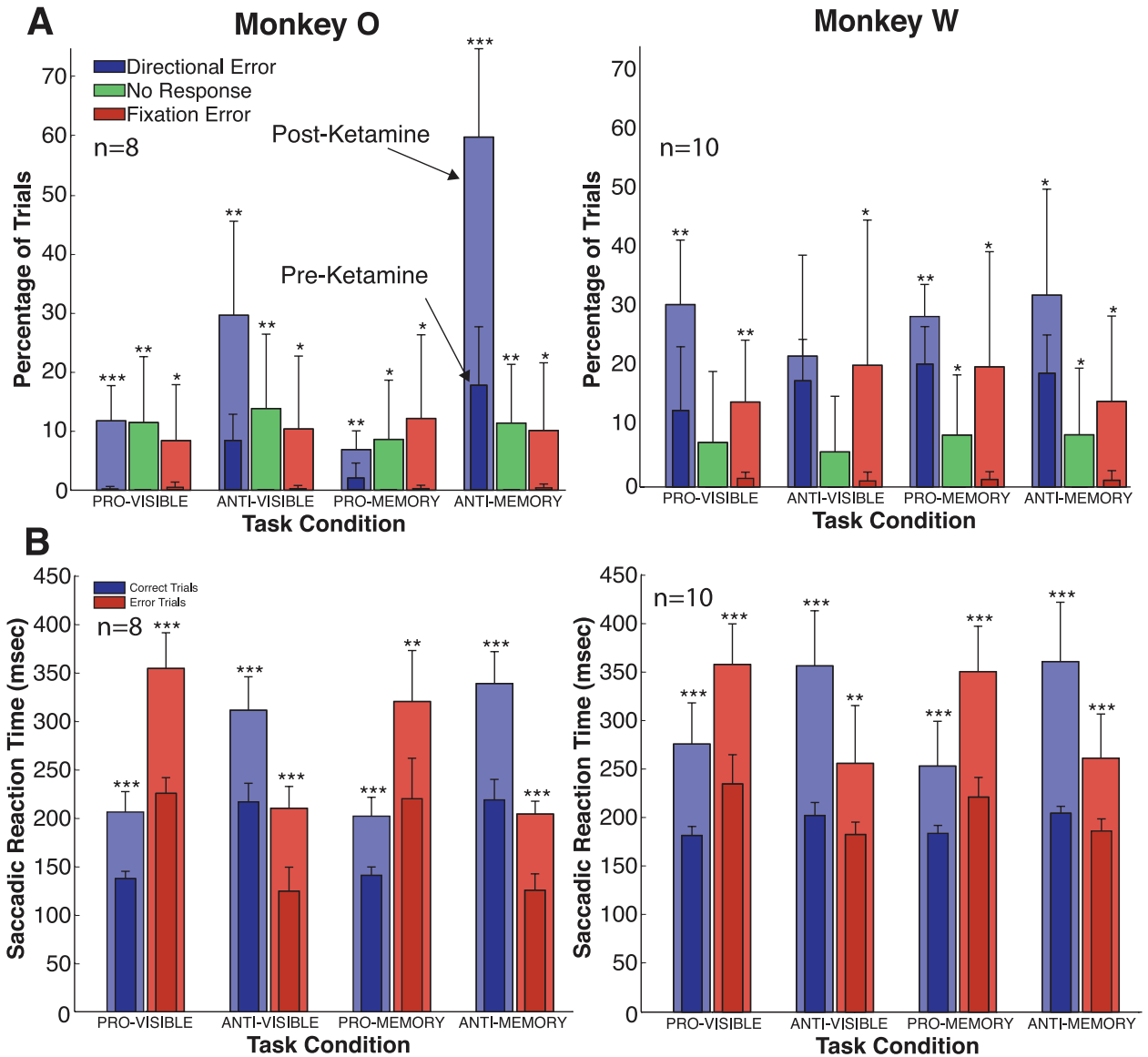


Figure 2.2: Behavioural effects of ketamine administration. *A*, Effect of ketamine on the directional error rates (blue bars), response rates (green bars), and fixation error rates (red bars). Darker shaded foreground bars represent pre-injection values and lighter background bars are post-injection values. Data for Monkey O was averaged from 8 experiment sessions; data for Monkey W was averaged from 10 experiment sessions. *B*, Effect of ketamine on saccade reaction times. Same format as *A* with blue bars representing saccade reaction times on correct trials and red bars representing saccade reaction times on (directional) error trials.

increased error rate on pro-saccade trials for both rule-visible and rule-memorized conditions reached a maximum of 10% in Monkey O and 30% in Monkey W. Ketamine caused a much stronger effect on the anti-saccade task in Monkey O, with response errors increasing 3-fold to 30% in the rule-visible task and to almost 60% in the rule-memorized task.

Error rates on the anti-saccade task highlighted the additional challenge that the rule-memorized paradigm introduced. Both monkeys made more errors in the rule-memorized condition than in the rule-visible condition. A similar separation between rule-visible and rule-memorized tasks was not observed on the pro-saccade task for either monkey. These changes were accompanied by a weak post-saccade nystagmus, similar to those reported in previous studies involving ketamine and saccadic eye movements (Radant et al., 1998; Condy et al., 2005; Shen et al., 2010).

Saccade reaction times (SRTs) were also increased across all conditions in both animals (**Figure 2.2B**). When analyzing the SRT for error trials only, we found that these were also increased and that the errors being made were not express saccades (Fischer et al., 1984). Ketamine had a similar lengthening effect on SRTs of both correct and error responses.

2.3.2 Ketamine increases activity but decreases task selectivity of PFC neurons

We recorded the activity of a total of 115 neurons in the lateral PFC from two monkeys (97 from O, 18 from W) in 18 recording sessions in which we injected subanesthetic doses of ketamine (**Figure 2.3**). **Figure 2.4** illustrates the effect of ketamine on a single PFC neuron. Pre-injection (left panel), the neuron exhibited higher levels of activity prior to peripheral stimulus onset on pro-saccade trials than on anti-saccade trials. These rule-related differences were present at the time of stimulus presentation on both rule-visible (**Figure 2.4A**, left panel) and rule-memorized trials (**Figure 2.4B**, left panel). In the post-injection period, the neuron displayed an overall increase in activity and a decrease in rule-selectivity at the time of stimulus presentation, which was especially evident on rule-memorized trials (**Figure 2.4B**, right panel).

Figure 2.5 shows a scatter plot comparing indexed values of the change in the overall neural activity with indexed values of the change in the task selectivity of each cell. A significant majority of neurons ($n=75$, 65%) were found to be located in the lower right quadrant, indicating an increase in neural activity but a decrease in task selectivity (chi-squared test, $p<0.001$). Calculations were also performed to define each neuron as either narrow spiking (*filled symbols*, $n=20$) or broad spiking (*hollow symbols*, $n=95$) neural activity, but there appeared to be no difference between the two groups in their distribution across the scatter plot.

To further examine the effects of ketamine on neural activity, we analyzed

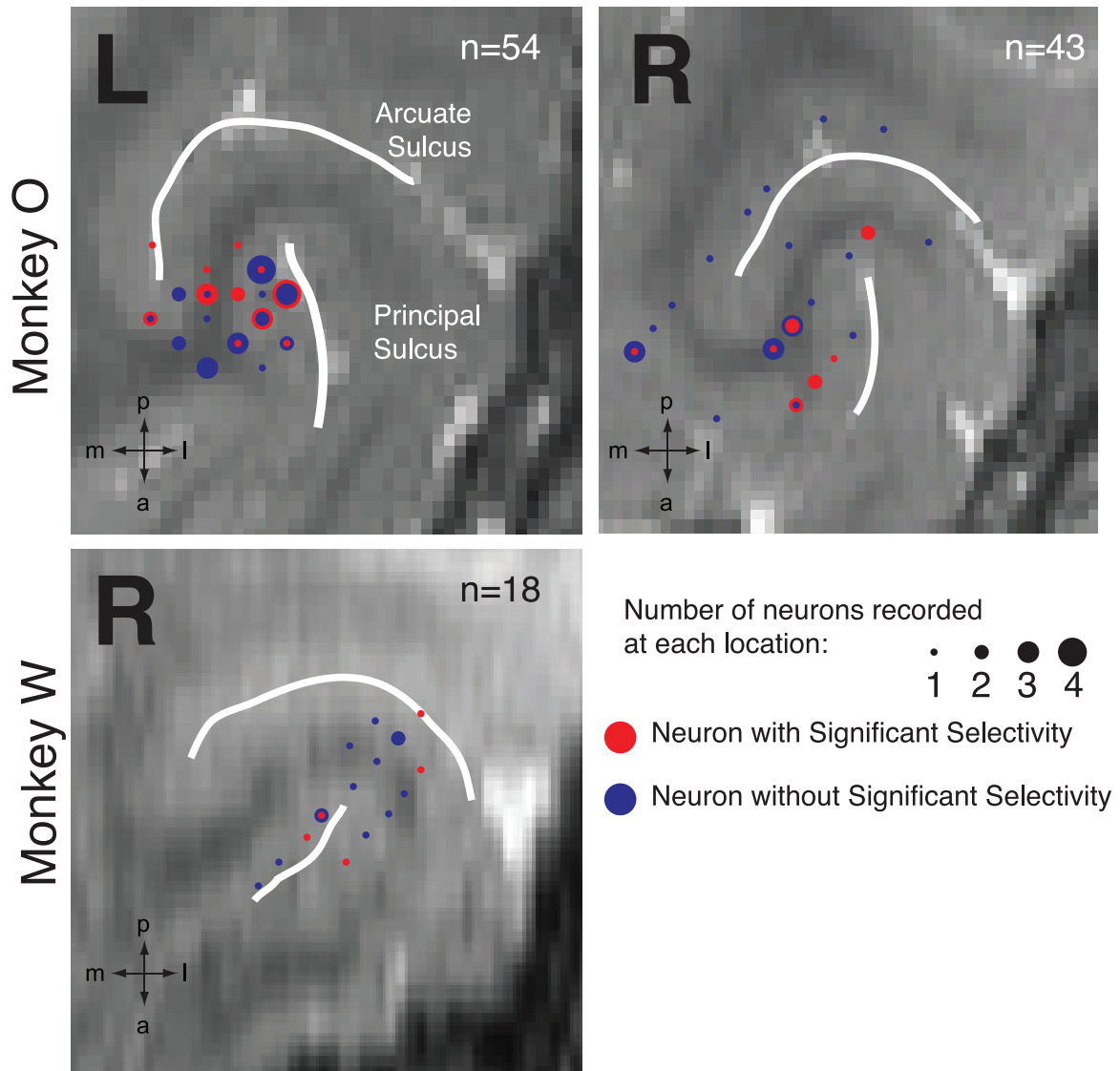


Figure 2.3: MRI reconstruction of recording locations. L, left hemisphere; R, right hemisphere; m, medial; a, anterior; p, posterior; l, lateral.

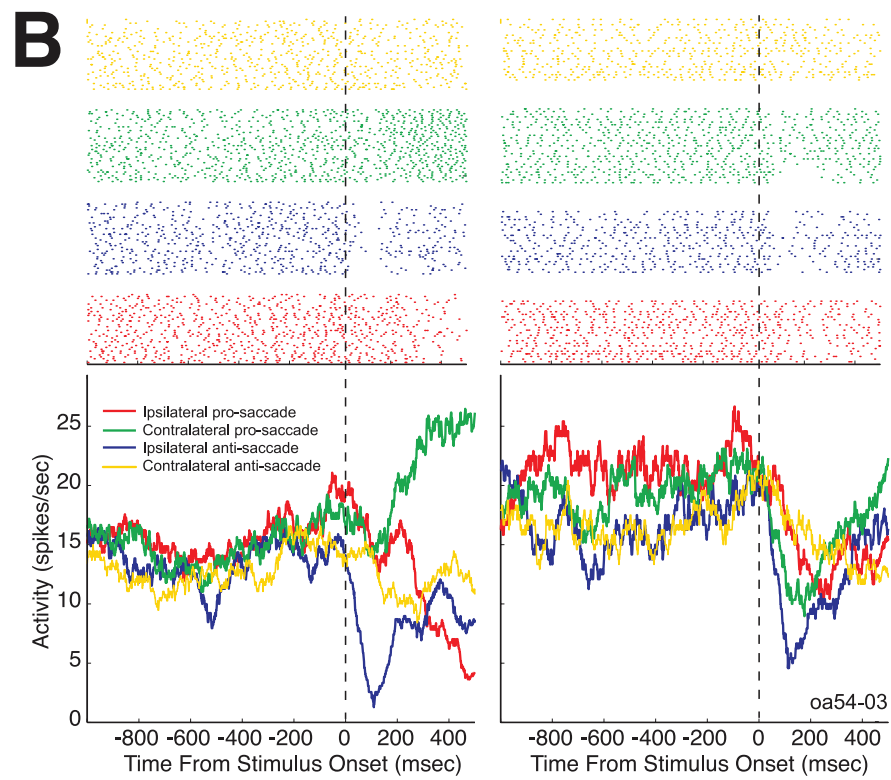
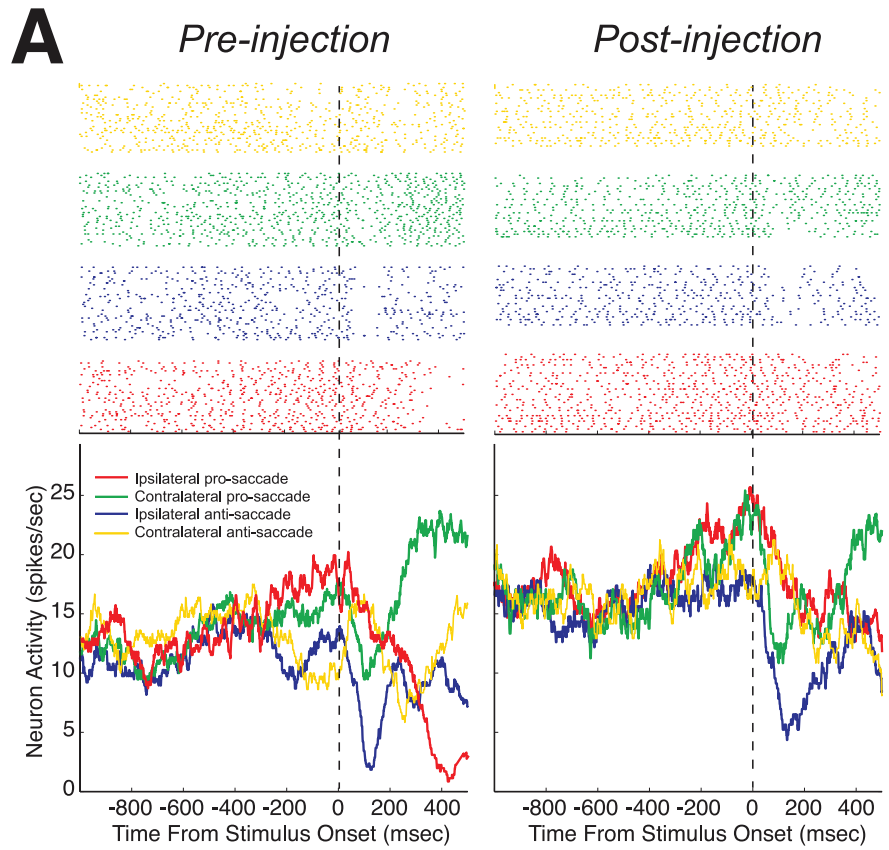


Figure 2.4 (previous page): Effect of ketamine on a single PFC neuron. The neuron was recorded from monkey O in the dorsal bank of the principal sulcus in the right hemisphere. *A*, Activity on rule-visible trials. *B*, Activity on rule-memorized trials. Upper panel shows a raster plot with each dot indicating the time of an action potential relative to stimulus presentation, and each row represents one trial.

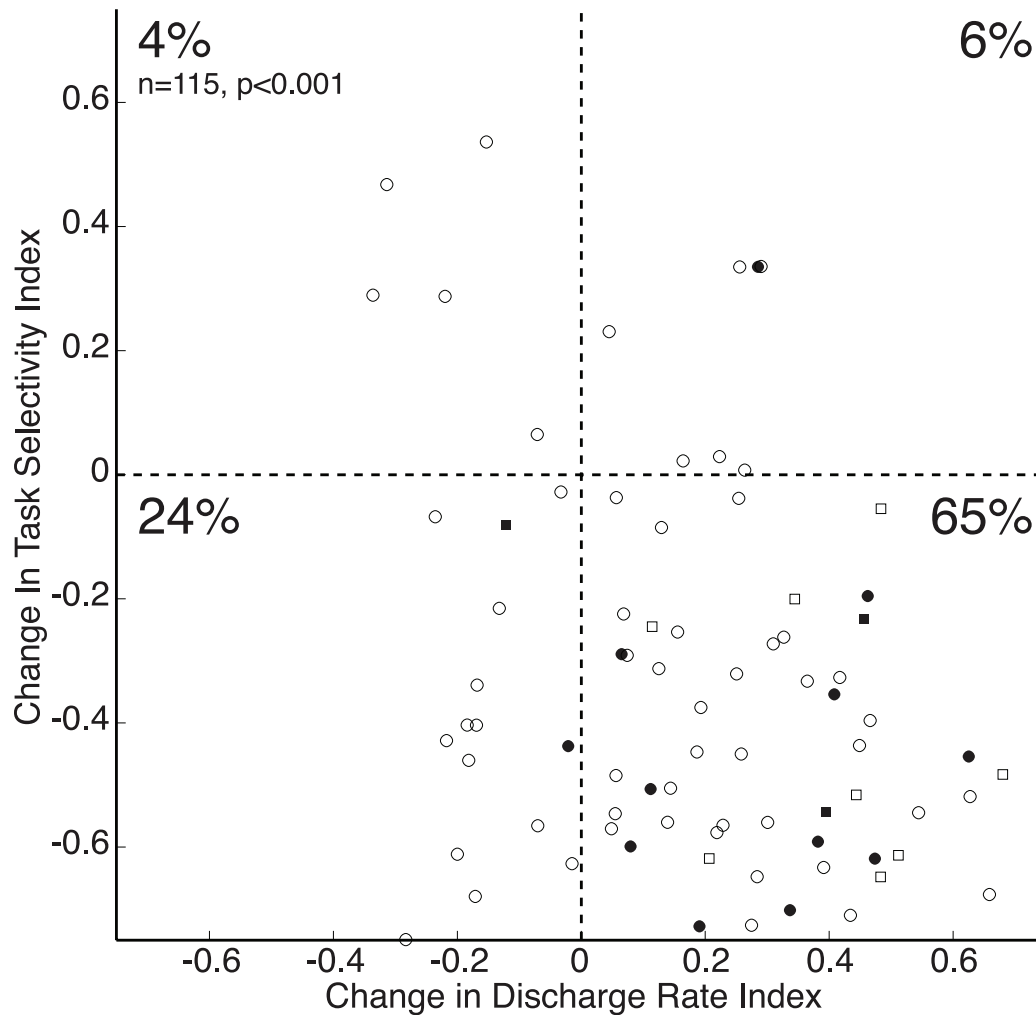


Figure 2.5: Effect of ketamine on change in discharge rate and change in task selectivity. Scatter plot depicts the change in discharge rates of neurons (interval from 1000 msec before to 500 msec after stimulus onset) after ketamine injection on the x-axis and the change in task selectivity (same analysis interval as x-axis) on the y-axis. *Circles* indicate neurons recorded from Monkey O, *squares* indicate neurons recorded from Monkey W, *filled* represent narrow-spiking neurons, and *hollow* points represent broad-spiking neurons.

mean discharge rates during the pre-stimulus period (500 msec before to stimulus onset) with a three-way ANOVA with the factors RUL (pro-saccade or anti-saccade), MEM (rule-visible or rule-memorized), and DRUG (pre-injection and post-injection). This analysis showed that the factor DRUG had the strongest effect on pre-stimulus activity, with ~60% (n=69) of neurons showing significant differences in activity between pre- and post-injection periods (**Figure 2.6A**). Further, 35 neurons (33 from Monkey O, 2 from Monkey W) displayed interaction effects of DRUG with MEM and/or RUL. Next, we examined the effects of ketamine on neural activity during the stimulus/response period (defined as the period 100 msec to 400 msec following stimulus onset) with a three-way ANOVA with the factors DIR (contralateral or ipsilateral stimulus location), RUL (pro-saccade or anti-saccade), and DRUG (pre-injection and post-injection). The results are shown in **Figure 2.6B**. Overall, 40% (n=46) of PFC neurons showed a main effect of DRUG. In addition, 49 neurons (43 from Monkey O, 6 from Monkey W) showed significant interactions of DRUG with DIR and/or RUL. For the remainder of the analysis, we focus on those PFC neurons that exhibited interaction effects of DRUG (pre- versus post-ketamine injection) during the pre-stimulus or stimulus/response period.

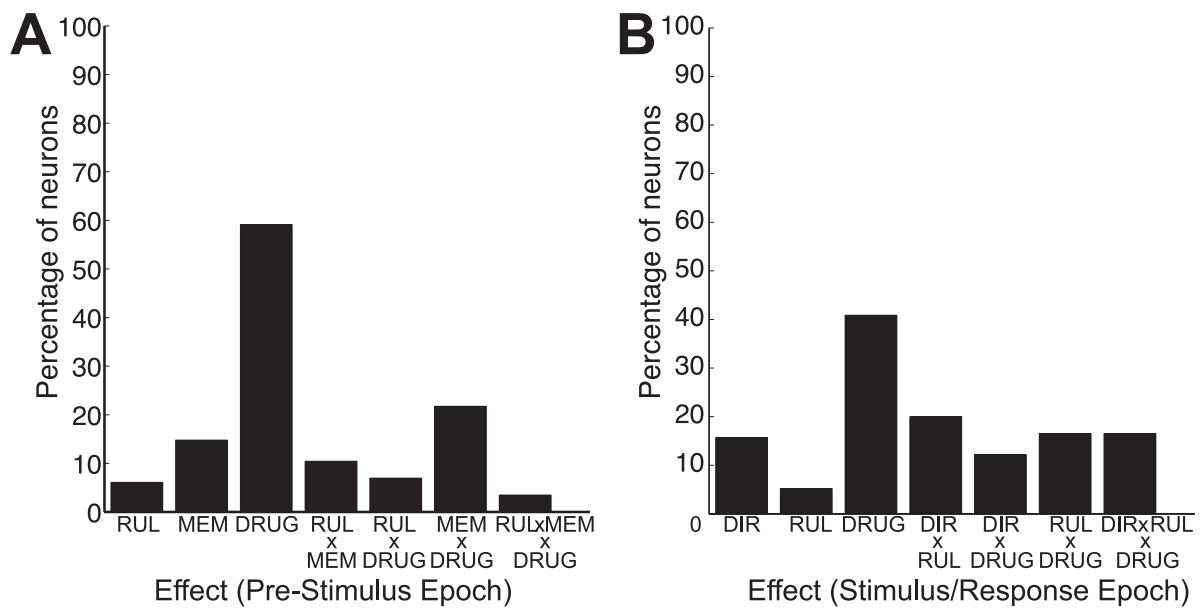


Figure 2.6: Results of three-way ANOVA on differences in neural activity. A, Results during pre-stimulus epoch. B, Results during stimulus/response epoch. RUL: rule (pro-saccade versus anti-saccade); MEM: memory condition (rule visible versus rule memorized condition); DIR: direction (ipsilateral versus contralateral stimulus location) DRUG: ketamine status (pre-injection versus post-injection).

