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An Investigation of the Neural Mechanism by which the Prefrontal Cortex Facilitates Anti-saccade Task Performance

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

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The thesis by

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Abstract

Cognitive control enables us to guide our behaviour in an appropriate manner, such as rapid eye movements (saccades) toward a location or object of interest. A well-established test of cognitive control is the anti-saccade task, which instructs subjects to look away from a suddenly-appearing stimulus. The dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) are part of a cortical saccade control network that influences the superior colliculus (SC), which sends saccade commands to the brainstem saccade generator. To compare and contrast the roles of the dlPFC and ACC in saccade control, the cryoloop method of reversible cryogenic deactivation was used to identify the effects of dlPFC and ACC deactivation on pro-saccades and anti-saccades. Both dlPFC and ACC deactivation increased the incidence of ipsilateral saccades, but only dlPFC deactivation impaired contralateral saccades. An inhibitory model of prefrontal function has been proposed by which the prefrontal cortex suppresses the activity of SC saccade neurons on anti-saccade trials, to inhibit an unwanted saccade toward the stimulus. A direct test of this inhibitory model was performed by deactivating the dlPFC and recording the activity of SC saccade neurons. Unilateral dlPFC deactivation delayed the onset of saccade-related activity in the SC ipsilateral to deactivation, which suggests that the dlPFC has an excitatory influence on SC saccade neurons. There was also an increase of activity in the contralateral SC, which suggests that unilateral dlPFC deactivation caused a neural imbalance at the SC. Bilateral dlPFC deactivation, on the other hand, should not cause a neural imbalance, and thus was used to identify the effects of dlPFC deactivation that were caused by cognitive control impairments. Bilateral dlPFC deactivation increased the stimulus-related activity, and decreased the saccade-related activity, of SC saccade neurons. An increase of anti-saccade errors was more substantial in a “rule memorized” condition, which suggests that the dlPFC plays an important role in rule maintenance. Given an excitatory influence of the dlPFC on SC saccade neurons, I propose that the dlPFC facilitates anti-saccade task performance by first maintaining the relevant rule in working memory, then implementing the rule by enhancing the saccade-generating signal at the SC.

Keywords

Anterior cingulate cortex

Anti-saccade task

Cryogenic deactivation

Dorsolateral prefrontal cortex

Monkey neurophysiology

Oculomotor control

Response inhibition

Superior colliculus

Saccadic eye movements

Co-Authorship

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Michael Koval performed the experiments, analysed the data, assisted in the preparation of manuscripts for publication, and wrote the remaining chapters of this dissertation. Dr. Stefan Everling designed the experiments, Dr. Steve Lomber designed the equipment that was used for the experiments, and both Drs. Everling and Lomber performed surgeries to prepare the animals for the experiments. Dr. Everling and Dr. Kevin Johnston wrote the original manuscript for Chapter 3, while Drs. Everling and Lomber wrote the original manuscript for Chapter 4. I have modified these manuscripts to provide consistency across all chapters, and include additional information that I felt was necessary for the purposes of my dissertation.

The results of the third experiment (Chapter 4) have been published in the Journal of Neuroscience as Koval MJ, Lomber SG, Everling S (2011) Prefrontal cortex deactivation in macaques alters activity in the superior colliculus and impairs voluntary control of saccades. *J Neurosci* 31:8659-8668. The results of the second experiment (Chapter 3) have been accepted for publication in Cerebral Cortex as Johnston K, Koval MJ, Lomber SG, Everling S (2013). Macaque dorsolateral prefrontal cortex does not suppress saccade-related activity in the superior colliculus. *Cerebral Cortex* (in press). Finally, it remains to be determined how the results of the first experiment (Chapter 2) will be included with the results of another experiment for the purposes of publication.

"Keep me safe and warm
When I'm riding out my storm…
A narrow bank, a narrow wall
There's room to hide here...
Calm never fails, it merely shines"

Dickinson, Rob. "The Storm."
Fresh Wine for the Horses.
Sanctuary Records Group, 2005.

I dedicate the completion of this, my PhD dissertation, both to my parents,
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Abbreviations

2DG – 2-deoxyglucose
ACC – anterior cingulate cortex
ANOVA – analysis of variance
BOLD – blood oxygenation level dependent
CEF – cingulate eye field
CFN – caudal fastigial nucleus
dlPFC – dorsolateral prefrontal cortex
dlPFC+ – dorsolateral prefrontal cortex active
dlPFC- – dorsolateral prefrontal cortex deactivated
EBN – excitatory burst neuron
ERP – event-related potential
FEF – frontal eye field
fMRI – functional magnetic resonance imaging
GABA – gamma-aminobutyric acid
IBN – inhibitory burst neuron
LLBN – long lead burst neuron
MLBN – medium lead burst neuron
MR – magnetic resonance
NRTP – nuclei reticularis tegmenti pontis
OPN – omnipause neuron
PET – positron emission tomography
PPC – posterior parietal cortex
PPRF – paramedian pontine reticular formation
rCBF – regional cerebral blood flow
RF – response field
riMLF – rostral interstitial nucleus of the medial longitudinal fasciculus
ROC – receiver operating characteristics
SEF – supplementary eye field
SC – superior colliculus
SCBN – superior colliculus burst neuron
SCBUN – superior colliculus buildup neuron
SCFN – superior colliculus fixation neuron
SNpr – substantia nigra pars reticulata
SR – stimulus-response
SRT – saccadic reaction times
WCST – Wisconsin card sorting test

Symbols

°	– degrees
°C	– degrees Celsius
>	– greater than
Hz	– Hertz (cycles/second)
K^+	– potassium
<	– less than
mm	– millimetres
mm^3	– millimetres cubed
ms	– milliseconds
Na^+	– sodium
%	– percent
±	– plus or minus
s	– seconds

Preface

Imagine yourself in the Stanley Cup Final. With the score tied and only a few seconds left on the clock, you find yourself with the puck in front of your opponent's net. While in this situation you would normally try to score a goal by shooting at the net, on this occasion you notice a teammate of yours in a better scoring position at the side of the net. Realizing there is now a better chance of scoring a goal by passing the puck to your teammate instead, you change your plan accordingly. The puck goes in and the crowd goes wild! Now back to reality, where this scenario illustrates that behavioral control requires high-level cognitive functions, such as identifying the context of the situation, selecting an appropriate behaviour, and suppressing unwanted alternatives; all of which requires coordination of an extensive neurophysiological network. A model that is used to study the cognitive control of behaviour is the oculomotor system, in which high-level control areas send commands to the motor structures that generate eye movements. The neural mechanisms for oculomotor control have been extensively studied, and furthermore both humans and non-human primates can be trained on identical oculomotor