2.3.3 Ketamine-induced decrease in neural task selectivity is synchronous with behavioural changes

Figure 2.7 shows the time-course of the behavioural changes as well as the task-selectivity of these PFC neurons during the recording sessions. Shortly after ketamine-administration, error rates (**Figure 2.7A**) and SRTs (**Figure 2.7B**) significantly increase from pre-injection levels. Following the same time course, task-selectivity (**Figure 2.7C**) decreased during the pre-stimulus period (*dashed line*) and the stimulus/response period (*solid line*). For each of the neurons selected from the ANOVAs we calculated a pre-injection and post-injection selectivity index for the pre-stimulus (**Figure 2.8A**) and stimulus/response period (**Figure 2.8B**). The population of neurons exhibited a significant decrease in memory-selectivity (rule-visible versus rule-memorized) ($p < 0.001$, Wilcoxon-signed rank test) and rule-selectivity (pro- versus anti-saccade) ($p < 0.01$, Wilcoxon-signed rank test) during the pre-stimulus period and in rule- ($p < 0.001$, Wilcoxon-signed rank test) and direction-selectivity (contra- versus ipsilateral) ($p < 0.001$, Wilcoxon-signed rank test) during the stimulus/response period. Neurons were again delineated as either narrow-spiking neurons (*filled symbols*) or broad-spiking neurons (*hollow symbols*). Both groups of neurons showed a reduction in task selectivity following ketamine injection.

If the decrease in performance is related to the decrease in task selectivity of prefrontal neurons following ketamine administration, one would expect that task-selectivity would be higher on correct trials than on error trials during this

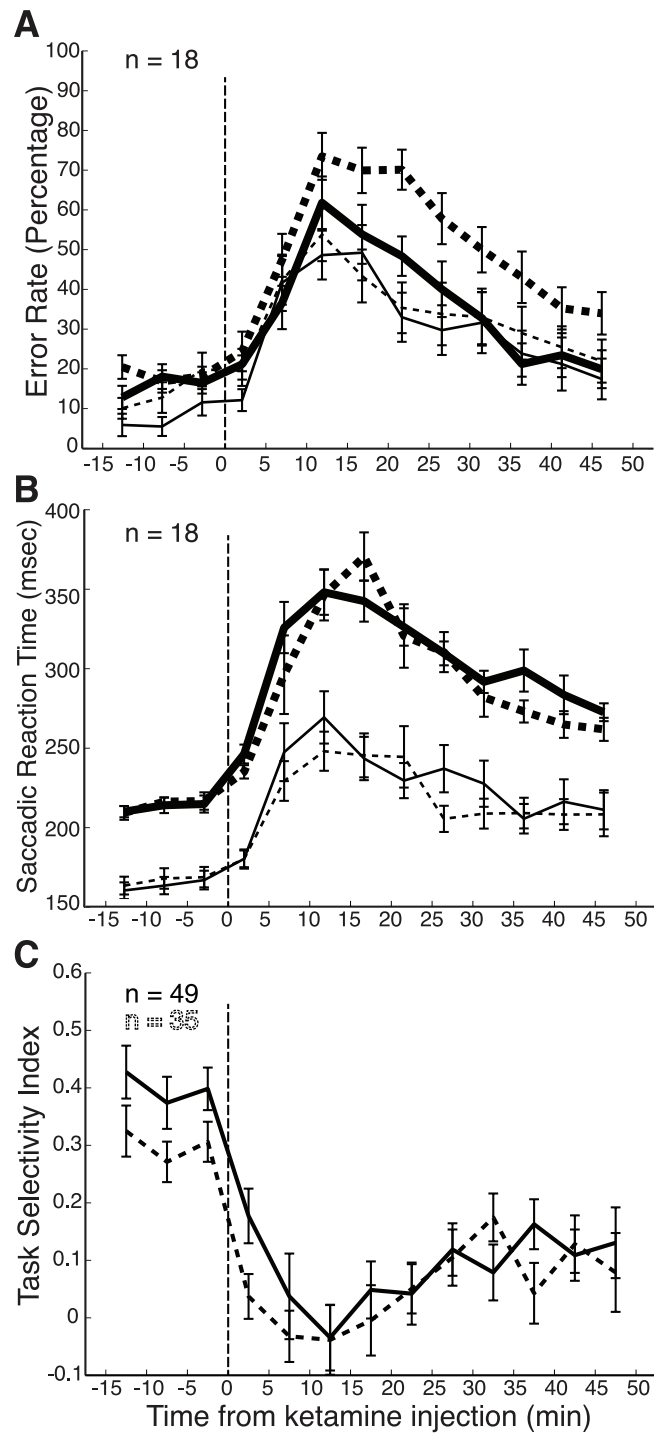


Figure 2.7: Time course of ketamine administration. *A*, Transient increase of error rates in pro-saccade (blue lines) and anti-saccade trials (red lines) in rule visible (solid lines) and rule memorized (dashed lines) conditions. *B*, Transient increase in saccade reaction times, same format as *A*. *C*, Reduction of task-selectivity following ketamine administration. Task-selectivity was calculated for significant neurons for the pre-stimulus (dashed line) and stimulus/response (solid line) epochs. Time of ketamine injection is indicated by a vertical dashed line.

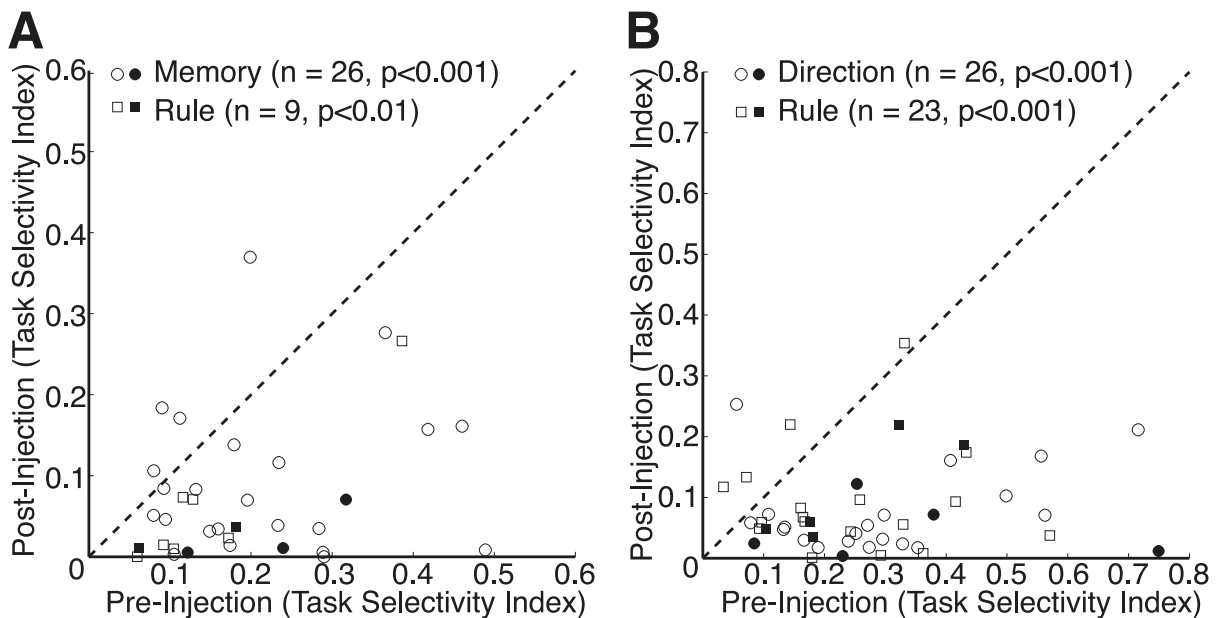


Figure 2.8: Effects of ketamine on task-selectivity. *A*, Task-selectivity for the individual 35 neurons is plotted during the pre- injection period against task-selectivity during the post-injection period. Rule indicates neurons (*squares*) that showed maximal task-selectivity between pro- and anti-saccades. Memory indicates neurons (*circles*) that showed maximal task-selectivity between the rule-visible and rule-memorized conditions. *Dashed line*, unity line (slope =1). *B*, Same as *A* but for the 49 neurons in the stimulus/response epoch. Rule indicates neurons (*squares*) that showed maximal task-selectivity between pro- and anti-saccades. Direction indicates neurons (*circles*) that showed maximal task-selectivity between ipsilateral and contralateral stimulus presentations. *Solid* points in both plots represent narrow spiking neurons, *hollow* points represent broad spiking neurons.

post-injection period. We tested this prediction by first identifying the preferred and non-preferred condition for each neuron (highest and lowest discharge rate, respectively) during the pre-injection period and then comparing task-selectivity following ketamine injection between correct trials and error trials. Neurons that were recorded from sessions in which the animal completed fewer than 5 of any task type correctly were excluded from this analysis. In both the pre-stimulus period (**Figure 2.9A**) and the stimulus/response period (**Figure 2.9B**), neurons showed a significantly lower task selectivity during erroneous trials than correct trials ($p < 0.01$, Wilcoxon-signed rank test). This analysis demonstrates that prefrontal task-selectivity during the pre-stimulus and stimulus/response period is correlated with the animals' task performance.

To illustrate the changes in task-selectivity following ketamine injection, we determined for each neuron that displayed interaction effects of DRUG during the pre-stimulus or stimulus/response period the neuron's preferred task (highest discharge rate) and non-preferred task (lowest discharge rate) during the pre-injection period and constructed mean population activity plots across the trial both before (*blue lines*) and after ketamine administration (*red lines*) (**Fig 2.10**). Although the mean discharge rate increased across the entire trial, the differences between the preferred (*solid lines*) and non-preferred condition (*dashed lines*) were reduced considerably following ketamine injection.

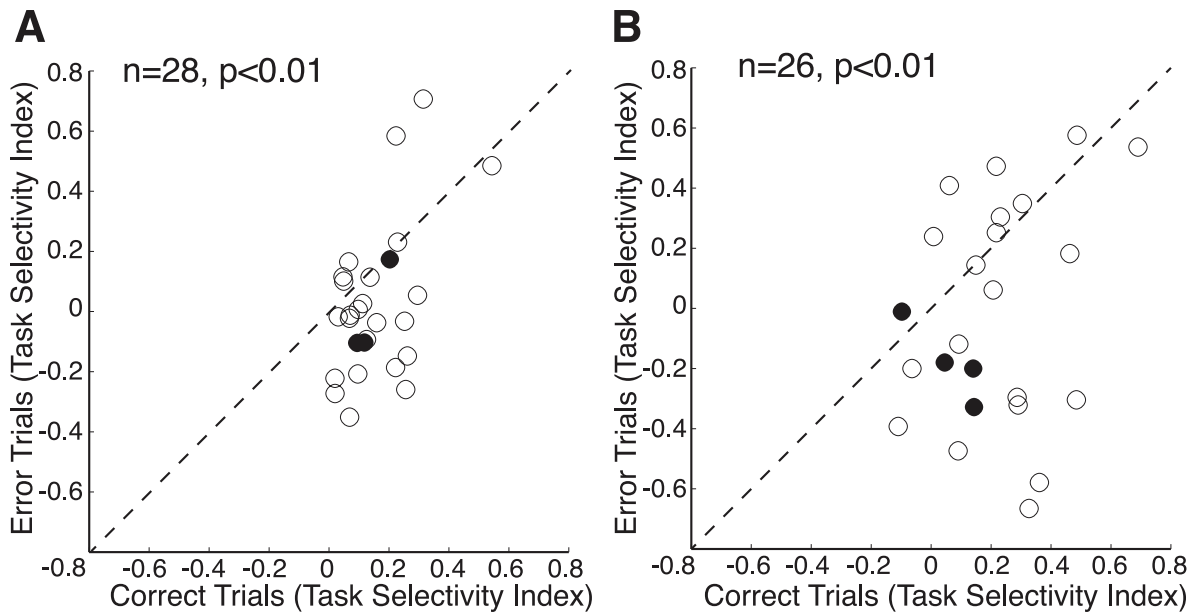


Figure 2.9: Changes in task-selectivity for error trials and correct trials. Change in task-selectivity of neurons during trials completed correctly after ketamine administration is plotted on the x-axis while changes in task-selectivity of neurons during error trials is plotted on the y-axis. *A*, Differences of correct trials versus error trials during the pre-stimulus epoch. Data points below the unity line (*dashed line*) indicate a greater loss in task selectivity during error trials. *B*, Differences of correct trials versus error trials during the stimulus/response epoch, same format as *A*. Solid points in both plots represent narrow spiking neurons, hollow points represent broad spiking neurons.

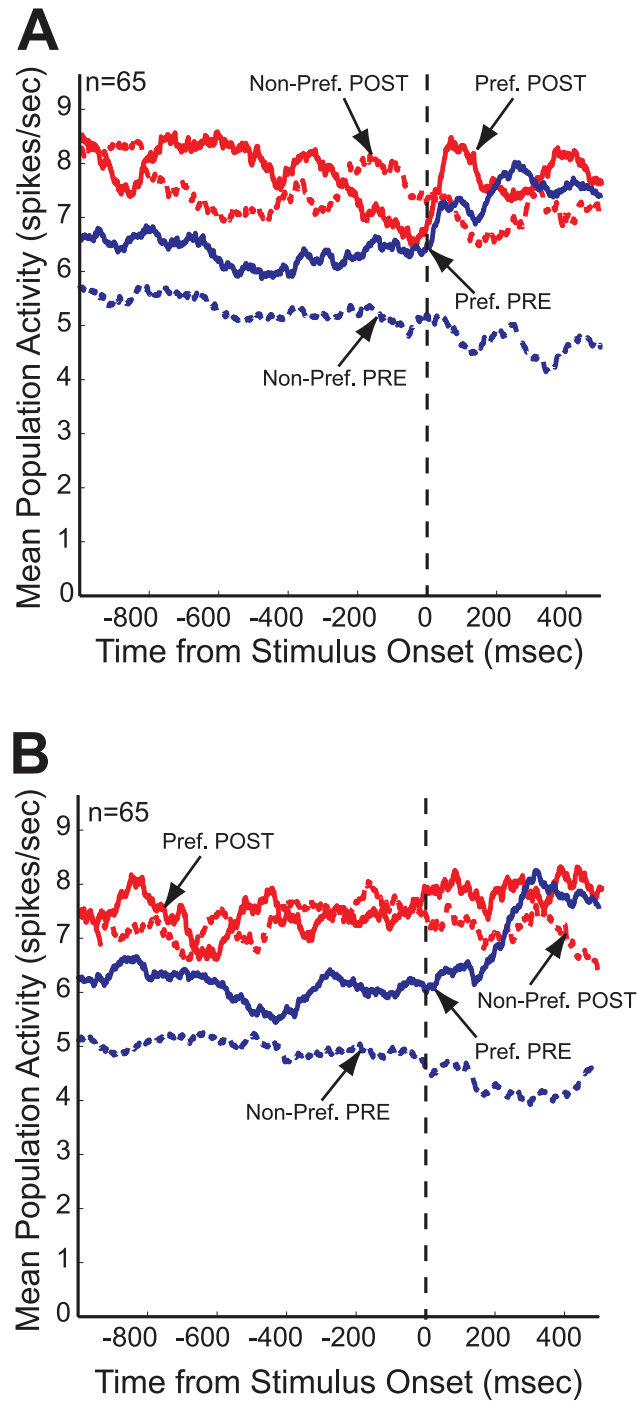


Figure 2.10: Effects of ketamine on population activity of task-selective neurons. In both rule-visible *A* and rule-memorized conditions *B*, the population activity show a large separation between the preferred and non-preferred condition before ketamine administration. This selectivity is reduced after ketamine.

Lastly, in order to highlight the importance of both epochs to our study, we constructed ROC curves testing task selectivity significance levels for rule-visible (**Figure 2.11A**) and rule-memorized (**Figure 2.11B**) tasks. The pre-stimulus period displayed multiple timepoints during which the selectivity achieved significance, however the strength of task selectivity in the stimulus/response period was much more prominent. The ROC values became non-significant following ketamine administration during both epochs.

2.4 Discussion

The ability of ketamine to induce a schizophrenia-like endophenotype was initially attributed to the antagonism of NMDA receptors in the PFC and an assumed downregulation of glutamatergic activity in this area (Krystal et al., 1994; Malhotra et al., 1996). Electrophysiological studies in rodents, however, have shown that acute, subanaesthetic doses of ketamine induce hyperactivity in the frontal cortex while still producing the cognitive deficits required for a model of this disease (Jackson et al., 2004; Homayoun and Moghaddam, 2007). Our results confirm this finding in the lateral PFC of nonhuman primates and demonstrate that subanaesthetic doses of ketamine reduce task-selectivity of PFC neurons. Our findings suggest that an increase in activity may be impairing task-selective outputs from the PFC by decreasing the task-selectivity of neurons and thus reducing the ability of the PFC to exert cognitive control.

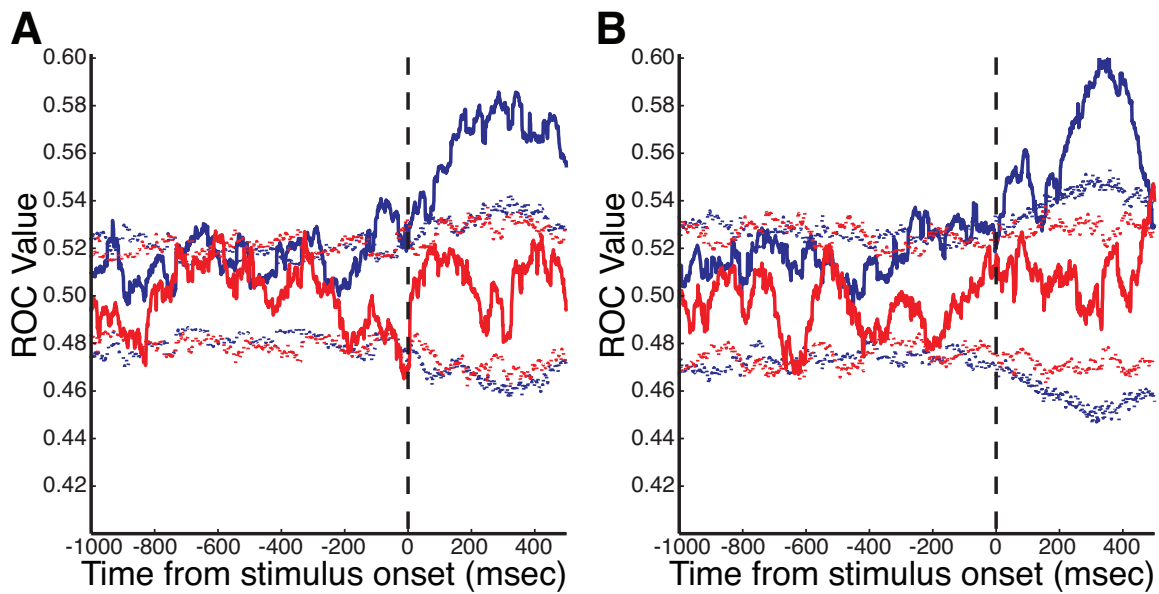


Figure 2.11: ROC curve of task-selectivity before and after ketamine administration. **A**, ROC values for rule visible trials. Pre-injection (blue solid line) and post-injection (red solid line) task selectivity ROC values with accompanying 5% and 95% (dashed lines) significance cutoff values as calculated by bootstrap analysis. **B**, ROC values for rule memorized trials, same format as **A**.

2.4.1 Ketamine impairs pro- and anti-saccades

A previous study that examined the behavioural consequences of acute ketamine administration in green monkeys found a dose-dependent increase in reaction times for both pro- and anti-saccades (Condy et al., 2005). Condy and colleagues (2005) tested pro- and anti-saccade trials in separate blocks and reported that ketamine increased errors by up to 60-70% on anti-saccade trials, whereas it did not impair the performance of pro-saccade blocks. Our data confirm the increases in SRTs and increased errors on anti-saccade trials following subanaesthetic doses of ketamine but also show that ketamine impairs the performance on pro-saccade trials when these are randomly interleaved with anti-saccade trials. Similar increases in SRT and error rates have been reported in a cued task-switch paradigm following ketamine administration (Stoet and Snyder, 2006).

The increased error rates by ketamine on anti-saccade trials have been interpreted as a loss of behavioural inhibitory control over the pre-potent stimulus-triggered saccade, potentially by interrupting dorsolateral PFC function (Condy et al., 2005). Our data do not support this hypothesis. While ketamine did increase the error rate on anti-saccade trials, it also led to a greater number of errors on pro-saccade trials, i.e. the monkeys generated anti-saccades on pro-saccade trials. The effects were prolonged on anti-saccade trials that contained a working memory component and following ketamine administration both monkeys

frequently discontinued fixation or did not respond. Furthermore, the errors on anti-saccade trials had reaction times well-above the range of automatic express saccades (80-125 msec) which are directly triggered by the incoming visual stimulus in the superior colliculus (Dorris et al., 1997; Everling et al., 1999). Interestingly, although often described as automatic or prepotent responses, errors of schizophrenic patients in the anti-saccade task are typically not in the express saccade range (Lencer, personal communication). We propose that ketamine increased errors on pro- and anti-saccade trials because it impaired the animal's ability to selectively maintain, or apply the two task sets. This type of deficit is common in patients with frontal lobe damage and has been termed goal neglect (Duncan et al., 1996). Duncan and colleagues have suggested that frontal and possibly parietal brain areas are involved in organizing relevant facts, rules, and requirements into a "task model" (Duncan et al., 2008). This idea is supported by many experiments on single neurons in the primate lateral PFC in behaving monkey. These studies have found that a large proportion of PFC neurons code various aspects of whatever task a monkey has been trained to perform (stimuli, responses, rules, rewards) and that the coding changes when the animal must perform a different task (Hoshi et al., 1998; Rainer et al., 1998; White and Wise, 1999; Asaad et al., 2000; Everling and DeSouza, 2005; Everling et al., 2006; Johnston and Everling, 2006a).

2.4.2 Ketamine increases activity of PFC neurons

To directly test whether ketamine interfered with the coding of task-relevant information in the PFC, we recorded the activity of single neurons in the lateral PFC before and after the administration of ketamine. Rodent studies showed that subanesthetic, systemic doses of ketamine are capable of increasing frontal cortex metabolic (Duncan et al., 1999; Dawson et al., 2013) and neural discharge activity (Jackson et al., 2004; Homayoun and Moghaddam, 2007). When administered to human subjects, acute doses of ketamine can also increase neural activity as measured by fMRI in working memory tasks (Honey et al., 2004) and regional cerebral blood flow (Holcomb et al., 2005). Here we found that subanaesthetic, systemic administration of ketamine in the behaving primate also acutely increases the activity of neurons in the lateral PFC. While the behavioural findings alone provide support for a ketamine-centric preclinical model of schizophrenia, there may be paradoxical reversal when the model is compared to the disease at the neurophysiological level.

The glutamate model of schizophrenia posits that many of the disease's symptoms (including the cognitive deficits) arise from a hypofrontality and hypoglutamatergic state in the PFC (Olney et al., 1999). While chronic administrations of NMDA antagonists can induce a similar hypofrontality (Morris et al., 2005; Mouri et al., 2007), the present study in primates and previous studies in rodents (Jackson et al., 2004; Homayoun and Moghaddam, 2007) found that acute treatments of NMDA antagonists *increase* frontal activity. It

should be noted here that the hypofrontality in the PFC of schizophrenic patients is inferred from EEG, PET, and fMRI studies but that it is unknown whether the activity of single PFC neurons is actually reduced in schizophrenic patients (Ragland et al., 2007; Marek et al., 2010).

It has been suggested that ketamine acts preferentially on PFC interneurons and therefore produces a localized disinhibition of pyramidal neurons (Homayoun and Moghaddam, 2007). This specificity for GABAergic interneurons parallels postmortem studies in humans that found patients with schizophrenia often exhibited reduced levels of interneuron markers (Benes et al., 1991; Lewis et al., 1999). However a more recent study has found that this selectivity may not be this straightforward, as NMDA antagonists have been shown to exhibit a lowered affinity for fast-spiking interneurons (Rotaru et al., 2011). This newer data supports the hypothesis that the mechanism behind the increased activity in the PFC may be an effect of NMDA receptor blockade in regions of the brain that have inhibitory projections to the PFC (Kiss et al., 2011) since localized injections of NMDA antagonists into the PFC were unable to increase PFC glutamatergic activity in rodents (Suzuki et al., 2002; Lorrain et al., 2003). To test whether ketamine had different effects on interneurons and pyramidal cells, we separated the recorded neurons based on their spike widths (Mitchell et al., 2007; Johnston et al., 2009). The results suggest that systemic ketamine administration had similar effects on the two groups, however, these

results should be interpreted with caution because of the low number of putative interneurons.

One might have expected that an increase in activity would increase task-selectivity of PFC neurons, but our data clearly show that the increase in activity was accompanied by a decrease in selectivity for the pro-/anti-saccade task and response direction. Moreover, we could show that task-selectivity was lower on error trials than correct trials following ketamine administration. This finding demonstrates that task-selectivity of PFC neurons correlates with the animal's performance. This non-discriminatory increase in neural activity - which decreases task-selectivity - would decrease the task-selective signals that the PFC sends to other areas like the superior colliculus (Johnston and Everling, 2006a, 2009). Without these task-selective signals, the flow of task-related activity in the saccade-generation network may be impaired. Interestingly, an increased prefrontal activation has recently been shown using fMRI in the dorsolateral PFC of schizophrenic patients during the performance of pro- and anti-saccades (Fukumoto-Motoshita et al., 2009).

It should be noted that the behavioural deficits in task performance are much stronger in this study that induced PFC hyperactivity than they were in a previous study, where we induced acute PFC hypoactivity via surgically implanted cryoloops in the principle sulcus (Koval et al., 2011). Animals

performing the anti-saccade task exhibited only mild impairments in the rule-visible task when the principal sulcus was cryogenically deactivated, however, performance in the rule-memorized task was impaired to a much greater degree. The strong impairments on the rule-visible task following ketamine injections seen here may have been caused by a disruption of task-selectivity in the ventral PFC, which is critical for cued stimulus-response associations (Bussey et al., 2001, 2002; Buckley et al., 2009).

Due to the systemic administration method, we cannot rule out that the behavioural effects by ketamine on pro- and anti-saccades are mediated by areas outside of the lateral PFC, as task-selectivity for pro- and anti-saccades has also been found in the globus pallidus, thalamus, lateral intraparietal area, frontal eye fields, and supplementary eye fields (Schlag-Rey et al., 1997; Gottlieb and Goldberg, 1999; Everling and Munoz, 2000; Zhang and Barash, 2000; Ford and Everling, 2009; Watanabe and Munoz, 2009; Yoshida and Tanaka, 2009; Kunimatsu and Tanaka, 2010). In fact, it has previously been demonstrated that ketamine can have a significant effect on superior colliculus neurons (Populin, 2005), however that study used doses of ketamine well within the anaesthetic range.

In summary, our data show that ketamine increases the activity of PFC neurons in primates, while it reduces at the same time task-selectivity in these

neurons. The non-discriminate increase in PFC activity may mask the efferent task-related signal required by downstream cortical and subcortical regions for correct task performance, effectively exporting irrelevant noise.

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Chapter 3

NMDA receptor antagonist ketamine impairs action-monitoring activity in the prefrontal cortex

A version of Chapter 3 is published as K Skoblenick and S Everling, (2014) NMDA receptor antagonist ketamine impairs action-monitoring activity in the prefrontal cortex. *The Journal of Cognitive Neuroscience*.

3.1 Introduction

Everyday life requires the ability to detect when one's actions are erroneous and to correct them if necessary. Electrophysiological recordings in nonhuman primates have shown that both lateral prefrontal cortex (LPFC) and anterior cingulate cortex (ACC) neurons are active when this action monitoring is required (Niki and Watanabe, 1979; Ito et al., 2003; Amiez et al., 2005). According to one prominent theory, the ACC detects the presence of conflict and monitors the outcome of actions and then provides a signal to areas like the LPFC, which then adjust the level of cognitive control for future actions (Botvinick et al., 2001; Carter et al., 2001; Miller and Cohen, 2001; Yeung et al., 2004; Carter and van Veen, 2007). The strongest support for a role of the ACC in action monitoring comes from studies of the error-related negativity (ERN) in human subjects (Yeung et al., 2004), an event-related potential that occurs after errors in reaction time tasks. However, the LPFC clearly interacts with the ACC in action monitoring as LPFC lesions abolish differences between error-trial ERN and

correct trial activity and affect corrective behaviour after a response error (Gehring and Knight, 2000).

Blunted brain responses to errors are characteristic for schizophrenic patients as indicated by reduced error-related scalp potentials and altered activation in the LPFC and ACC on response error trials in patients (Gehring et al., 1995; Kopp and Rist, 1999; Polli et al., 2005; Polli et al., 2008; Mathalon et al., 2009). In fact, it has been proposed that failures in self-generated action monitoring and internal monitoring of speech output contribute to delusions of alien control (Frith and Done, 1989) and formal thought disorder (Feinberg and Guazzelli, 1999), respectively. Deficits in action and internal speech monitoring (Stone et al., 2011), together with other positive, negative, and cognitive symptoms typical for schizophrenia, also occur following acute subanesthetic doses of ketamine (Luby et al., 1959; Domino et al., 1965; Gunduz-Bruce, 2009), a noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonist. Therefore, it has been suggested that NMDAR dysfunction may underlie self-monitoring deficits and psychotic symptoms in schizophrenia (Stone et al., 2011) and that ketamine is a promising pharmacologically induced model of schizophrenia in nonhuman primates (Condy et al., 2005; Skoblenick and Everling, 2012; Blackman et al., 2013; Gil-da-Costa et al., 2013).

A link between prefrontal cortex function and the action of ketamine has been found in rats (Jackson et al., 2004) and nonhuman primates (Skoblenick and Everling, 2012), where low systemic doses of NMDAR antagonists potentiate the firing of most neurons. We have shown recently that LPFC neurons also lose their selectivity for the task rule following administration of a single subanesthetic dose of ketamine (Skoblenick and Everling, 2012), consistent with the hypothesis that NMDAR-antagonists disrupt frontal lobe function by decreasing the signal-to-noise ratio of LPFC neurons (Jackson et al., 2004).

Here we examined the effects of ketamine on post-response activity and inter-trial activity in the LPFC by recording single neuron activity in macaque LPFC neurons before and after the injection of a subanesthetic dose of ketamine, while the animals performed randomly interleaved trials of prosaccades and antisaccades (Munoz and Everling, 2004). Prosaccade trials required the animals to simply look towards a peripheral stimulus, whereas antisaccade trials required the animals to suppress a saccade towards the stimulus and instead to look away from it to the opposite direction. Antisaccade errors, which are elevated in patients with prefrontal lesions (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991; Ploner et al., 2005), schizophrenia patients (Fukushima et al., 1988; Sereno and Holzman, 1995), and in nonhuman primates following ketamine administration (Condy et al., 2005; Skoblenick and Everling, 2012), elicit robust error-related scalp potentials in healthy human subjects (Nieuwenhuis et al.,

2001). Analysis of error-related activity was restricted to antisaccade trials since nonhuman primates (Bell et al., 2000), like human subjects (Dafoe et al., 2007), produce few prosaccade errors in this paradigm.

3.2 Materials and Methods

We collected data in two male adult macaque monkeys (*Macaca mulatta*) following guidelines of the Canadian Council of Animal Care policy and a protocol approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care. All experimental procedure have been described in detail previously (Skoblenick and Everling, 2012).

3.2.1 Behavioral Task

Monkeys were seated in a primate chair within a sound-attenuating chamber with their heads restrained and a juice-spout placed at their mouth for computer-controlled reward delivery. Stimuli were presented on a 21" CRT monitor 42 cm in front of the animals. Behavioural control and stimulus generation were accomplished with the CORTEX and MonkeyLogic software package, while eye movements were recorded at 120 Hz with an infrared video eye tracker (ISCAN, Boston, MA). Each trial started with the presentation of a white fixation point (FP), which changed colour to either red or green in a pseudorandom order after an initial 100 ms fixation period. In monkey O, a red FP instructed an antisaccade and a green FP a prosaccade in response to the

ensuing peripheral stimulus. The mapping was reversed for monkey T. In half of the trials, the initial central FP changed back to white after a 200 ms period. This manipulation was included for a previous study in which we investigated ketamine effects on working memory for the task rule (Skoblenick and Everling, 2012). A peripheral stimulus appeared 8° to either the left or right following a 1000-1200 ms fixation period. To obtain a juice reward, the animal was required to look to the correct target location within a target window, i.e. towards the stimulus on prosaccade trials and diametrically opposite of the peripheral stimulus on antisaccade trials. The reward was delivered as soon as the monkey looked into target window (8° circular window in MonkeyLogic and 8°x 8° in CORTEX). Error trials were defined as those in which the monkey performed the incorrect saccade: a prosaccade during a trial with the antisaccade rule or vice versa.

3.2.2 Recording Method

Extracellular recordings were conducted with a semichronic screw microdrive system (Neuronitek, London, ON) equipped with 32 individually moveable tungsten microelectrodes (FHC Inst., Bowdoinham, Maine), guided by anatomical MR images to ensure targeting the area in and around the posterior principal sulcus. Initially, all individual electrodes were lowered through both a thin silicone membrane and the monkey's dura until multiple neurons were well isolated on a maximal number of electrodes. On each subsequent recording day, the 32

channel headstage (Plexon Inc., Dallas, TX) was reconnected and electrodes were moved to isolate new neurons for that day's experimental session. To ensure a relatively unbiased sampling of LPFC neural activity, we did not pre-screen neurons for task-related responses. The microdrive system remained implanted for up to 2 weeks before it was removed for cleaning and maintenance.

Data acquisition and filtering were performed with a multi-acquisition processor (MAP) system (Plexon Inc., Dallas, TX). Recorded neurons were sorted off-line using 2-D and 3-D principal component analysis using Offline Sorter (Plexon Inc., Dallas, TX). Horizontal and vertical eye positions and the occurrence of behavioural events (e.g. start of trial, onset of fixation, stimulus presentation) were also stored in the Plexon MAP system.

3.2.3 Drug administration

Each experimental session began with a 10 min (monkey T) or 15 min (monkey O) period of baseline activity during which the animal performed blocks of trials in a pseudo-randomized order. After the baseline period, the experiment was briefly paused and the animal received either a single subanesthetic intramuscular injection of 0.4 ml ketamine (0.4mg/kg, diluted in sterile saline), which previously has been found to elicit cognitive deficits with minimal anaesthetic effects in rhesus monkeys (Condy et al., 2005; Stoet and Snyder, 2006; Shen et al., 2010; Blackman et al., 2013), or 0.4 ml of sterile saline into

their right tricep brachii muscle. The injection process only interrupted the animal's experimental session for less than 15 sec after which the monkeys would continue the paradigm. We recorded neural activity for at least 40 min following ketamine or saline injection throughout these sessions. Ketamine injection sessions were separated by at least 4 d to avoid cumulative dosing effects and potential neuronal injury (Liao et al., 2011). Saline injections were performed on some of the days on which no ketamine was administered.

3.2.4 Data Analysis

Data analysis was performed using custom-designed software programmed in Matlab (Mathworks). Saccade onset of the initial saccade was defined as the time at which the horizontal velocity exceeded $30^\circ/\text{s}$ after peripheral stimulus onset and the end of the saccade was defined as the time when the velocity fell below $30^\circ/\text{s}$. The onset of the return or correction saccade was defined as the time when the horizontal velocity exceeded $30^\circ/\text{s}$ after the end of the initial saccade. An antisaccade error was defined as an initial saccade toward the peripheral stimulus. Fixation breaks or no responses were not included in error rate calculations. All trials were visually-inspected after automatic saccade detection and corrected if necessary.

3.2.5 Effect of previous trial performance

Several studies have reported post-error slowing of antisaccades (Polli et al., 2006; Klein et al., 2007). For this analysis, we only included correct antisaccade trials that were preceded by a correct response (pro- or antisaccade) or an error (pro- or antisaccade) on the previous trial. Correct antisaccade trials that were preceded by a broken fixation or no response trial were excluded from this analysis. The low number of correct antisaccades that were preceded by errors required us to pool the reaction times from all 10 sessions. To do this, we first z-normalized the reaction times of correct antisaccades before and after ketamine for each session and then combined the z-normalized reaction times from the individual sessions. There were not enough trials for this analysis in the five saline control sessions.

3.2.6 Performance-selectivity

Neurons were classified as performance-selective, based on a two-way analysis of variance (ANOVA) on neural activity in the interval 200-700 ms after saccade onset during the pre-injection period, evaluated at $p < 0.05$. The factors were Performance (correct or error antisaccades) and Saccade Direction (ipsilateral or contralateral to recorded hemisphere). Only neurons that showed a main effect of Performance but no significant effect of Saccade Direction and no significant Performance x Saccade Direction interaction were classified as performance-related. A neuron's preferred performance was defined as the performance (correct or error) that was associated with the maximal spiking

activity. As a measure of performance selectivity (preferred vs. nonpreferred performance), the selectivity index was defined as: $S = (p - np) / (p + np)$ where p = activity on preferred trials in the interval 200-700 ms after saccade onset; np = activity on nonpreferred in the same interval.

3.2.7 Selectivity during intertrial period

According to several cognitive control models (Botvinick et al., 2001; Miller and Cohen, 2001; Yeung et al., 2004; Carter and van Veen, 2007), the activity in the LPFC is increased after response conflict or response errors to increase cognitive control for the following trial. To test whether ketamine affected the activity during the intertrial period, we classified neurons as outcome selective during the intertrial period if they exhibited significant differences (t-test, $p < 0.05$) in the period 1500-2500 ms following saccade onset between correct and error trials. This period was just before the fixation point was presented for the following trial. A neuron's preferred outcome was defined as the outcome (correct or error) for which the neuron displayed the maximal spiking activity during this period.

3.2.8 ROC analysis

To evaluate how well LPFC neurons could discriminate between correct and error responses, we computed receiver operating characteristic (ROC) values for the discharge during the performance-monitoring and the intertrial

periods for selective neurons. An ROC analysis measures the degree of overlap between two distributions. For each neuron, the activity on correct trials was compared with the activity on error trials, yielding two distributions of neuronal activity. Each point on the ROC curve was created by plotting the proportion of the distribution with the higher mean activity against the proportion of the distribution with the lower mean activity. To generate the entire ROC curve, the criterion level was incremented from zero to the maximal discharge rate in 20 steps. The area under the ROC curve is a quantitative measure of the separation of the two distributions. A value of 0.5 indicates that the two distributions completely overlap. Values of 0 and 1.0 indicate that the two distributions are completely separate.

3.2.9 Fano factor

As a measure of neural reliability, we computed the fano factor (variance divided by mean) for the discharge rates during the performance-monitoring period and the intertrial period.

3.2.10 Waveform analysis

To test whether ketamine had different effects on narrow-spiking (putative interneurons) and broad-spiking neurons (putative pyramidal neurons), we computed mean trough-to-peak times for the extracellular waveform of each neuron using previously described procedures (Mitchell et al., 2007; Johnston et

al., 2009) Based on the results of the previous study from our lab we use used the same recording system and the same type of microelectrodes in the LPFC, we defined neurons with waveform durations of $270 \mu\text{s}$ as narrow-spiking neurons and any neurons with waveform durations of $270 \mu\text{s}$ as broad-spiking neurons.

3.3 Results

We administered subanesthetic doses of ketamine in 10 experimental sessions (5 in each monkey) and control injections of saline in 5 sessions (4 in monkey O and 1 in monkey T), while the animals performed randomly interleaved pro- and antisaccade trials. We recorded the activity of 343 LPFC neurons (111 in monkey O, 232 in monkey T) during the ketamine sessions and of 99 LPFC neurons during the saline injections (51 in monkey O, 48 in monkey T).

3.3.1 Reaction times and error rates

Figure 3.1 shows the effects of ketamine and saline injections on error rates and reaction times of prosaccades (**Figure 3.1A**) and antisaccades (**Figure 3.1B**). In line with several previous reports, both animals had longer reaction times for antisaccades than prosaccades and made very few prosaccade errors prior to ketamine administration, but generated a 10-20% error rate on antisaccade trials (Amador et al., 1998; Bell et al., 2000). Following ketamine administration (thick lines), reaction times of prosaccades (**Figure 3.1A**, top

panel) and antisaccades (**Figure 3.1B**, top panel) increased. At the same time, error rates on antisaccade trials (**Figure 3.1B**, bottom panel) and to a lesser degree on prosaccade trials (**Figure 3.1A**, bottom panel) increased. Reaction time and performance effects started about 5 min following ketamine injections. Performance returned to pre-injection levels after about 20 min, whereas the effects on reaction times persisted for the duration of the sessions. Saline injections (thin lines) neither influenced reaction times nor performance of pro- or antisaccades. For all following behavioural and neural analyses, we compared a pre-injection period (10 min interval before injection) with a post-injection period (5-20 min after injection).

3.3.2 Reaction times of correct responses and errors on antisaccade trials

We next examined the effects of ketamine on reaction times of correct responses and errors separately for each monkey. **Figure 3.2A** shows cumulative distributions of correct antisaccade reaction times for ketamine and saline sessions. In monkey O (**Figure 3.2**, top panel) reaction times increased from 193 ± 2 ms to 252 ± 5 ms (t-test, $p < 0.00001$) following ketamine injections. No effects were found for saline injections (196 ± 5 ms versus 198 ± 3 ms; t-test, $p = 0.65$). Similarly, in monkey T (**Figure 3.2**, bottom panel) antisaccade reaction times increased from 188 ± 2 ms to 237 ± 4 ms (t-test, $p < 0.00001$) after ketamine injections. Saline injections also did not increase antisaccade reaction times in this animal (180 ± 5 ms versus 178 ± 3 ms; t-test, $p = 0.18$).

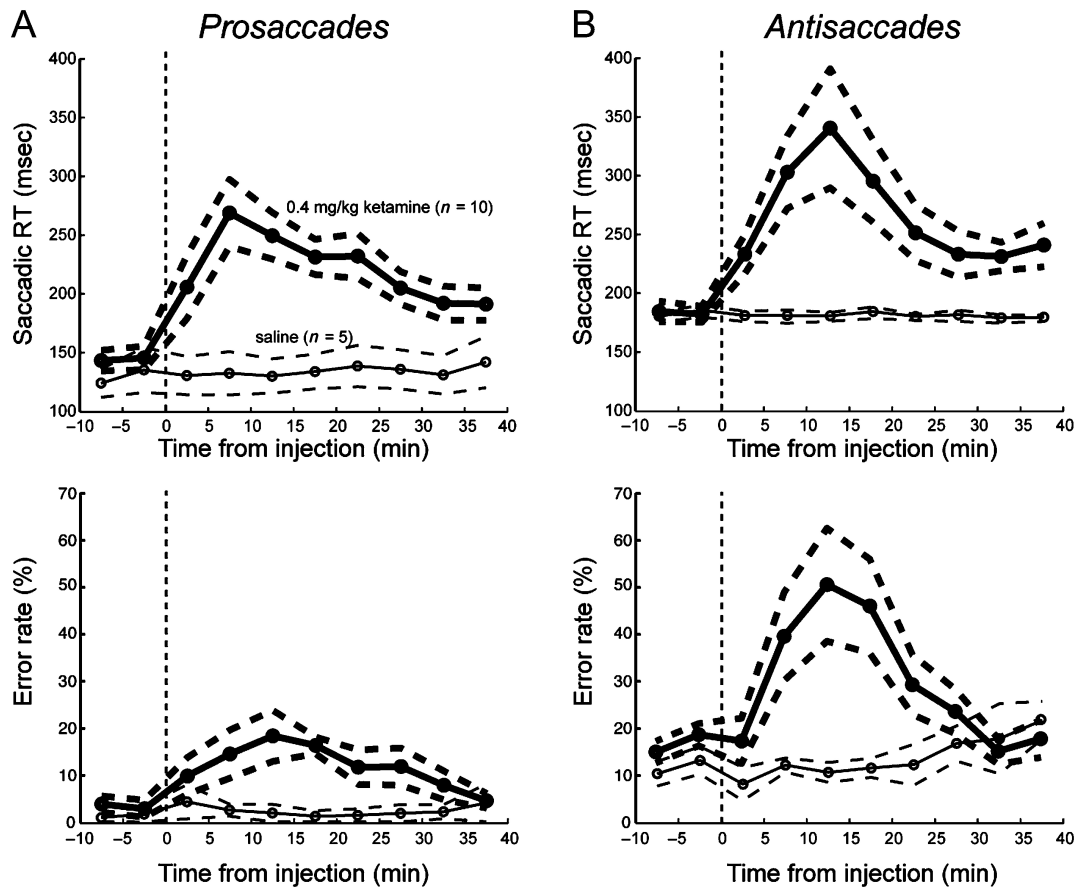


Figure 3.1: Time course of ketamine and saline injections on behaviour. A, Effects of ketamine (thick line) and saline (thin lines) injections on mean \pm standard error of the mean saccadic reaction times (top panel) and error rates (bottom panel) on prosaccade trials. The time of injection is indicated by a vertical dashed line. B, Same as A for antisaccade trials.

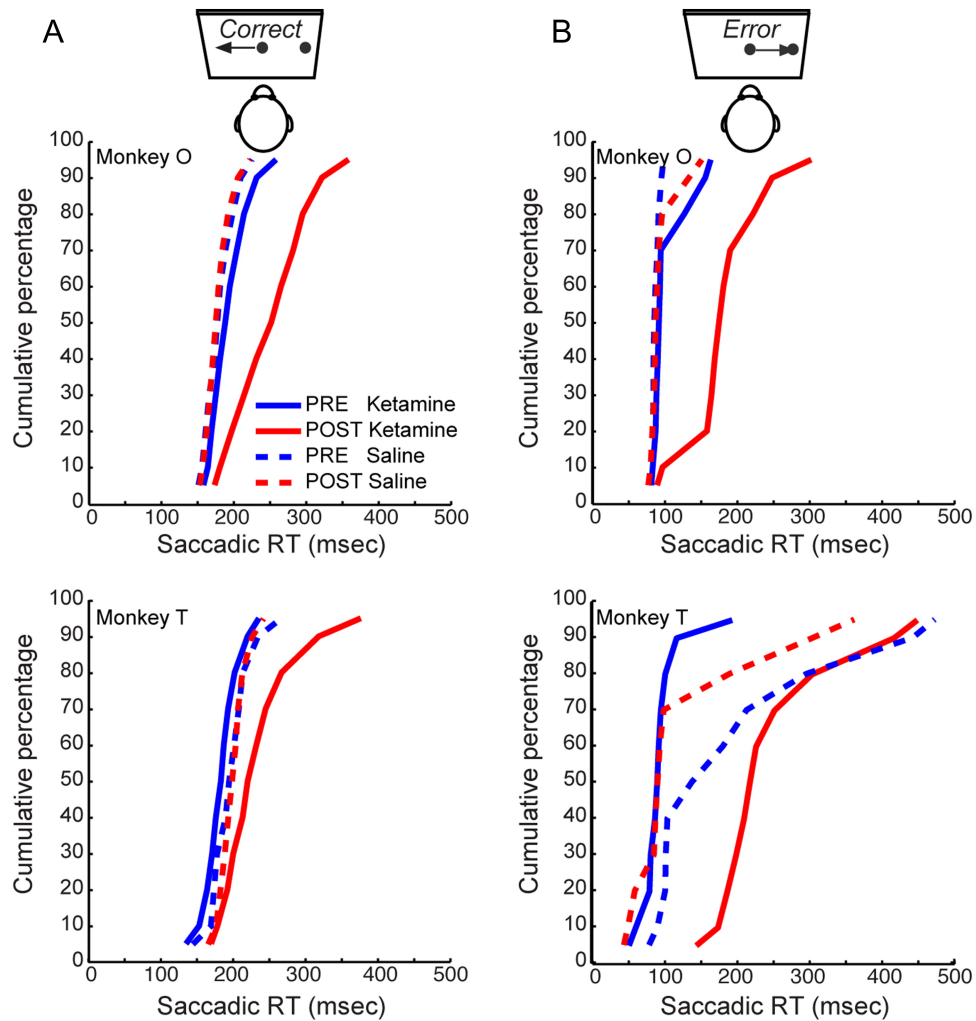


Figure 3.2: Antisaccade reaction times before and after injections. A, Cumulative distribution of correct responses on antisaccade trials for monkey O (top panel) and monkey T (bottom panel). Ketamine increased antisaccade reaction times in both animals. B, Cumulative distribution of error responses on antisaccade trials monkey O (top panel) and monkey T (bottom panel). Ketamine increased error reaction times in both animals.

The cumulative reaction time plots in **Figure 3.2B** show that monkeys mainly generated errors in the range of express saccades (Boch et al., 1984) before ketamine injections, while post-ketamine errors had considerably longer reaction times. In monkey O (**Fig. 3.2B**, top panel), error reaction times increased from 103 ± 4 ms to 185 ± 8 ms (t-test, $p < 0.00001$) and in monkey T (**Figure 3.2B**, bottom panel) from 96 ± 4 ms to 248 ms (t-test, $p < 0.00001$). We found a small increase in error reaction times following saline injections (from 87 ± 1 ms to 94 ± 3 ms; t-test, $p < 0.05$) in monkey O and no differences in monkey T (from 199 ± 32 to 128 ± 22 ms; t-test, $p = 0.07$).

3.3.3 Behaviour after initial saccade

Next, we investigated whether ketamine had effects on the monkeys' behaviour after the saccade. **Figure 3.3A** shows the cumulative reaction times of the saccade away from the initial antisaccade fixation location. On these correct trials, the animals received a liquid reward immediately after the antisaccade entered the target window (see Methods). In both monkeys, the reaction times of return saccades did not differ between the pre-injection (solid blue line) and post-injection period (solid red line) in ketamine session (Monkey O: 398 ± 13 ms vs. 360 ± 17 ms, respectively; $p = 0.11$; Monkey T: 981 ± 20 ms vs. 948 ± 20 ms, respectively, $p = 0.24$). Similarly, the reaction times of return saccades did not differ before (dashed blue line) and after (dashed red line) saline injections

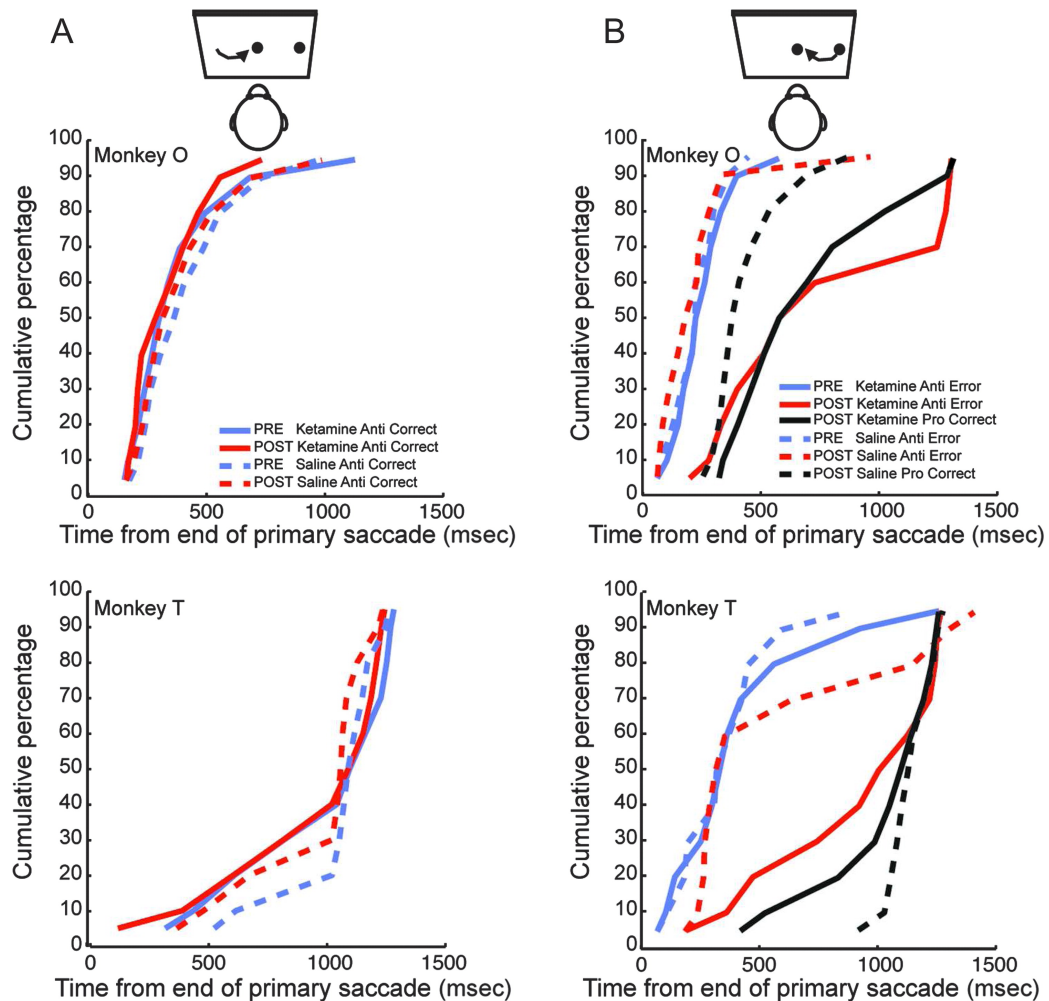


Figure 3.3: Reaction time of the return saccade. A, Cumulative distribution of reaction times of return saccades on correct antisaccade trials for monkey O (top panel) and monkey T (bottom panel). There were no differences in reaction times before (blue lines) and after (red lines) ketamine (solid lines) or saline (dashed lines) injections. B, Same as A but for return saccades following an error on antisaccade trials. Ketamine increased the reaction times of return saccades following a response error, rendering them similar to the reaction times following correct prosaccades (black lines).

(Monkey O: 429 ± 13 ms vs. 399 ± 13 ms, respectively; $p=0.09$; Monkey T: 1041 ± 29 ms vs. 975 ± 27 ms, respectively, $p=0.11$).

On antisaccade error trials (**Figure 3.3B**) on which the animal looked towards the stimulus and did not receive a reward, the reaction times of the return saccades were significantly longer in the post-injection (solid red line) compared with the pre-injection period (solid blue line) in ketamine sessions in monkey O (730 ± 51 ms vs. 248 ± 20 ms, respectively; $p=3.2 * 10^{-12}$) and monkey T (904 ± 36 ms vs. 416 ± 37 ms, respectively; $p=2.2 * 10^{-42}$). The reaction times of return saccades after antisaccade errors were similar to the reaction times of return saccades after correct prosaccades (solid black line) after ketamine (Monkey O: 685 ± 51 ms; Monkey T: 1057 ± 15 ms). In both cases, the animals looked towards the stimulus, but they were only rewarded on correct prosaccades trials and not on antisaccade error trials. Saline injections (dashed lines) had no effect on the reaction times of the return saccade after an error (Monkey O: 225 ± 19 ms vs. 235 ± 33 ms, $p=0.81$; Monkey T: 347 ± 54 ms vs. 557 ± 96 ms, $p=0.07$) and the reaction times of these return saccades post saline injections were significantly shorter than the reaction times of return saccades after correct prosaccades (Monkey O: 445 ± 11 ms, $p<0.00001$; Monkey T: 1159 ± 13 ms, $p=2.2 * 10^{-20}$). These findings show that ketamine did not influence the time when monkeys made a return saccade after a correct antisaccade, but it

significantly increased the reaction time of return saccades after an error, rendering them similar to return saccades after correct prosaccades.

Although these data suggest that ketamine impaired the animals' ability to detect that they made a mistake on antisaccade error trials, it is also possible that the presence of the stimulus on the fovea after the response errors attracted fixation and prevented the animals from looking back. In this case, ketamine would impair the disengagement of fixation and not performance monitoring. If the deficit is due to an attraction to visual stimuli following ketamine, then one would expect that errors on prosaccade trials would be corrected faster after ketamine. Our data do not support this possibility. Monkey O made a return saccade after 433 ± 41 ms prior to ketamine injections and after 611 ± 32 ms post ketamine injections on prosaccade error trials (i.e. an antisaccade) ($p < 0.005$). The same trend was found in monkey T (300 ± 47 ms pre injection and 451 ± 55 ms post ketamine injections). This was not significant due to the small number of trials (5 pre ketamine and 38 post ketamine trials). Therefore the pattern of reaction times is inconsistent with the disengagement explanation in both animals, but it cannot be definitely ruled out due to insufficient power.

3.3.4 Reaction times on the following trial

The reaction times of correct antisaccades were significantly longer when they were preceded by an antisaccade error trial compared with a correct antisaccade trials (z-scores 0.39 ± 0.15 versus -0.04 ± 0.04 ; $p < 0.005$, t-test) before ketamine was injected. These differences disappeared after ketamine administration (z-scores 0.03 ± 0.12 versus -0.01 ± 0.05 ; $p = 0.98$, t-test).

3.3.5 Performance-related neural activity

Consistent with previous reports (Niki and Watanabe, 1979), we observed that many LPFC neurons had differences in post-response activity between correct and error trials. Of the 343 neurons recorded during the 10 ketamine injection sessions, 63 neurons (18.4%) showed a main effect of performance, 30 (8.6%) showed an effect of saccade direction, 16 neurons (4.6%) showed both main effects, and 47 neurons (13.5%) showed an interaction effect. The results were comparable for the 99 neurons that we recorded in the 5 saline injection sessions. Here, 16 (16.2%) showed a main effect of performance, 10 (10.1%) showed a main effect of direction, 5 (5.1%) showed both effects and 17 neurons showed an interaction effect (17.2%). For those neurons that had a main effect of saccade direction, there was a mild direction preference for contralateral saccades (54% in the ketamine sessions and 60% in the saline sessions were more active after contralateral saccades).

For the following analyses, we defined a neuron as performance-related, if it had a significant main effect of performance and no main effect of saccade direction or interaction. Of the 63 performance-related neurons recorded during the ketamine injection sessions, 44 neurons (69.8%) were more active after errors and 19 neurons were more active after correct responses (30.2%). This proportion was similar for the saline injection sessions, where 13 of 16 neurons (81.3%) were more active for errors than correct responses.

Figure 3.4 shows single neuron examples of performance-related LPFC neurons on correct and error antisaccade trials (**Figure 3.4A**) before (left panel) and after the administration of 0.4 mg/kg ketamine (right panel). The neuron in **Figure 3.4B** was more active after errors during the pre-injection period, whereas the neuron in **Figure 3.4C** was more active after correct responses during this period. Following ketamine, the differences in activity between correct and error responses disappeared in both neurons. For the population of recorded neurons, the effects were very similar for neurons that preferred errors and those that preferred correct responses (see **Table 3.1**). Therefore we combined the responses for the two types of performance-related neurons by defining each performance-related neuron's preferred outcome as the response (correct or error) that yielded the maximal response during the pre-injection period.

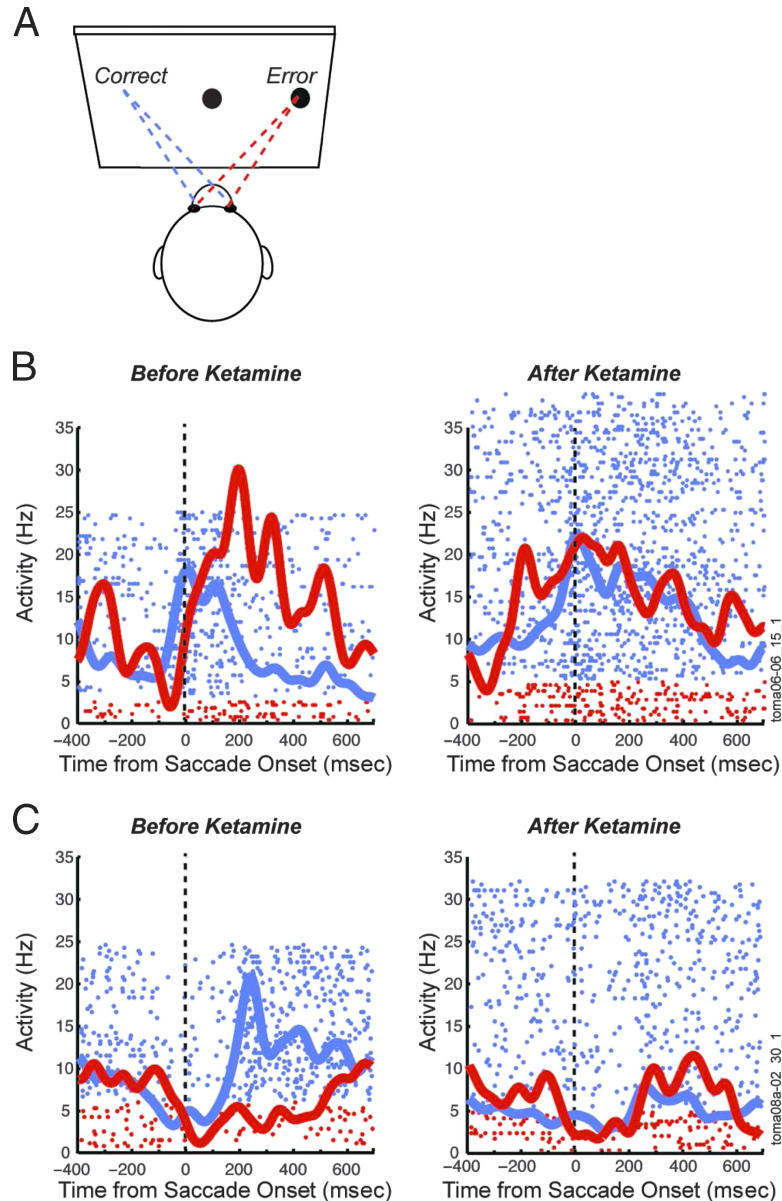


Figure 3.4: Schematic of correct responses and errors in the antisaccade task and single neuron activity. A, Correct responses were defined as saccades away from the peripheral stimulus and error responses were defined as saccades toward the stimulus. B, Activity of a single neuron in the prefrontal cortex that exhibited increased post-response activity on error trials (red) compared with correct trials (blue). Dots indicate the time of an action potential following saccade onset. Each row is the activity on one trial. Superimposed are the spike density functions for correct and error trials. C, Same as B but for a neuron that exhibited increased post-response activity on correct trials (blue) compared with error trials (red). The differences between correct and error trials were reduced in both neurons after administration of 0.4 mg/kg ketamine.

Table 3.1 Effects of Injections on Neural Activity

| Type | Ketamine | | Saline | |
|-----------|----------------------------|-----------------------------|----------------------------|-----------------------------|
| | PRE Activity (spikes/s) | POST Activity (spikes/s) | PRE Activity (spikes/s) | POST Activity (spikes/s) |
| Correct < | ** 3.2±0.5 | 6.1±0.8 | 7.4±1.8 | 9.3±2.8 |
| Error | 6.4±0.8 | 6.7±0.9 | 13.8±3.4 | 11.8±2.3 |
| Correct > | 10.2±2.1 | 11.1±2.9 | 4.6±2.0 | 5.0±2.2 |
| Error | * 5.6±1.5 | 11.3±3.1 | 2.7±1.6 | 3.3±1.7 |

* p<0.01 ** p<0.00001

Figure 3.5A shows the activity of the population of performance-related neurons before ketamine (dashed lines) and after ketamine (solid line). The neurons' activity increased prior to saccade onset and began to decline 100 ms after saccade onset for nonpreferred trials (blue dashed line). On preferred trials (red dashed line), the activity decreased later. Following ketamine administration (solid lines), the pre-saccade firing rates increased in the population of neurons, consistent with the known effects of NMDAR antagonists on LPFC neurons in primates (Skoblenick and Everling, 2012) and rodents (Jackson et al., 2004). Ketamine did not seem to affect the activity on preferred trials following the saccade (red solid line), but it clearly increased the activity on nonpreferred trials (blue solid line).

To quantify these effects, we compared the activity before and after ketamine for preferred and nonpreferred responses in the period 200-700 ms after saccade onset. On preferred trials (**Figure 3.5B**), no significant effect ($p=0.30$; paired t-test) was found between the activity before and after ketamine (7.6 ± 0.8 and 8.0 ± 1.1 spikes/s, respectively). On nonpreferred trials (**Figure 3.5C**), performance-related activity increased from 3.9 ± 0.6 to 7.6 ± 1.1 spikes/s ($p<0.0001$; paired t-test). For saline injections (**Figure 3.5D**), no differences in activity were found before and after injections for preferred trials (**Figure 3.5E**, 11.9 ± 2.9 and 10.6 ± 2.0 spikes/s, respectively; $p=0.30$, paired t-test) and

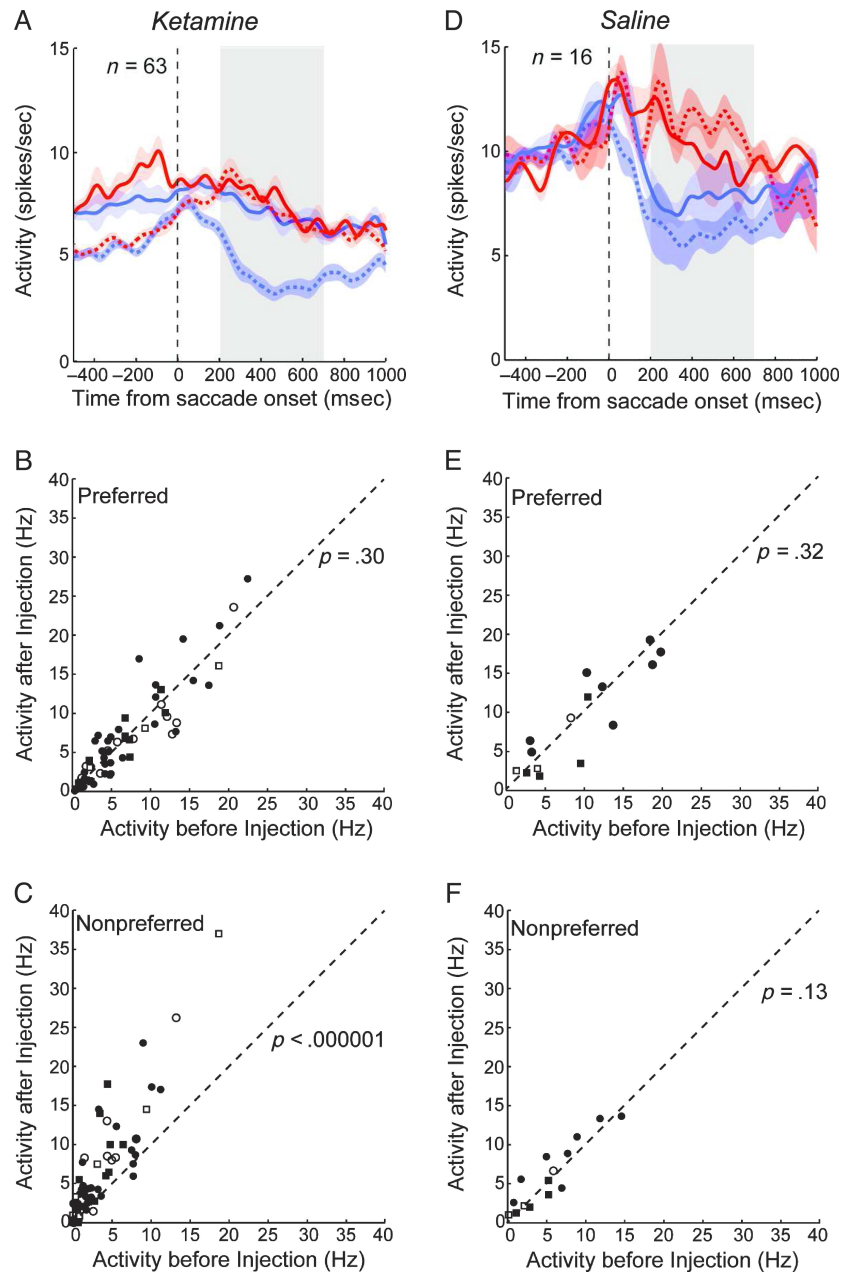


Figure 3.5: Activity of LPFC neurons with performance-selectivity. A, Mean \pm within-subject standard error of the mean spike density of the population for preferred (red) and nonpreferred responses (blue) before (dashed lines) and after ketamine (solid lines). B, Activity levels in the shaded region in A of individual LPFC neurons are plotted for preferred trials before ketamine (abscissa) against the activity levels after ketamine (ordinate). Circles and squares indicate neurons that were recorded from monkey O and monkey T, respectively. Filled symbols indicate neurons that preferred error responses and unfilled symbols indicate neurons that preferred correct responses. C, Same as B but for nonpreferred trials. D, E, F, Same as A, B, C but for saline sessions.

nonpreferred trials (**Figure 3.5F**, 6.5 ± 1.6 and 8.2 ± 2.4 spikes/s, respectively; $p=0.13$, paired t-test).

The behavioural finding that ketamine increased reaction times of error antisaccades raised the possibility that the ketamine effects on neural activity in the LPFC might have been related to the different onset times of return saccades. To test this possibility, we aligned neural activity on the onset of the return saccade (**Figure 3.6**). There was no decline in neural activity before the return saccade, ruling out the possibility that differences in reaction times caused the differences in neural activity of LPFC neurons before and after ketamine.

3.3.5(i) Discriminability

To evaluate the effects of ketamine on the ability of neurons to discriminate between correct and error trials, we compared the ROC values before and after injections. For the population, the ROC value dropped from 0.73 ± 0.01 to 0.53 ± 0.02 ($p=6.59 \times 10^{-16}$) after the administration of ketamine (**Figure 3.7A**). There was also a decrease in ROC value, i.e. in the discriminability between correct and error trials, from 0.73 ± 0.02 to 0.64 ± 0.03 ($p<0.01$) following the injection of saline (**Figure 3.7B**) which might be related to fatigue or motivation effects. The reduction in ROC-value, however, was significantly larger following ketamine compared with saline injections (-0.19 ± 0.02 versus -0.09 ± 0.03 ; $p<0.01$, paired t-test) (**Figure 3.7C**).

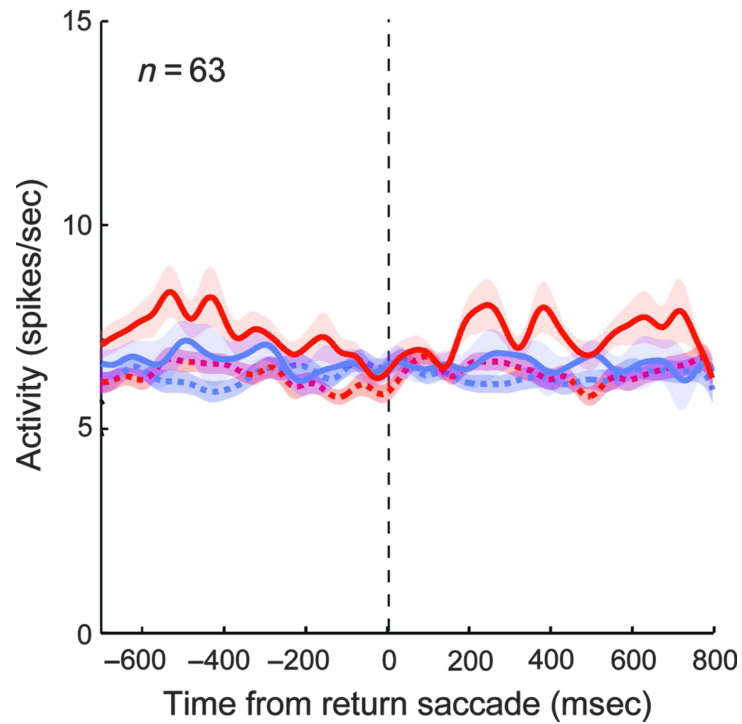


Figure 3.6: Activity of LPFC neurons that showed performance-selectivity aligned on return saccade onset. Mean \pm within-subject standard error of the mean spike density of the population for preferred (red) and nonpreferred responses (blue) before (dashed lines) and after ketamine (solid lines). The activity did not decline prior to the return saccade, indicating that the ketamine-mediated effects on neural activity in Fig. 4 are not due to differences in reaction times of return saccades.

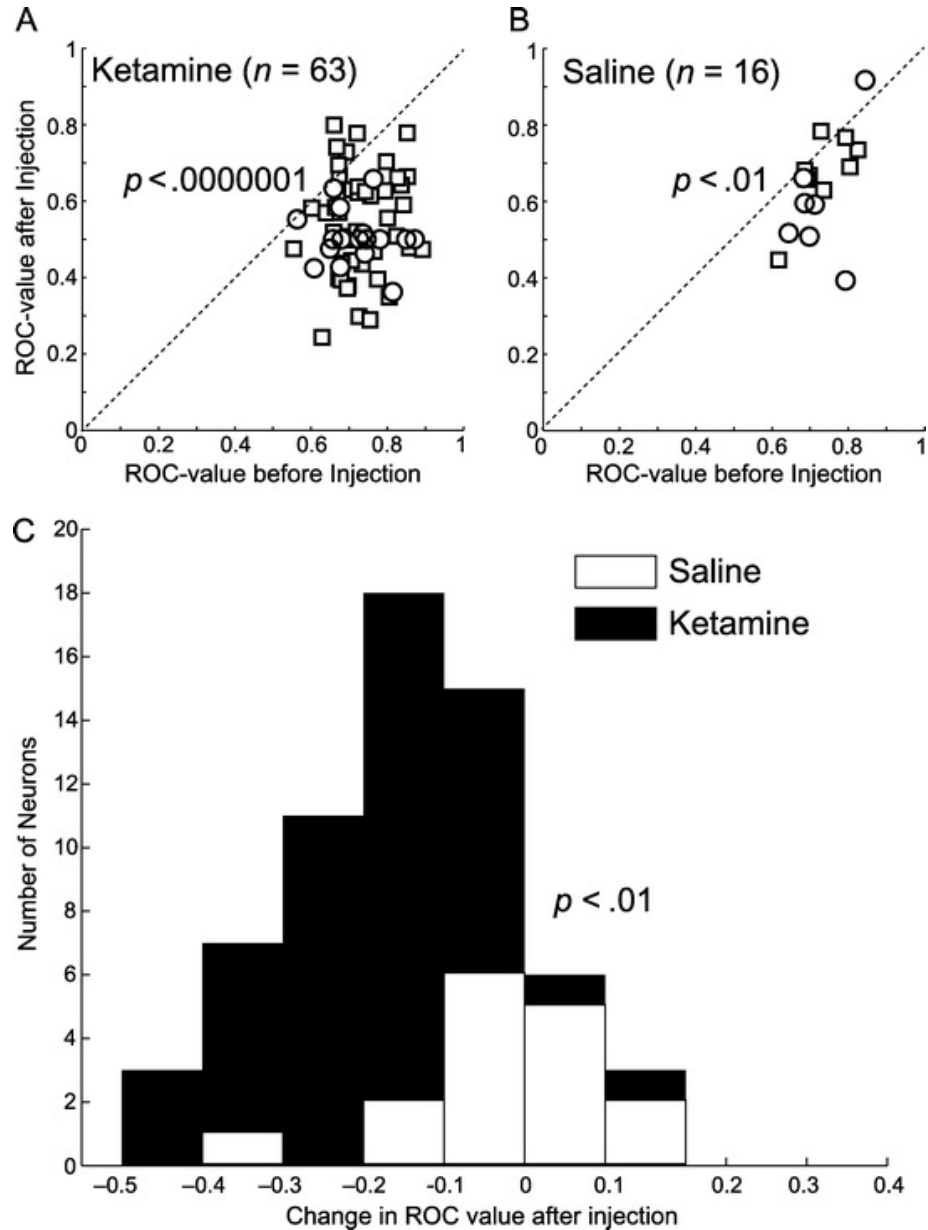


Figure 3.7: ROC analysis of performance-related activity. A, ROC-values for performance-selective neurons are plotted before ketamine (abscissa) against ROC-values after ketamine (ordinate). Circles and squares indicate neurons that were recorded from monkey O and monkey T, respectively. B, Same as in A but for saline sessions. C, Distribution of changes in ROC-values for individual neurons after ketamine (black bars) and saline (white bars) injections.

3.3.5(ii) Reliability

While we observed changes in the neural activity after ketamine administration, there were no differences in response variability as measured by the fano factor before and after ketamine injections for the preferred (3.01 ± 0.17 versus 3.03 ± 0.20 ; $p=0.98$, paired t-test) or nonpreferred condition (3.06 ± 0.17 versus 3.11 ± 0.24 ; $p=0.91$, paired t-test). There were also no changes in the fano factor for the saline sessions.

3.3.6 Narrow-spiking versus broad-spiking neurons

Of the 63 performance-selective neurons recorded during the ketamine injections, 10 were classified as narrow spiking, i.e. putative interneurons, and 44 neurons were classified as broad-spiking, i.e. putative pyramidal neurons, based on their waveforms (see Methods). Of the 10 narrow-spiking neurons, four were more active after correct responses and 6 were more active after errors. **Figure 3.8** shows the effects of ketamine on broad-spiking and narrow-spiking neurons. Broad-spiking neurons exhibited no differences in activity before and after ketamine (6.5 ± 0.8 and 6.8 ± 1.0 spikes/s, respectively; $p=0.51$, paired t-test) for the neurons' preferred condition. For the nonpreferred condition, broad-spiking neurons increased their activity from 3.3 ± 0.5 to 6.5 ± 0.9 spikes/s ($p<0.000001$, paired t-test). The responses of narrow-spiking neurons were similar. No significant effect ($p=0.41$; paired t-test) was found between the activity before and after ketamine (13.2 ± 3.1 and 14.7 ± 4.3 spikes/s, respectively) for the neurons'

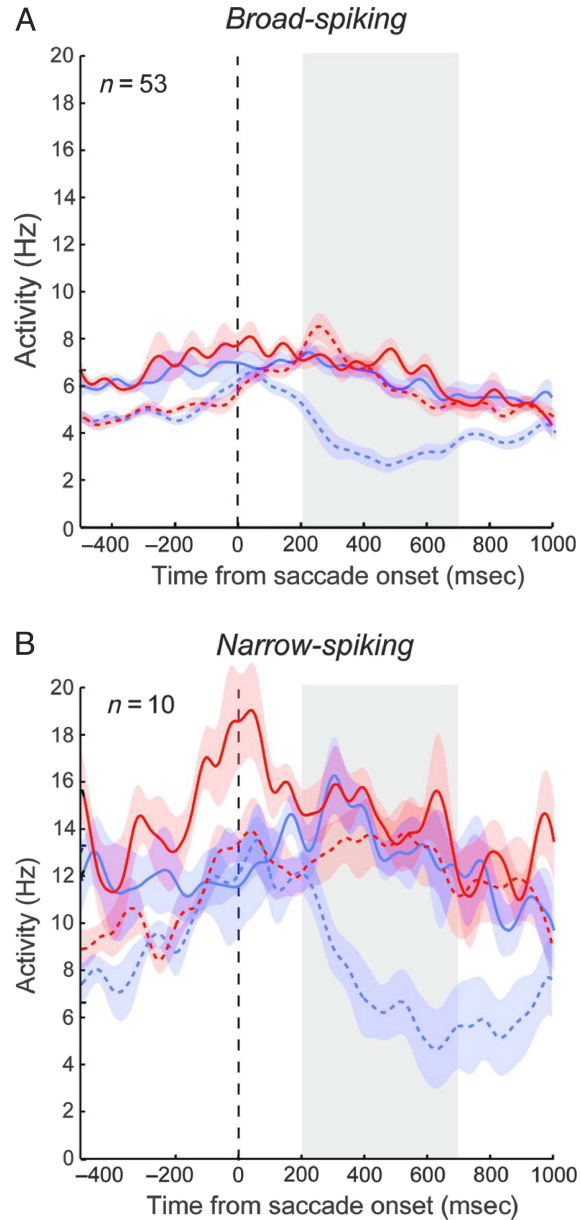


Figure 3.8: Effects of ketamine on broad-spiking and narrow-spiking neurons with performance-selectivity. A, Mean \pm within-subject standard error of the mean spike density of broad-spiking neurons (putative pyramidal neurons) for preferred (red) and nonpreferred responses (blue) before (dashed lines) and after ketamine (solid lines). B, Same as in A but narrow-spiking neurons (putative interneurons).

preferred condition, but their activity increased from 7.3 ± 2.2 to 13.7 ± 4.7 spikes/sec ($p < 0.05$; paired t-test) for the neurons' nonpreferred condition.

3.3.7 Timecourse of performance selectivity

To investigate the timecourse of the ketamine effect on LPFC activity, we computed mean normalized selectivity indices (see Methods) for consecutive 10 min time bins for the ketamine and saline injection sessions (**Figure 3.9**). Performance selectivity decreased in the 10 min following ketamine administration and reached its minimum 10-30 min following the injection. Although performance selectivity slowly increased afterwards, it did not return to its pre-injection level during our recording sessions. For saline injections, there were some fluctuations in performance selectivity, but the levels never fell below those after ketamine injections and performance selectivity was similar at the end and beginning of the recording sessions.

3.3.8 Intertrial neural activity

In 24.8% (85/343) of the neurons recorded during the ketamine sessions, the intertrial activity was significantly different depending on whether the previous trial was correct or a response error ($p < 0.05$, t-test). The majority of the neurons (83.5%) were more active following a response error on the previous trial than a correct response. Ketamine decreased the activity for the neurons' preferred trial outcome from 8.42 ± 0.69 spikes/s to 7.26 ± 0.74 spikes/s ($p < 0.02$; paired t-test).

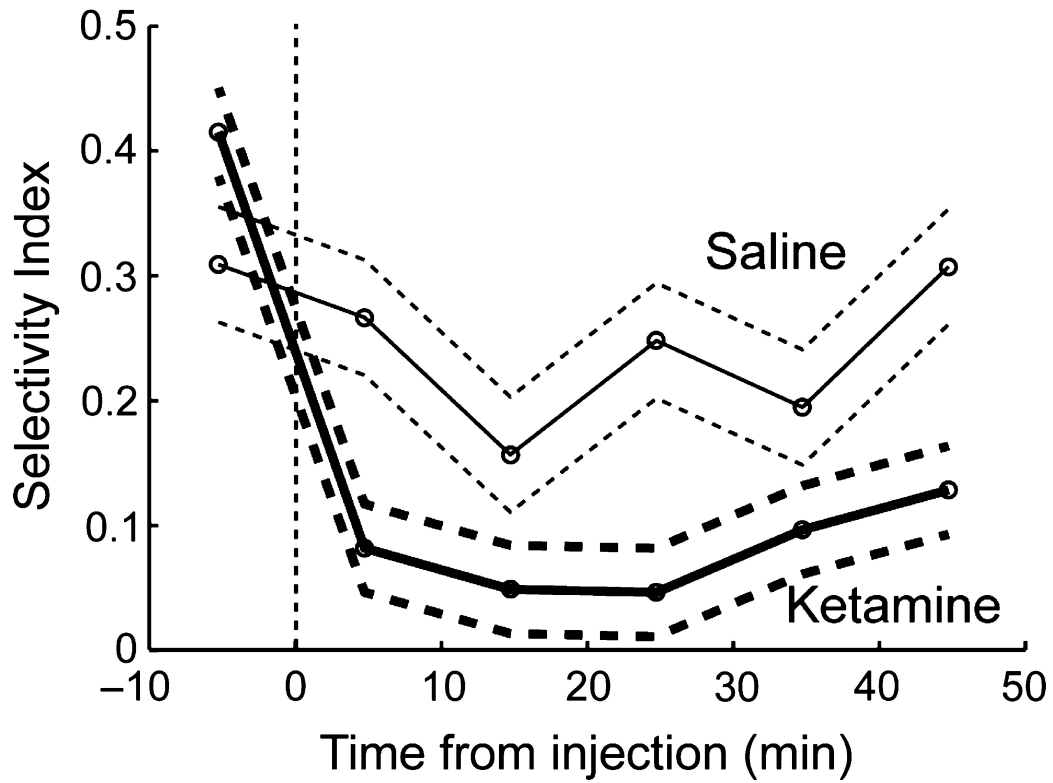


Figure 3.9: Time course of performance selectivity of LPFC neurons. Thick lines and thin lines indicate the mean \pm standard error of the mean selectivity on ketamine sessions and saline sessions, respectively. The time of injection is indicated by a vertical dashed line.

For the nonpreferred outcome, the activity increased from 4.77 ± 0.5 to 5.87 spikes/s ($p < 0.0001$; paired t-test) following ketamine administration (**Figure 3.10A**).

A similar proportion of neurons exhibited differences in intertrial activity between previous correct and error trials in the saline sessions (28% or 28/99 neurons). Like in the ketamine sessions, more neurons were active for errors than correct response (64.3%, 18/28). The activity for the preferred condition did not differ before and after saline injection (10.29 ± 1.28 versus 9.73 ± 1.39 spikes/s, respectively; $p = 0.28$, paired t-test), but the activity for the nonpreferred condition increased in the period after the injection (6.24 ± 0.9 versus $7.28.12$ spikes/s, respectively; $p < 0.05$, paired t-test) (**Figure 3.10C**).

3.3.8(i) Discriminability

There was a significant decrease in the ROC for the population from 0.74 ± 0.01 to 0.58 ± 0.01 ($p = 8.1 \times 10^{-17}$) after the administration of ketamine (**Figure 3.10B**). Although there was also a decrease in ROC value from 0.72 ± 0.01 to 0.63 ± 0.02 ($p < 0.001$) following the injection of saline (**Figure 3.10D**), the reduction in discriminability was significantly larger for ketamine compared with saline injections (-0.16 ± 0.02 versus -0.09 ± 0.02 ; $p < 0.02$, paired t-test) (**Figure 10E**). This finding shows that ketamine decreased the discriminability during the intertrial period between previous correct and erroneous response trials.

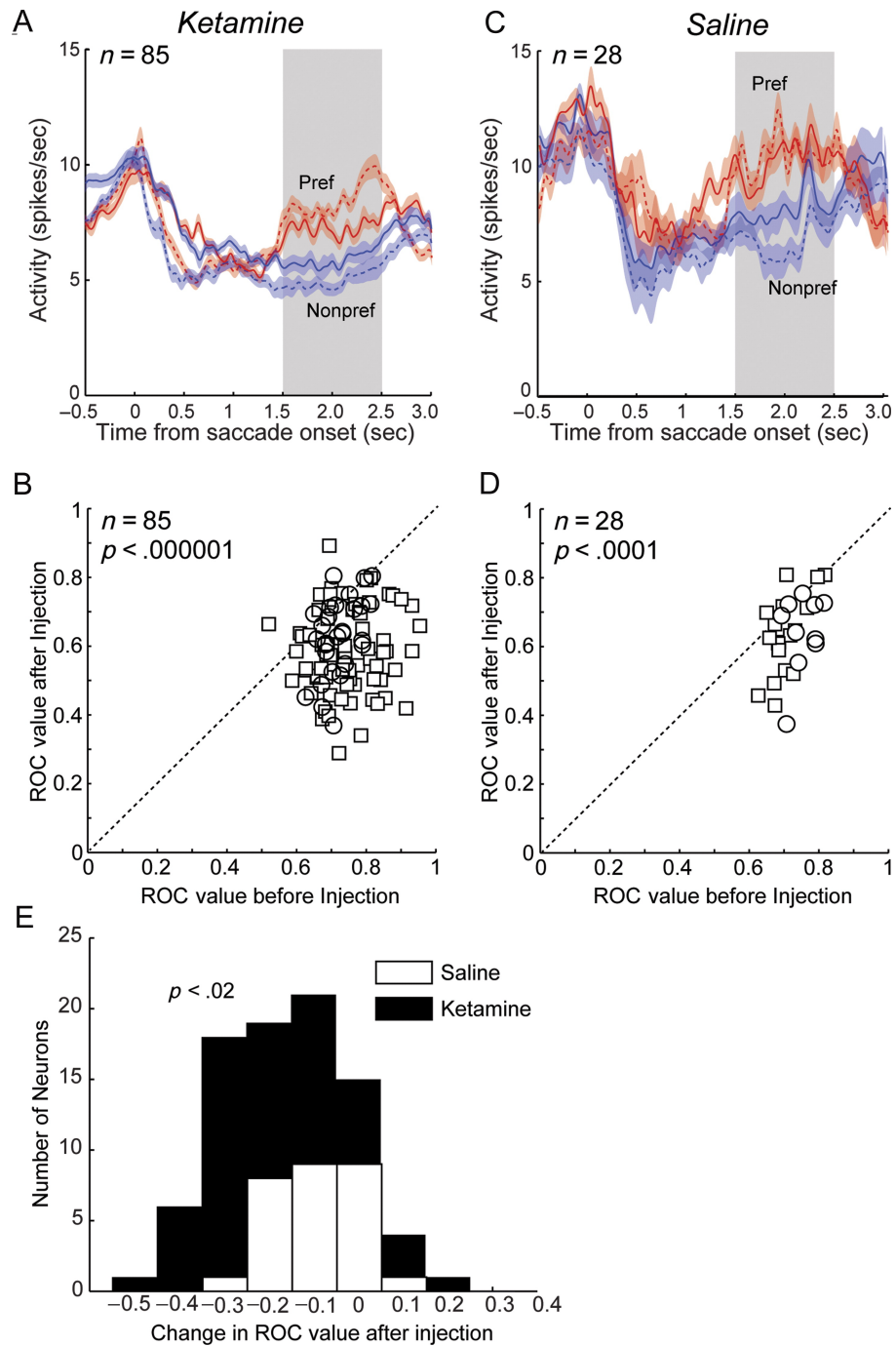


Figure 3.10: Activity of LPFC neurons that showed performance-selectivity during the intertrial period. A, Mean \pm within-subject standard error of the mean spike density of the population for preferred (red) and nonpreferred responses (blue) before (dashed lines) and after ketamine (solid lines). B, ROC-values individual neurons are plotted before ketamine (abscissa) against ROC-values after ketamine (ordinate). Circles and squares indicate neurons that were recorded from monkey O and monkey T, respectively. C, Same as in A and B but for saline injections. D, ROC-values individual neurons are plotted before saline (abscissa) against ROC-values after saline (ordinate). E, Distribution of changes in ROC-values for individual neurons after ketamine (black bars) and saline (white bars) injections.

3.3.8(ii) Reliability

The fano factor did not differ between before and after ketamine injections for the preferred (2.21 ± 0.20 versus 2.63 ± 0.26 ; $p=0.07$, paired t-test) or nonpreferred condition (1.94 ± 0.15 versus 2.11 ± 0.17 ; $p=0.12$, paired t-test) during the intertrial period. The findings were similar for the saline injections for the preferred (2.29 ± 0.50 versus 1.65 ± 0.5 $p=0.09$, paired t-test) and nonpreferred condition (1.98 ± 0.37 versus 1.98 ± 0.3 ; $p=0.93$; paired t-test). This finding indicates that ketamine did not affect the reliability of neural responses during the intertrial period.

3.4 Discussion

Our data demonstrate that disruption of NMDARs impairs error-processing in nonhuman primates: a single systemic subanesthetic dose of ketamine reduced the differences in post-response activity between correct and error trials of LPFC neurons in an antisaccade task. For those neurons that had higher activity following an error than a correct response, ketamine increased the post-response activity on correct trials only. Ketamine had no effect on the activity following response errors in these neurons. Similarly, neurons that exhibited higher post-response activity for correct trials, increased their activity following ketamine on error trials but not on correct trials. The effect of these performance-dependent increases in neural activity was that performance selectivity was significantly reduced in LPFC neurons following ketamine administration.

Consistent with the finding that ketamine impaired performance-related activity of LPFC neurons, we also found that animals fixated longer on the stimulus on error trials following ketamine, thereby resembling the fixation durations after correct prosaccades. The absence of any behavioural or neural effects after saline injections demonstrate that the effects were due to the action of ketamine and not related to a decrease in task performance and performance-selectivity of LPFC in response to the injection process.

Consistent with previous reports (Condy et al., 2005; Skoblenick and Everling, 2012), we found that ketamine impaired the overall performance of antisaccades and to a smaller degree also of prosaccades. We have previously argued that this decline in performance might be related to impairments in the animal's ability to selectively maintain, or apply the appropriate task set (Skoblenick and Everling, 2012). Such an impairment in context processing, i.e. the ability to produce a different response to the same stimulus depending on the goal or rule (Cohen and Servan-Schreiber, 1992; Miller and Cohen, 2001), has also recently been described for the Dot Pattern Expectancy Task after ketamine injections (Blackman et al., 2013). Here we found some evidence that ketamine also affected the monkeys' behaviour after a saccade. While ketamine did not affect the behaviour after a correct response, both animals maintained much longer fixation on the stimulus after a response error following ketamine administration. In fact, fixation durations after a response error on an antisaccade

trial resembled the fixation duration after a correct prosaccade. After both types of responses, monkeys fixated the peripheral cue, but they were only rewarded on the correct prosaccade trials. The finding that ketamine did not affect the behaviour after correct antisaccades indicates that the delivery of the reward was sufficient to signal that the trial was performed correctly even under ketamine and that ketamine did not lead to a general increase in fixation durations after a saccade. Instead, the data suggest that ketamine impaired the animal's ability to detect that their saccade was a response error, which would be consistent with its effects on performance-related activity of LPFC neurons. This would also explain the finding that post-error slowing after an antisaccade response error disappeared after ketamine administration since post-error slowing has only been found after aware but not unaware antisaccade errors in human subjects (Klein et al., 2007).

In addition to its effect on action monitoring activity of LPFC neurons, ketamine also altered neural activity during the intertrial period. Consistent with the conflict-monitoring model (Botvinick et al., 2001; Miller and Cohen, 2001; Yeung et al., 2004; Carter and van Veen, 2007), we found that a large proportion of LPFC exhibited differences between correct and error trials in the following intertrial period. This finding is reminiscent to the observation that some LPFC neurons maintain conflict-related signal throughout the intertrial period (Mansouri et al., 2007). Ketamine reduced the differences between previous correct and

previous error trials by decreasing the activity for the neurons' preferred condition and increasing the activity for their nonpreferred condition.

Although ketamine binds to other receptors besides the NMDAR, there is strong evidence that its behavioural effects are mediated primarily by NMDAR (Byrd et al., 1987; Ginski and Witkin, 1994; Duncan et al., 1999). Our data show that ketamine increased the activity of LPFC neurons. This findings seems surprising given that non-competitive NMDAR antagonists block the NMDA channel (Huettner and Bean, 1988) and decrease the firing rate of neurons in anesthetized animals (Moghaddam et al., 1997). However, increased LPFC activity following a subanaesthetic dose of noncompetitive NMDAR antagonists has also been found in rodent LPFC neurons (Jackson et al., 2004). The authors hypothesized that the blockage of the NMDAR may lead to a compensatory overactivation of AMPA receptors. Alternatively, ketamine has been shown to block NMDAR in fast-spiking inhibitory interneurons more effectively than in pyramidal neurons in rodents (Olney et al., 1999; Homayoun and Moghaddam, 2007; Seamans, 2008). We have recently also found increases in LPFC activity following ketamine administration in nonhuman primates, but these effects were similar for putative interneurons and pyramidal neurons (Skoblenick and Everling, 2012). Here we also found that ketamine had similar effects on putative interneurons and pyramidal neurons. It is critical to note that we did not observe an overall increase in performance-related LPFC activity here, but a selective

increase in a neuron's post-response activity for its nonpreferred performance. The finding that some LPFC neurons were active after response errors and other neurons that were more active after correct responses supports the notion that opponent coding is a general feature of prefrontal decision making (Kusunoki et al., 2010; Lennert and Martinez-Trujillo, 2013). We also show here that these two response patterns are likely not mediated by different classes of neurons as putative interneurons and pyramidal neurons were found in both populations. This result is reminiscent to the absence of waveform differences between ipsilateral and contralateral LPFC neurons (Lennert and Martinez-Trujillo, 2013).

Although we have found that ketamine altered action monitoring-activity of LPFC neurons, we cannot assume that ketamine acted specifically on these neurons. Resting-state functional MRI studies have demonstrated that ketamine leads to an increase in global connectivity throughout the brain (Driesen et al., 2013) and alters the relationship between task-positive and task-negative neural systems (Anticevic et al., 2012), indicating that it does not selectively act on the LPFC. Further, single neuron recordings in monkeys (Parent and Hazrati, 1995; Phillips and Everling, 2012) combined with functional imaging studies and lesion studies in humans suggest that a cortico-basal ganglia-thalamic circuit is involved in action monitoring. In particular the ACC and LPFC are closely interconnected (Bates and Goldman-Rakic, 1993; Paus, 2001) and it is possibly that the effects were mediated by projections from the ACC to the LPFC. Note, however, that in

humans with LPFC damage, the correct-trial ERN activity is equal to the error-trial ERN (Gehring and Knight, 2000), which clearly demonstrates that the LPFC interacts with the anterior cingulate cortex in performance monitoring.

According to an influential model of error processing and reinforcement learning (Holroyd and Coles, 2002), dopaminergic neurons in the ventral tegmental area and the substantia nigra pars compacta send a negative reinforcement signal to the anterior cingulate cortex. Support for this model has come from subjects suffering from Parkinson's disease, which is characterized by a severe degeneration of dopaminergic neurons in the substantia nigra pars compacta. It has been shown that individuals with Parkinson's disease exhibit a reduced ERN and deficits in performance monitoring (Jocham and Ullsperger, 2009). However, it has been argued by others that the mesoprefrontal dopamine signal lacks the temporal precision required to generate the fast ERN (Jocham and Ullsperger, 2009). In addition, more recent studies have also pointed towards a role for serotonin, norepinephrine, GABA, and adenosine in performance monitoring (Jocham and Ullsperger, 2009). NMDAR signalling interacts with all of these systems, so it might be impossible to trace the effects of ketamine on prefrontal action-monitoring activity to a single neuropharmacological mechanism.

Our results demonstrate that a low dose of ketamine alters action-monitoring activity of LPFC neurons in nonhuman primates. These changes in neural activity could explain the deficits in action-monitoring found in humans after ketamine administration (Stone et al., 2011). Taken together, the findings also support the hypothesis that an NMDAR dysfunction may mediate self-monitoring deficits and ultimately leads to the psychotic symptoms in schizophrenia (Bickel and Javitt, 2009).

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Chapter 4

Decreased outcome-sensitive beta-band activity accompany gamma-band changes in the monkey prefrontal cortex following ketamine administration

A version of Chapter 4 will be submitted for publication as KJ Skoblenick, T Womelsdorf, and S Everling, Decreased outcome-sensitive beta-band activity accompany gamma-band changes in the monkey prefrontal cortex following ketamine administration. To *Biological Psychiatry*.

4.1 Introduction

Schizophrenia is a debilitating mental illness affecting nearly 1% of the world's population and causes significant strain on many nations' healthcare systems (Knapp et al., 2004). The disease manifests itself in varying degrees of three symptomatic categories. Positive symptoms which include hallucinations and delusions, negative symptoms such as social withdrawal and anhedonia, and cognitive symptoms including declining executive function and action planning (Insel, 2010).

Older, preclinical animal models of schizophrenia focused on the dopamine theory of schizophrenia (Seeman and Lee, 1975; Seeman, 1987; Cohen and Servan-Schreiber, 1992) and produced animals with a clear positive symptom phenotype. More recent theories that incorporate the role of aberrant

glutamatergic signaling have led to the ketamine model of schizophrenia, which generates animals that express behaviours analogous to all three symptomatic categories (Javitt and Zukin, 1991; Olney and Farber, 1995; Olney et al., 1999). Additionally, this model is able to induce a transient schizophrenia-like behavioural phenotype in normal human subjects (Krystal et al., 1994; Adler et al., 1999).

The assortment of symptoms experienced by a patient with schizophrenia has implicated many brain regions and networks as potentially contributing to the disease state. It is possible that a disconnect in communication between these regions underlies all of the individual regional deficits (Friston, 1998; Phillips and Silverstein, 2003). Inter-regional communication is facilitated by neural oscillations (Salinas and Sejnowski, 2000, 2001; Tiesinga and Sejnowski, 2004), with two distinct frequency ranges being of particular interest in schizophrenia. Both beta-band activity (15-30Hz) and gamma-band activity (30+ Hz) have been shown to be altered in both schizophrenia patients and animal models of the disease (Uhlhaas and Singer, 2010). Non-human primates challenged with ketamine exhibited similarities to schizophrenic patients in their sensory processing event-related potentials (ERPs) (Gil-da-Costa et al., 2013) and their performance in the anti-saccade task (Condy et al., 2005; Skoblenick and Everling, 2012, 2014). Rodents tested with the ketamine model of schizophrenia have consistently shown increased gamma-band activity (Pinault, 2008; Hakami

et al., 2009). Although beta-band activity has not been studied as thoroughly as gamma-band oscillations, electroencephalograph (EEG) recordings have also shown decreased beta-band activity in human schizophrenic patients (Krishnan et al., 2005; Uhlhaas et al., 2006; Hirano et al., 2008).

One consistent finding in EEG recordings of the PFC in patients with schizophrenia is a change in the error related negativity (ERN) (Kopp and Rist, 1999). Normally, this phenomenon appears as a brief negative deflection of the event related potential immediately following an erroneous response to a cue. The ERN is reliable and reproducible in the healthy human and non-human primate population and can be measured even when the subject is unaware that a mistake was performed (Falkenstein et al., 2000; Godlove et al., 2011; Murphy et al., 2012; Phillips and Everling, 2014). It has been hypothesized that this contributes to the performance monitoring network of the PFC and helps the subject disengage from erroneous behaviours so that they may find a correct behavioural response instead (Walsh and Anderson, 2012). In patients with schizophrenia, the ERN amplitude is reduced (Bates et al., 2002). It is possible that this decrease in ERN represents a failure of the PFC to recognize the patient's mistakes and consequently cannot engage the neuronal mechanisms required to switch their behavioural set (Polli et al., 2008).

This study aimed to examine the oscillatory properties of the primate prefrontal cortex through intracranial recordings during a cognitively demanding task and search for changes to this oscillatory activity induced by an acute administration of the NMDA-antagonist, ketamine.

4.2 Materials and Methods

4.2.1 Animals

The experiments were performed in accordance with the Canadian Council of Animal Care policy on the use of laboratory animals and all procedures were approved by the Animal Use Subcommittee of the University Western Ontario Council on Animal Care. For this study two male rhesus monkeys (*Macaca mulatta*) weighing 5kg (Monkey T) and 7kg (Monkey O) performed the behavioural tasks. The animals were implanted with a recording chamber located above their lateral PFC and a plastic head restraint, as previously described (Johnston and Everling, 2006). Post-surgical treatments included analgesics, prophylactic antibiotics, and oversight by the university veterinarian. Following surgical recovery, animals had cranial MR imaging to obtain anatomical localization of the recording chambers (**Figure 4.1**).

4.2.2 Behavioural Task

Both animals were trained to perform the anti-saccade task in order to

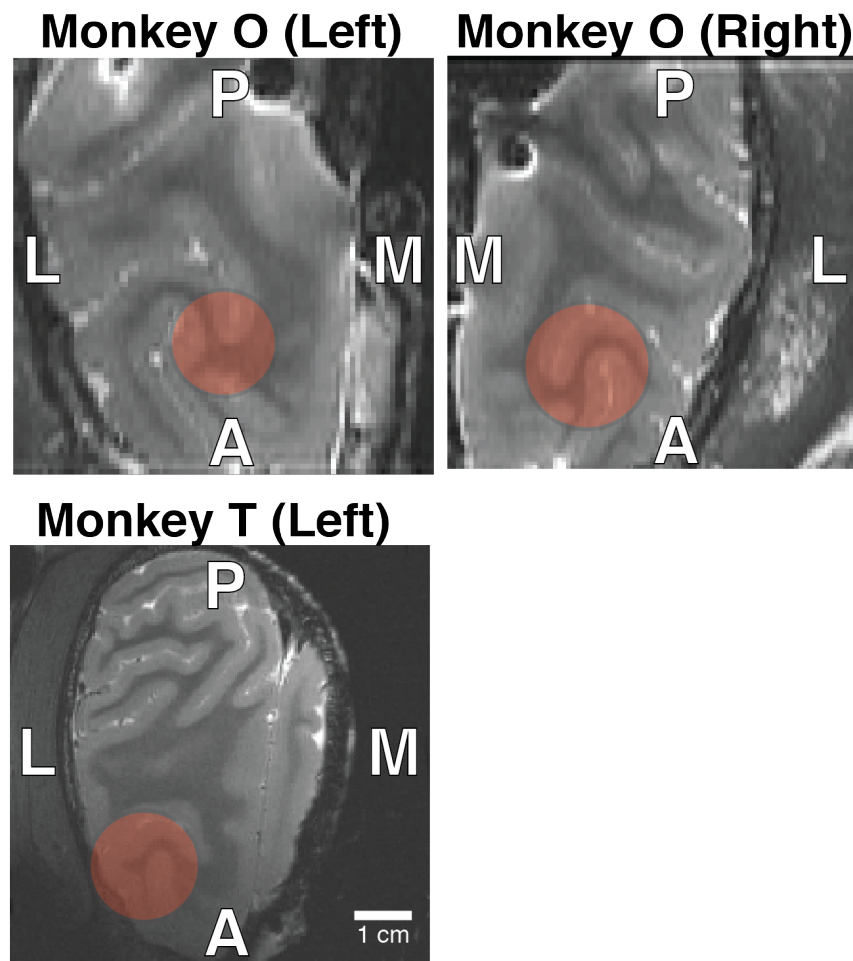


Figure 4.1: Anatomical MR images of electrode recording locations in both animals. Monkey O had bilateral recording chamber implantation and had two sessions recorded from the right hemisphere and three sessions recorded from the left hemisphere. Monkey T had all three sessions recorded from his unilateral left hemisphere chamber. The red circle indicates the area that the multi-electrode grid covered. P:Posterior; A: Anterior; L: Lateral; M: Medial.

receive a liquid reward, as described previously by our laboratory (Skoblenick and Everling, 2012). Briefly, the animal was enclosed in a light and sound attenuated chamber with their head fixed and oriented towards a computer monitor displaying the task. Each trial began with a fixation stimulus which was replaced with a rule cue after fixation for 100ms. Monkey O was trained that a green coloured rule cue indicated a pro-saccade task and a red-coloured cue indicated an anti-saccade task. The colour rules were reversed for training Monkey T. After a 1000-1200ms instruction period, a white, circular, peripheral stimuli appeared either 8° to the left or right of the central fixation cue. Pro-saccade trials required the animal look at this stimuli, while anti-saccade trials required the animal to look towards a blank location on the screen diametrically opposite to the stimuli's position. Following correct trials, a liquid reward was delivered to the monkey's mouth through a sipping tube immediate after a saccade to the correct target location. Eye gazes in any direction other than towards or away from the stimuli ended the trial immediately. Following the completion of a trial the screen was blanked and a new trial began after a 200ms inter trial interval. Trials were presented in a randomized order such that each task variant occurred equally. The animal's eye position was recorded and digitized at either 120Hz using an ISCAN infrared pupillary tracking system for Monkey O (ISCAN, Woburn MA) or at 1000Hz using an Eyelink 1000 infrared pupillary tracking system for both Monkey O and Monkey T (SR Research, Mississauga ON).

4.2.3 Electrode Recording

The setup for multi-electrode recording sessions began a day before the experimental session with the installation of the semi-chronic multi-electrode grid (Neuronitek, London ON) into the recording chamber. The 36 tungsten electrodes (FHC, Bowdoin ME) were lowered through a silicone membrane and into the monkey's lateral PFC, providing 32 recording channels and 4 reference channels. Each electrode was lowered manually using a micro-screwdriver until background activity was observed on a maximal number of recording channels. The animal was returned to its cage until the next day so that the electrodes had time to settle in the cortical tissue. On an experimental session day, the animal was returned to the sound-attenuated chamber and the electrode grid was connected to a head stage and amplifying unit. Neuronal spiking activity and LFP activity from each channel were combined with performance and eye-tracking data in a multi acquisition processor system (Plexon, Dallas TX) and sorted offline using 2D and 3D principal component analysis. Subsequent recording days only required reconnecting the head stage and a minimal adjustment to each electrode's depth before spiking neuronal activity was observed. The multi electrode grid was left implanted for two weeks, after which it was removed for cleaning and sterilization of the recording chamber.

4.2.4 Drug Administration

Each session began with a 15 minute pre-injection period, during which the animal performed 200-250 trials with accompanying PFC recordings. The animals both averaged less than 15% error rate on both pro- and anti-saccade trials. At the 15 minute mark, the experimental paradigm was paused briefly and the animal was given a 0.4mg/kg intramuscular injection of ketamine diluted in saline to 0.4ml into their right triceps brachii muscle. The experiment booth was closed and the experimental task resumed. In total, the injection process introduced less than a 20s pause and the animal began performing the task again immediately. Both animals had their dose titrated between 0.2mg/kg and 1.0mg/kg to optimize a dose that elicited a behavioural deficit but had no appreciable anesthetic effect (Condy et al., 2005). The animals' behaviour continued at baseline performance levels for approximately 5 minutes before the behavioural effects of the ketamine injection became apparent. Sessions would then continue for another 45 minutes to monitor the effects of ketamine. Control experiments with injections of saline did not produce any significant changes between pre- and post-injection periods in the animals' behavioural performance or neuronal activity. Experiment sessions with ketamine were spaced at least 3 days apart to ensure there were no cumulative effect of the drug or changes to the pre-injection baseline activity.

4.2.5 Data Analysis

Electrophysiological data was analyzed using custom scripts for Matlab (Mathworks) that made use of the FieldTrip toolbox developed by the Donders Institute for Brain, Cognition and Behaviour. For LFP analysis, the continuous analog signal was divided into discrete trials using event time markers provided by the Plexon recording system. Data was filtered with a low-pass filter at 150Hz and line noise was removed at 60Hz and 120Hz using a discrete Fourier transform. Z-score thresholding, and component analysis were used to detect and discard any additional mechanical artifacts in the analog signal. In order to remove the reward artifact, the data was first downsampled to 1/100th the polling rate after which component analysis was run to manually identify those components that contained the artifacts. The component analysis was then reperformed on the original data without downsampling to remove the artifact components from the final data set. To determine time-locked LFP power, frequency analysis was performed using the multitaper method with a discrete prolate spheroidal sequence taper set around a 0.667s window every 50ms for the low frequency range (1-50Hz) and a 0.33s window every 50ms for the high frequency range (45-150Hz). The LFP data for each trial was normalized to the oscillatory activity on the same channel during the preceding intertrial interval (500ms preceding fixation onset) resulting in a Z-score that could be compared between channels, sessions, and animals. Statistical analysis on the resulting time-frequency-LFP Power maps used a nonparametric cluster-based analysis that created a T-value map for the significance level between two conditions and

highlighted time-frequency epochs with statistical significance (Maris and Oostenveld, 2007). Neuronal spiking activity was analyzed as previously described (Skoblenick and Everling, 2012).

4.3 Results

Data was collected from 8 testing sessions (5 with Monkey O and 3 with Monkey T) yielding 158 LFP channels and 215 neurons for analysis. Channels without usable signals were removed from the analysis. Behavioural effects were similar to those previously described (Skoblenick and Everling, 2012, 2014) and are summarized in **Table 4.1**.

4.3.1 Prefrontal cortex exhibits outcome-dependent beta-band LFP activity

To examine how the beta-band range of LFP activity may be involved in a task requiring explicit cognitive control, we recorded the LFP signal in 2 monkeys. After lower frequency analysis was performed (1-50 Hz), a clear time and frequency locked epoch emerged following the animal's saccade response to the trial stimulus. The response occurred most strongly between 15-30 Hz which corresponds to beta-band oscillations. Further, this response appeared to be outcome specific, showing a stronger effect following trials in which the animal made a correct response (**Figure 4.2**). After the effect was visualized the statistical strength of these findings were challenged with a cluster-based analysis. The resulting T-score map highlighted a cluster between 0.3s to 0.75s

after saccade onset that showed a significant difference in the LFP activation between correct and error trials. To better illustrate the evolution of this difference, the mean LFP power of the relevant oscillatory range (15-30 Hz) was plotted as mean \pm SEM over the course of the trial (**Figure 4.2B**).

| | Monkey O | | | Monkey T | | |
|---------------|-------------------|--------------|----------------------|-------------------|--------------|----------------------|
| | SRT | Error Rate | Return Time Δ | SRT | Error Rate | Return Time Δ |
| Pre-Ketamine | 193 \pm 2 ms | 16 \pm 10% | -150 \pm 33 ms | 188 \pm 2 ms | 17 \pm 2% | -575 \pm 57 ms |
| Post-Ketamine | 252 \pm 5 ms | 58 \pm 17% | +370 \pm 68 ms | 237 \pm 4 ms | 51 \pm 12% | - 44 \pm 56 ms |

Table 4.1: Effect of ketamine on performance measurements in the anti-saccade task. SRT: Saccade response time; Error Rate: percentage of trials when a pro-saccade was performed instead of an anti-saccade or vice-versa; Return Time Δ : Difference in time after saccade completion that that animal remained focused on their target location (Error – Correct).

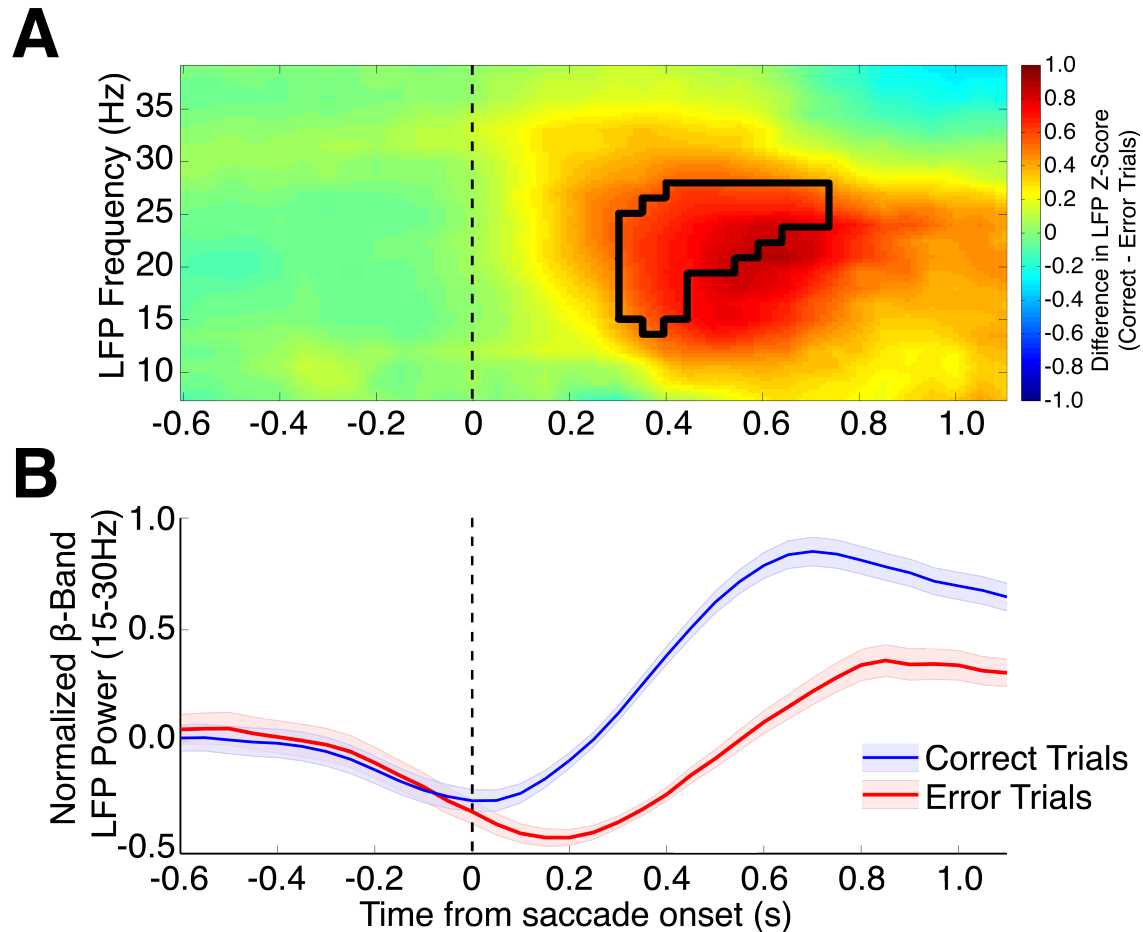


Figure 4.2: Performance-selective differences in beta-band LFP power. A heatmap displaying the trial time from saccade onset (x-axis) and frequencies (y-axis) shows the difference between the mean activity following a correct trial versus an error trial (Panel A). The colour bar indicates the z-score value obtained from (correct trials – error trials) baseline normalized z-score. Positive values indicate stronger activity during correct trials while negative values show time-frequency points with stronger activity for error trials. The black outline indicates the region in which statistical significance was found via cluster-based analysis. Panel B shows the mean Z-score \pm SEM for the beta-band frequency range (15-30Hz) aligned on saccade onset for correct (blue) and error (red) trials.

4.3.2 Ketamine reduces performance selectivity of beta-band LFP activity

After observing the selectivity in the beta-band response following task completion we next analyzed how these changes were affected by the administration of a subanesthetic dose of ketamine. In previous studies we have described the effect of this drug on response time and error rates (Skoblenick and Everling, 2012, 2014). The findings were once again similar in this study. In regards to beta-band activity however, a large decrease in performance selectivity was observed (**Figure 4.3A**). The overall beta-band activity was decreased (pre-ketamine mean Z-score: 0.132 ± 0.267 , post-ketamine mean Z-score: -0.278 ± 0.205 , $p < 0.001$) to the detriment of the correct-selective epoch beginning 200ms after saccade onset. The differences in (correct – error) LFP power were compared for statistical significance and the cluster-based analysis once again found a time-frequency epoch significantly different between pre- and post-ketamine conditions. The evolution of this change in selective over the course of the trial was also plotted as mean power (15-30Hz) +/- SEM to illustrate how pre- and post-ketamine conditions differed (**Figure 4.3B**).

4.3.3 No Significant Gamma-Band Selectivity

With the gamma-band of oscillatory activity also implicated in schizophrenia, we next examined how the higher frequencies responded to the task before and after ketamine administration. Initial difference maps were generated between (correct – error) task outcomes and a clearly defined error-

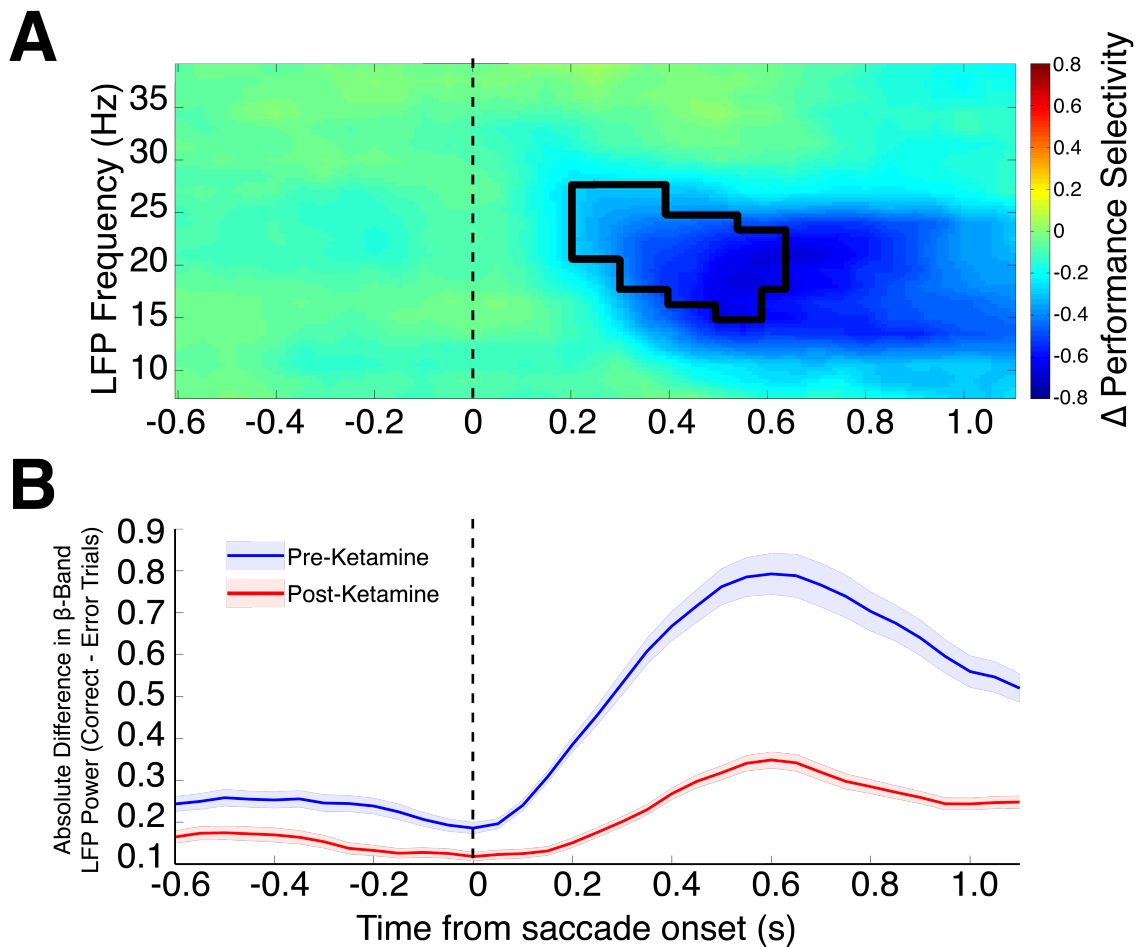


Figure 4.3: Difference in performance selectivity following ketamine administration. The heatmap in Panel A displays the trial time from saccade onset (x-axis) and frequencies (y-axis) with the colour bar indicating the difference in absolute selectivity calculated as (absolute difference between correct and error trials after ketamine) – (absolute difference between correct and error trials before ketamine). Thus a positive value indicates a time-frequency area in which the difference between correct and error trials was greater after ketamine injection, whereas a negative value indicates the performance selectivity was greater before ketamine. The epoch found to be statistically significant through cluster-based analyses is outlined in black. In Panel B the mean difference between correct and error trials \pm SEM in the beta-band range (15-30Hz) is plotted for both pre-ketamine values (blue) and post-ketamine values (red).

selectivity before ketamine was visible, however clustered-based comparisons between the correct and error heatmaps did not reach significance values in any relevant areas (**Figure 4.4**). Overall mean gamma power (40-120Hz) during the trial was significantly increased following ketamine administration (pre-ketamine mean Z-score = -0.1584 ± 0.3521 post-ketamine mean Z-score = 0.8535 ± 0.5539 , $p < 0.000001$ Student t-test).

4.3.4 Gamma-Band Activity Follows Neural Spiking Patterns

Lastly, similarities were observed between the gamma-band LFP patterns and neural spiking activity changes following ketamine administration. By comparing the mean LFP powers in the high gamma range (60-120Hz) across the duration of a trial to the mean spiking power, we found correlations between these two measurements (**Figure 4.5**). Indeed, pre-ketamine high-gamma LFP powers follow a very similar pattern of evolution and peak-time to the average spiking activity of neurons in the PFC. Further validation of these parallels can be observed post-ketamine. The pre-ketamine LFP power was found to be significantly correlated with the mean neuronal spiking rate in both correct ($r=0.58$, $p < 0.001$) and error ($r=0.84$, $p < 0.0001$) trials as well as post-ketamine in correct ($r=0.37$, $p < 0.05$) and error ($r=0.87$, $p < 0.0001$) trials.

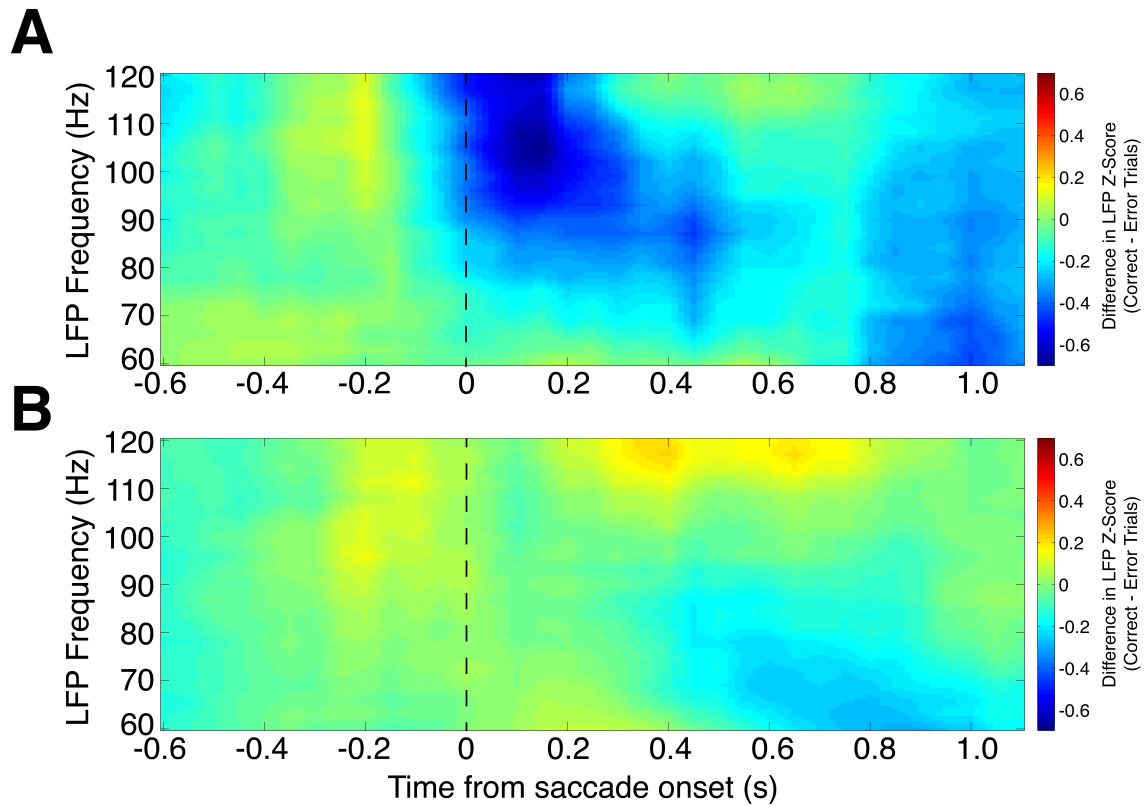


Figure 4.4: Outcome specific heat maps for gamma-band frequencies. The mean difference between correct and error trials for pre-ketamine trials (**Panel A**) and post-ketamine trials (**Panel B**) are displayed, with positive values indicating stronger activity for correct trials and negative values showing stronger activity for error trials. No significance was found through the cluster based analyses for the pre-ketamine selectivity map, however overall gamma-band activity was significant increased between pre-ketamine and post-ketamine values.

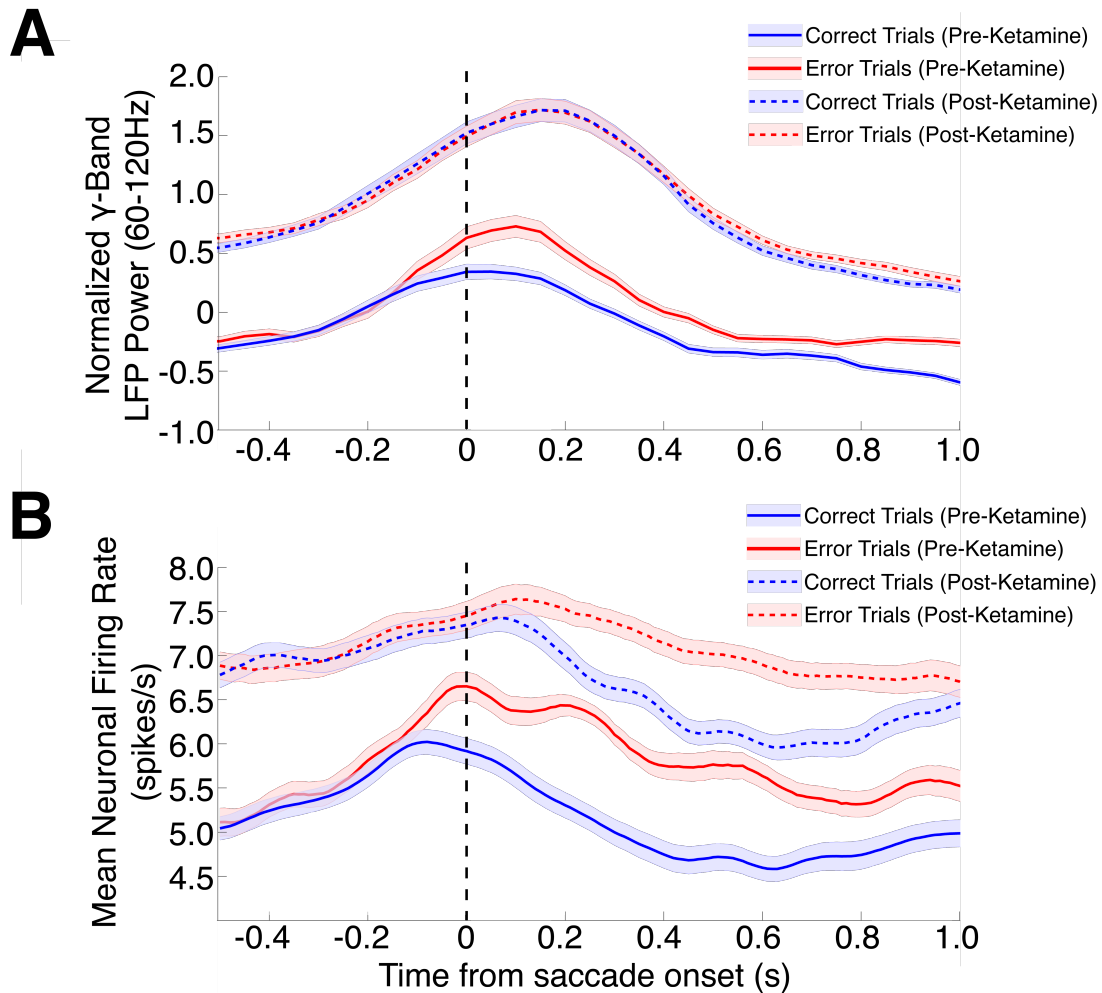


Figure 4.5: Gamma-band LFP power and corresponding neuronal firing rates. The mean high gamma-band (60-120Hz) activity \pm SEM is plotted in Panel A contrasting both pre-ketamine (solid lines) correct trials (blue) and error trials (red) with post ketamine (dashed lines) values. The pattern of activity is notably similar to the mean spiking activity simultaneously measured from neurons in the PFC (Panel B) in both their peak activity epoch and their response to ketamine administration.

4.4 Discussion

This experiment set out to determine if the LFP events observed during other tasks that challenge a subject's cognitive control over behaviour may also be measured in relevant epochs of the anti-saccade task. Further, we wanted to test if these events are altered by ketamine in a manner similar to the aberrant oscillatory activity found in human patients with schizophrenia (Uhlhaas and Singer, 2010, 2013). Our strongest findings were in the beta-band frequency range (15-30Hz) and were sensitive to the outcome of the trial. The PFC in conjunction with the anterior cingulate cortex have been shown to have an important role in performance monitoring during tasks requiring the cognitive suppression of automatic responses (MacDonald et al., 2000; Swick and Turken, 2002; Ridderinkhof et al., 2004; Walton et al., 2004). Our data show that the beta-band may be involved in this feedback monitoring, as the timing occurs only after the animal's response has been made and is dependent on whether or not the trial was performed correctly.

Alternatively, the beta-band range of oscillations has been demonstrated in other brain regions to have a very strong impact on motor control (Androulidakis et al., 2006; Swann et al., 2009). Increased beta activity decreases the ability of a subject to initiate a new movement or to disengage from a current action (Gilbertson et al., 2005; Pogosyan et al., 2009). It has also been shown in human EEG studies to increase in tasks during which a muscle

must maintain a specific action against an unpredictable amount of force (Androulidakis et al., 2007). Thus the beta activity we observe in the PFC may be an efferent signal to the saccade system to hold the eye's position at the correct location. This maintenance at a correct location has been hypothesized to allow the organism to gain more information and increase attention to a desirable outcome or to process the stimuli that resulted in a reward (Engel and Fries, 2010). Incorrect trials, with their decreased beta-band activity, allow for a quicker disengagement from the peripheral stimulus because of its unrewarding properties.

The introduction of ketamine to this pathway appears to disrupt the outcome specific signal causing the animals to no longer display beta-band activity that is selective for correct trials. The beta signal is elicited in the same epoch for both correct and error trials, indicating that the animal either does not recognize the trial was erroneous or that the PFC is no longer able to send outcome specific efferents to downstream locations. These results corroborate our previous findings with regards to a change in the animal's post-response reaction time (Skoblenick and Everling, 2014). In those studies we discovered that pre-ketamine, the animal's gaze would remain fixed on the target location longer after making a correct pro- or anti-saccade when compared to error trials. After ketamine, the animal's gaze lingered much longer on the stimulus for an incorrect anti-saccade trial, similar to their post-response reaction after making a

correct pro-saccade (**Table 4.1**). Our beta-band findings showed that after ketamine, the beta-band response was similar for both correct and error trials. This supports the hypothesis that beta-band activity can maintain a current motor state, in this case a saccade target location. Evidence for this beta-band influence on behavior has also been demonstrated in hypodopaminergic states as Parkinsons disease (Limousin et al., 1995; Brown, 2003). It is suspect that the increased beta-band activity observed in Parkinsons patients may contribute to the decreased ability to initiate motor movements based on internal or cognitive cues (Kuhn et al., 2004; Chen et al., 2007; Kuhn et al., 2008). Taken together, these results show that the similarities in beta-band activity after ketamine administration (**Figure 4.2B**) may act as a physiological basis for the similarities between correct and error trial post saccade response times (**Table 4.1**). This epoch specific increase in beta-band activity may also correspond with task-specific prefrontal hypodopaminergic states observed in schizophrenia (Dworkin and Opler, 1992; Abi-Dargham and Moore, 2003; Stone et al., 2007). Additionally, we found that the generalized beta power was decreased, which corresponds both with the increased PFC dopamine release caused by ketamine (Lorrain et al., 2003) and the resting-state beta power decrease in schizophrenia (Krishnan et al., 2005; Uhlhaas et al., 2006). Combining these findings reinforces the critical interplay between the glutamatergic and dopaminergic systems in schizophrenia and its associated animal models.

Gamma-band oscillations have been studied extensively in schizophrenia for many years as well but have been debated whether they are increased or decreased in the disease (Kocsis et al., 2013). NMDA antagonism in humans (Hong et al., 2010) and animal models of schizophrenia (Pinault, 2008; Kocsis, 2012) have demonstrated an increase in gamma-band activity whereas human studies had been showing a decrease in gamma-band signal (Gandal et al., 2012). Recently, studies that have revisited human schizophrenia data found two trends that may explain these discrepancies. Firstly, there appears to be an overall increase in gamma activity resulting in more background noise and thus a relatively decreased signal (Kikuchi et al., 2011; Spencer, 2011; Suazo et al., 2014) and secondly, that antipsychotic medications decrease the gamma-band activity (Jones et al., 2012).

Our study found that there was indeed an overall increase in gamma-band activity. This increase mirrored the increase in spiking activity that we had previously described (Skoblenick and Everling, 2012). The increased spiking activity was found to be reducing the signal to noise ratio of task specific neurons in the prefrontal cortex because of their increased activity due to ketamine administration. It may be that the increase in gamma-band activity we are observing in the primate PFC may be another manifestation of this increase in background noise and a loss in an additional dimension of information conveyance. Further, these similarities lend credence to the idea that oscillatory

activity in this high gamma range may be reflective of the net spiking activity of nearby neurons in the macaque brain (Ray et al., 2008; Whittingstall and Logothetis, 2009). Additional investigation is required to support this idea but this opens the possibility to measure gamma-band power as a proxy measurement for spiking activity.

In summary, beta-band activity appears to play an important role in the PFC for determining how to respond to correct and erroneous trial outcomes. This performance-sensitive frequency and time-locked pattern is vulnerable to disruption in the ketamine model of schizophrenia. The NMDA antagonist removes any differentiation between correct and error trials following saccade response. In addition, ketamine also increased the overall activity in the gamma-band range, potentially reflecting decreased cognitive control in the PFC and similar increases in background noise as observed in the neuronal spiking activity. These results further validate the neural similarities between humans with schizophrenia and the ketamine model of schizophrenia in non-human primates.

4.5 References

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Chapter 5

Discussion

5.1 Summary of Findings

5.1.1 Ketamine increases error rates and reaction times in the anti-saccade task

Ketamine administration in healthy human subjects can induce a schizophrenia-like phenotype as demonstrated in a number of different behavioural experiments (Krystal et al., 1994; Malhotra et al., 1996). The anti-saccade task is particularly useful in this situation because a relatively simple training procedure yields a robust data set that describes a number of cognitive functions (Everling and Fischer, 1998). Extensive studies have repeatedly demonstrated that patients with schizophrenia show a significant impairment in this task (Fukushima et al., 1988; Fukushima et al., 1990; Mazhari et al., 2011). Although a single study showed a non-significant decrease in the ability for normal human subjects to perform this task after being administered ketamine (Radant et al., 1998), a non-human primate study showed a statistically significant effect of ketamine (Condy et al., 2005). Prior to the ketamine injection, monkeys exhibited error rates and reaction times similar to normal healthy humans. After the ketamine injection had taken effect however, these values regressed and instead resembled reaction times and error rates of human patients with schizophrenia (Condy et al., 2005).

The results across all three chapters and in all three monkeys that performed the task corroborated these findings. We saw a baseline error rate of approximately 20% in the anti-saccade task prior to ketamine, which transiently increased to up to 80% error rate at the height of the ketamine effect. To build upon these findings, we first looked in detail at the types of errors made by the subjects.

Through the inclusion of trials with a rule-memorization component, I identified a separate and significant effect that ketamine induced on the monkeys' working memory. Error rates were increased in all anti-saccade tasks but those trials that required the retrieval and maintenance of the rule from working memory were affected to a greater degree. This significant separation between the rule-visible and rule-memorized trials represents the negative effect ketamine exerts specifically on working memory, similar to other reports on impaired working memory in the ketamine model of schizophrenia (Taffe et al., 2002). After finding a high error rate in rule-visible trials, it became evident that the cognitive deficits being induced by ketamine are occurring at an early stage of the neural pathways involved in this task. Even with rule information present in the monkeys' visual field, they were still unable to saccade away from the stimulus in an anti-saccade trial. Possible explanations include either a total inability to perform rule-identification or a lack of feedback indicating that a

correct trial produces a more favourable outcome in the form of a reward. The electrophysiological changes in the performance monitoring component of PFC spiking activity strongly implicates the latter possibility. Similarly in the LFP results, we saw changes in the beta band specifically at the post-response epoch. The reduced selectivity of the beta band LFP power for correct trials over error trials also reflects a decrease in the PFC sensitivity to rewarded versus unrewarded outcome. Patients with schizophrenia may be experiencing a similar deficit in outcome monitoring (Silver et al., 2006). When these patients fail to recognize that an error has occurred there may be no signal to change their behavior or recall the relevant rule, which would aid them in getting back to correct trial outcomes. A correlate of this effect is also already observed in some fashion as the decreased error related negativity and decreased feedback negativity compared to normal subjects (Morris et al., 2008).

This impaired error response also gives a physiological basis for the impaired learning associated with schizophrenia. Feedback-related negativity is critical for learning (Walsh and Anderson, 2012) so a lifetime of reduced feedback would accumulate a number of cognitive impairments over experiences that would normally teach unimpaired individuals a wide range of behaviours. Immediate feedback learning is also important for adapting to challenges that evolve after the initial rules have been established, like the Wisconsin Card Sorting Task. The decreased ability for schizophrenia patients to adapt to

changes in the card sorting rules over time may be reflective of this impaired performance monitoring feedback in the PFC (Everett et al., 2001; Ohrmann et al., 2008).

The speed at which the monkeys made this erroneous action provided an insight into the potential neuronal pathways responsible. Prior to ketamine administration, the few errors being made by the subjects had very short saccadic reaction times (SRTs). Reaction times lower than 100ms are classified as express saccades (Boch et al., 1984) and at this speed of reaction time the saccade is being triggered by a direct pathway from the visual input to the saccade generating system in the superior colliculus (Freund et al., 1986; Dorris et al., 1997; Sommer and Wurtz, 2001). This implies that the errors are behaviourally distinct from those observed after ketamine administration. The long SRTs found on error trials after ketamine indicate that these errors are no longer associated with the triggering of the express saccade pathway. Instead, it suggests that there may be a breakdown in the processes that the subject uses to select the saccade target location. Automatic saccades caused by the express saccade pathway become incorrectly selected saccade targets due to the ketamine-induced deficits in cognition. This change in reaction times was associated with changes in the activity of the PFC in human schizophrenia patients as well (Bender et al., 2013).

For the first time in non-human primates, we simultaneously recorded single unit neuronal activity and local field potentials in the PFC while the animal performed an anti-saccade task both before and after the administration of ketamine. This allowed us to gain information from a region in brain often implicated for the cognitive dysfunction observed in schizophrenia. Similar to previous studies of neuronal activity in the PFC, we found neurons exhibiting rule-selectivity, where the selective neuron would produce more spiking activity for their preferred task - either pro- or anti-saccade (Everling and DeSouza, 2005; Johnston and Everling, 2006, 2009). The changes in firing rate we observed in the PFC directly correlated with the onset and offset of the behavioural changes observed. Additionally, the changes observed in the local field potential at both the beta and high gamma band ranges also aligned with the behavioural deviations. With this data, we can confirm that data previously obtained from non-invasive methods with poorer resolution both spatially and temporally may have electrophysiological correlates within relevant epochs during the experimental tasks (Driesen et al., 2013).

It is important to acknowledge that an increase in reaction time after ketamine administration is not always associated with a decrease in performance. Other visual tasks such as the visual search task exhibited different results. In this task, monkeys were trained to search in their visual field for a target stimulus amongst distractor stimuli that were present at a lower luminance.

Here, it was found that although ketamine was associated with an increase in SRT towards the target stimuli, this increase in reaction time led to a decrease in the error rate, presumably due to a longer window within which to select their target (Shen et al., 2010).

Our results are congruent with these results, as the difference in task requirements challenges a different set of cognitive processes in the PFC. In the visual search task used by Shen et al. (2010), the monkey must recognize a brighter luminance stimulus in a field of dimmer stimuli, termed perceptual discrimination. In our task, the monkey was able to identify the target stimulus but difficulties arose in selecting a behavioural response to this stimulus. Rather, these results for the visual search task further support the ketamine model of schizophrenia. Studies have found that patients with schizophrenia are less able to integrate separate elements into a global Gestalt (Place and Gilmore, 1980; Rief, 1991). Thus, when a number of individual distracting or background stimuli are presented in their visual field, patients with schizophrenia are better at processing the items individually. This is in contrast to a control subject's visual system, which attempts to integrate the noise together as a single item then break it down into parts for the search task (Butler et al., 2008).

5.1.2 Ketamine increases background activity to the detriment of task specific signals

By far the strongest novel finding of this project is ketamine's ability to induce a shift in the signal:noise ratio of task selective activity in the PFC. The effect was observed in both the spiking activity of individual neurons as well as in the LFP amplitude of two distinct frequency bands. In the spiking activity we found that two distinct types of neuron selectivity emerged. Activity in the pre-stimulus epoch showed rule-based selectivity for the type of trial being performed (pro- or anti-saccade). Post-saccadic response activity showed that there were neurons with selectivity for either correct or error trial outcomes, firing more for their preferred outcome during a specific epoch. Similarly, in the LFP results, beta band activity showed a significant increase in amplitude more so after correct trial outcomes than errors and only during the post-response epoch. There was also a non-significant trend visualized in the gamma band data during the post-response epoch. In contrast to the beta band results, gamma band amplitudes showed a non-significant increase for error trials over correct trials.

The selectivity properties of neurons and LFP amplitudes were recorded before ketamine administration and their disruption coincides with the onset of the behavioural symptoms. Most importantly, across both recording modalities and both trial epochs the cortical function was always disrupted in the same manner by ketamine; a decrease in the selectivity exhibited by neurons or frequency bands and an increase in irrelevant non-specific activity.

For the single cell recordings, this was demonstrated as an increase in the net firing rate of most neurons to the detriment of the selective signal that they are responsible for generating. A net increase in neuronal firing rates after ketamine administration has previously been described (Rowland, 2005), but the implications of this change have not. The neurons no longer fired more for their preferred condition and instead fired at an increased rate equally for all conditions. Further, since the non-epoch baseline firing rate was also increased, the critical time-locked burst of activity is proportionally smaller in comparison to the basal rate. The end result of this can be generalized across different neuron types as a failure to output the selective signals required of them for the expected PFC functions.

The LFP data can be thought of as a measurement of neuronal coherence at a single point. When more neurons are firing in an oscillatory pattern, the amplitude for that oscillatory frequency will be increased at that electrode recording location. After ketamine administration the post-response beta band amplitude was decreased to a greater degree after correct trials than error trials, which had the end result of creating identical post-response activity in an epoch previously capable of describing the trial outcome. This decreased beta band amplitude signifies fewer neurons synchronizing their firing rates to the beta band frequency range and most likely an impaired ability for the brain to generate signals normally associated with re-enforcement of correct trial behaviour and the

inhibition of error trial behavior. These results are similar to the ERN changes described in schizophrenia patients (Kopp and Rist, 1999). In these situations the EEG signal that normally differentiates between correct and error trials instead has a similar response for both outcomes.

It is possible that the prefrontal cortex in this ketamine model of schizophrenia may still be sending the same signals through the appropriate networks but ketamine increases the global baseline activity of neurons in the brain which impairs sensitive time-locked bursts of activity. Without this selectivity in PFC neurons, the ability to code contingent behavioural responses is greatly reduced and thus the normal flow of information becomes lost in the background noise. This inefficient activity of the PFC has already been demonstrated in human patients with schizophrenia. During tasks that challenge the subjects working memory, fMRI studies have shown that schizophrenia subjects require a stronger activation (as measured by the BOLD signal) of their PFC in order to obtain equal or poorer performance rates compared to healthy controls (Callicott et al., 2003). This excessive workload to only reach levels of normal functioning may be representing the extra effort exerted in order to create a strong enough signal to overwhelm the increased background noise. Ramping up neuronal activity like this would cause significant changes in the BOLD signal of this region. Even in unaffected healthy subjects, gamma band activity increases

proportionally in the PFC with the level of cognitive control that the subject requires to complete a task similar to the anti-saccade task (Cho et al., 2006).

Paradoxically, schizophrenia is usually associated with a hypofrontality profile and more often demonstrates a decrease in the overall activity in the PFC. Corroborating this is a recent study that suggests ketamine induces a hypofrontal state on selective neurons by preferentially inhibiting a select population of neurons in the primate PFC, which are responsible for advanced cognitive functions (Wang et al., 2013a). Unfortunately, this study used incomparable levels of ketamine which induced a 30 minute dysfunctional state in the non-human primates, where excessive levels of nystagmus prevented trial performance. It is possible that at these higher levels of drug administration, ketamine overwhelms the brain and no longer selectively binds to the NMDA receptors of clinically significant Pv+ interneurons (Beasley and Reynolds, 1997). Newer human studies have also started to find that patients with schizophrenia exhibit heightened levels of baseline gamma band activity, potentially referring to the increased activity and increased noise of the PFC after ketamine. This heightened baseline necessarily then causes all task and epoch analyzed activity to appear lower after within subject normalization (Kikuchi et al., 2011; Spencer, 2011; Suazo et al., 2014). Antipsychotic medications may be suppressing these levels as well, which has previously led researchers to classify these regions as hypoactive (Jones et al., 2012).

Potential therapeutic interventions incorporating these glutamateric discoveries are already being investigated. We have previously discussed the sensitivity of PFC neurons to increases in noise and how this poor information conductance negatively affects behaviour. As such, simplistic drugs that only act as agonists or antagonists may not rectify the problem. The poor signal inside the noise needs to be enhanced and if the glutamateric signaling system is disrupted through the NMDA receptor, a potential workaround is through the metabotropic glutamateric receptor (mGluR). Agonists for the mGluR receptor such as LY379268 are showing promising results in clinical trials. By increasing the glutamateric signal amplitude (but not the frequency of excitatory post-synaptic potentials), LY379268 facilitates the conveyance of existing signals, particularly within the PFC (Wang et al., 2013b). A phase 2 clinical trial has demonstrated that a similar mGluR agonist is as effective as current atypical antipsychotic medications but is not associated with the metabolic side-effects that normally accompany these pharmacological interventions (Patil et al., 2007).

5.1.3 Ketamine has opposing effects on beta band and high gamma band LFP activity in PFC

In the fourth chapter, I demonstrated that beta band LFP activity is sensitive during the post-response epoch to the monkeys performance in that trial. Correct outcomes elicited a higher amplitude beta band response than

incorrect outcomes and ketamine both decreased the overall beta band activity while also eliminating the outcome selective response. This has an interesting implication when observed in light of changes documented in the brains of patients with Parkinson's disease. This degenerative neurological disorder is characterized by a progressive inability to generate voluntary movements in the later stages of the disease. These changes are associated with an increase in neuronal oscillations in the beta band frequency, as measured by EEG (Chen et al., 2007). In healthy individuals the beta band oscillations help the brain to favor an existing motor state and prevent new unwanted movements (Gilbertson et al., 2005). Voluntary movements can even be slowed by artificially inducing beta band activity through transcranial current inductions (Pogosyan et al., 2009). Newer treatment methods of deep brain stimulation (DBS) aim to disrupt these increased oscillations by delivering asynchronous electrical pulses. Clinical trials with DBS-based beta band disruption has shown immense promise as once the pathological beta band activity has been disrupted patients saw a decrease in their akinesia symptoms (Kuhn et al., 2008).

If the increased beta band activity is associated with a pathological level of movement or behavioural suppression in the Parkinsonian brain, then the decreased beta band activity in this ketamine model of schizophrenia may be representative of the decreased level of cognitive control exhibited by patients with schizophrenia (Green, 1996). Indeed, patients with schizophrenia show

decreased beta band synchrony during cognition challenging tasks (Uhlhaas et al., 2006). This has been implicated in the decreased interregional communication found in patients with schizophrenia (Uhlhaas and Singer, 2010), resulting in a disconnected brain and poor PFC interactions with other regions (Tu et al., 2010).

Compounding these changes are the non-specific changes that we observed in the high gamma range of LFP amplitude. Gamma band activity has been associated with the initiation of behavioural responses that require motor movement. Human EEG studies demonstrated peaks in gamma band activity just before movement initiation (Shibata et al., 1999). In the diseased state, tests of working memory have shown increased gamma band activation in patients with schizophrenia despite poorer performance on the task (Barr et al., 2010) and the normalization of this heightened gamma band activity recovers performance back to normal levels (Farzan et al., 2012). Therefore it is possible that the increase in gamma band amplitude is predisposing the brain to the erroneous initiation of behavioural responses. With a higher baseline resting state in the gamma band, the neuronal activation required to reach a threshold for movement initiation can be reached much more easily. The correlation of this in humans would be an increased impulsivity, something that has been demonstrated to be increased in schizophrenia with significant neuronal correlates (Kaladjian et al., 2011). With both the decreased beta band unable to maintain the current motor set and the

increased gamma band predisposing the subject to impulsive, poorly planned behavioural responses, the LFP data provides insight from two different directions on how aberrant neuronal oscillations and their relation to the disconnected schizophrenic brain can predispose a patient to have poor cognitive control over their actions.

5.2 – Caveats and Limitations

5.2.1 Systemic administration of ketamine

One of the problematic areas of working on this project was ketamine's action occurring simultaneously on the entire brain instead of only on our region of interest, the PFC. Debate is still ongoing regarding ketamine's mechanism of action for the hyperactivity it induces despite antagonizing a receptor for an excitatory neurotransmitter (Moghaddam et al., 1997). Since the PFC acts as a hub with reciprocal connections to many other brain regions, the downstream effects of the NMDA antagonism in other brain regions may be what is measured in our studies in the PFC. Yet the infusion of NMDA antagonists into the frontal cortex of rats has still produced similar behavioural deficits (Murphy et al., 2005). For its relevance as a model of schizophrenia, ketamine needs to affect the entire brain to better model the global changes observed in these patients. Although the effects of ketamine may also be occurring in other brain regions, the PFC acts as the critical juncture through which the changes in neuronal activity are manifesting as decreases in cognitive control.

5.2.2 Single sensory modality

All of our studies made use of the anti-saccade task, which only requires visual sensory input and behavioural responses in the form of eye movements. This task was chosen because the neuronal circuitry of these responses is well understood and has been described in great detail over the years (Everling and Fischer, 1998). Although patients with schizophrenia exhibit a great many number of measurable changes in multimodal sensory perception and behavioural responses, the anti-saccade task has shown replicable results with a clear impairment in patients (Sereno and Holzman, 1995; Hutton and Ettinger, 2006). Aside from visual behaviour abnormalities, others often employ tasks that use auditory cues and stimuli to test and schizophrenia-specific EEG findings have been described in relation to these auditory changes (Blackwood et al., 1991). For the purposes of this study however, our laboratory is well equipped for the visual-centric tasks and the training paradigm for the monkeys has been refined to produce competently performing animals in a minimal amount of time.

5.3 Future directions

5.3.1 Effect of antipsychotic drugs on PFC activity and anti-saccade performance in the ketamine model of schizophrenia

While we have described strong findings that link the ketamine model of schizophrenia and potential sources of cognitive dysfunction, a stronger

connection could be made if the findings observed with antipsychotic medications in human patients with schizophrenia are also found in this ketamine model. Traditional typical and atypical antipsychotic medications are unable to produce changes in the schizophrenic person's cognitive domain of symptoms, but the newer mGluR agonists are showing some promise in cognitive benefits (Moghaddam, 2004; Conn et al., 2009). A study that looked at how these classes of drugs affect both the single unit recording changes and the LFP changes that we observed would be very interesting. Being able to strengthen the signal or decrease the noise would hopefully restore the information that is being lost in the chaotic activity of the ketamine-treated (and possibly schizophrenic) brain. Additional non-invasive interventions such as transcranial direct current stimulation that either enhances the lost beta band activity or decreases the excessive gamma band activity may also have behavioural implications.

5.3.2 – Changes in LFP-LFP and LFP-spike coherence following ketamine administration

The work I presented here discussed changes in the LFP amplitude as recorded from single electrodes. This represents the coherence of neurons at a specific point but by simultaneously recording from multiple electrodes we can take these results further. The coherence of LFP activity across multiple sites both intra and interregional have strong implications when discussing a potential model of schizophrenia. I have previously mentioned the hypothesis that

schizophrenia is a disease of disconnect, where through poor anatomical connections and poor communications between regions the brain becomes progressively impaired because of an inability to synchronize. We could verify these hypotheses better by looking at how the brain communicates through neural oscillations that synchronize activity, the frequency ranges this occurs at, and how these coherent oscillations are disrupted by ketamine. An additional dimension could be added by examining how the single unit spiking activity correlates with the LFP activity. It is possible that the NMDA receptor-inhibiting actions of ketamine disengage neurons from following the neural oscillations that normally encourage or discourage spiking activity, thus leading to the noisy and irrelevant activity pattern.

5.4 Concluding Remarks

Through chapters two through four I have described in detail the behavioural effects of a subanesthetic dose of ketamine on non-human primates ability to perform the anti-saccade task. The changes I observed align with shortcomings in the performance of human patients with schizophrenia who are tested on this task. These changes were accompanied by decreased task-specific signals in single unit activity as well as LFP amplitude that also follow neuronal abnormalities observed in human patients with schizophrenia.

While schizophrenia is a uniquely human disease, progress with the ketamine model of schizophrenia will hopefully aid us in determine the true physiological basis of this debilitating disease. It is my hope that with enough understanding of the neuronal basis of this disease, optimal interventions can be made and these patients can enjoy a life enriched by normal cognitive function.

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APPENDIX A – Ethics Approval



2008-125::5:

AUP Number: 2008-125

AUP Title: Role of Frontal Cortex in Cognitive Control

Yearly Renewal Date: 02/01/2014

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2008-125 has been approved, and will be approved for one year following the above review date.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Kinchlea, Will D
on behalf of the Animal Use Subcommittee

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 PhD Student, Department of Anatomy and Cell Biology
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 Investigated the effects of ketamine on working memory in non-human primates
 Completed course requirements for the graduate program
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2004 – 2009 **McMaster University** Hamilton, Ontario
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 Researched the effects of various antipsychotic drugs on proteins in the brain
 Performed *in vitro* experiments including immunocytochemistry, confocal
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KEVIN SKOBLINICK**PAGE 2**

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 Led weekly problem-based learning tutorial sessions for multiple groups of 15 students
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PUBLICATIONS:**Peer Reviewed Journal Articles:**

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Decreased expression of medial prefrontal cortex synapsin II in schizophrenic specimens from the Stanley Foundation Neuropathology Consortium. Tsang E, Chong VZ, Gabriele J, Thomas N, Castellano JM, Dyck BA, **Skoblenick KJ**, Ki K, and Mishra RK. Society for Neuroscience. Washington, DC, Oct. 2005.

Singh AN, Castellano JM, Sondhi S, Thomas N, Dyck BA, **Skoblenick KJ** and Mishra RK. Increased gene expression and plasma levels of glucose dependent insulinotropic polypeptide following clozapine treatment. British Association for Psychopharmacology. Harrogate, UK, July 2005.

Modulation of dopamine receptor activity by L-Prolyl-L-Leucyl-Glycinamide peptidomimetics. Dyck BA, Mann A, Verma V, Pontoreiro GF, Thomas N, Castellano JM, **Skoblenick KJ**, Dolbeare KB, Johnson RL and Mishra RK. Society for Neuroscience. San Diego, CA, Oct. 2004.

Rogoza RM, N-Marandi S, Castellano JM, **Skoblenick KJ**, Dyck BA, Doering LC, and Mishra RK. Differential effects of typical and atypical antipsychotic drugs on the release of apoptosis inducing factor from the mitochondria. Society for Neuroscience. San Diego, CA, Oct. 2004, 34, 908.20.

Patents:

Mishra RK, Johnson RL, Sharma S, **Skoblenick KJ**, Castellano JM and Dyck BA. PLG analogue #47 as a treatment for schizophrenia. Final stages of patent preparation.

Platform Presentations:

Skoblenick KJ. Changes in behaviour and neural activity in a non-human primate ketamine model of schizophrenia. Anatomy and Cell Biology

Seminar Series, Western University. June 2012.

Skoblenick KJ. Ketamine and Neural Correlates of Cognition: Studies in non-human primates. University of Western Ontario MD/PhD Research Day. Nov. 2011.

Skoblenick KJ. Neuropharmacology research at McMaster University. Invited presentation by Dr. Sam Weiss at the Hotchkiss Brain Institute, University of Calgary, May 2008.

Skoblenick KJ and Mishra RK. Role of AP-2 alpha transcription factor in the regulation of synapsin II expression by dopamine D1 and D2 receptors. McMaster Biology Undergraduate Symposium, Apr. 2006.

Skoblenick KJ and Mishra RK. Role of AP-2 alpha transcription factor in the regulation of synapsin II expression by dopamine D1 and D2 receptors. McMaster Psychiatry Department Research Day, Apr. 2006.

Reviewer:

Trevizo F et al. Comparative study between acute reserpine and sub-chronic haloperidol and its implications for tardive dyskinesia: Beneficial effects of pecan nut shells extract (*Carya illinoensis*). Submitted to *Behav Brain Res*, March 2010.

McNamara et al. Reductions in prefrontal cortex docosahexaenoic acid (DHA) composition increase the serotonin 5-HT_{2A}:Dopamine D2 receptor binding ratio: Normalization by DHA repletion but not chronic risperidone treatment. Submitted to *J Neurosci*, March 2008. Invited reviewer by Dr. Ram Mishra.

Authors hidden. Effect of alpha lipoic acid on the tardive dyskinesia and oxidative stress induced by haloperidol in rats. Submitted to *J Neural Transm*. November 2008. Invited reviewer by Dr. Ram Mishra.

SUMMARY:

Peer-Reviewed Publications: 15 (+1 submitted). **Abstracts:** 14.
Presentations: 5. **Articles Reviewed:** 3. **Patents:** 1.

AWARDS:

Dean's Honour List, McMaster University (2004-2006)
Best Poster (Cell/Molecular Biology Division) BUS (Plaque Award - 2006)
McMaster Medical Sciences Graduate Scholarship (\$9,000 - 2007)

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NSERC PGS-M Scholarship (\$17,300 - 2008)
OGS Scholarship (\$15,000 - 2008, Declined)
CIHR CGS-D Scholarship (\$35,000 x 3 years – 2009)
OGS Scholarship (\$15,000 – 2009, Declined)
Suzanne Bernier Publication Award for Anatomy & Cell Biology (Publication of the Year in the Department of Anatomy & Cell Biology \$400 - 2013)
Drs. Madge and Charles Macklin Fellowship for Publication (Best Student Publication of the Year in Schulich School of Medicine & Dentistry \$3000 - 2013)

TRAINING:

WHMIS (2004, Updated 2012)
Biohazard Safety (2004, Updated 2011)
Animal Handling & Injections (2004, Updated 2009)
Fire Safety (2004, Updated 2012)
Radioisotope Safety (2004, Updated 2008)
Human Venipuncture (2008)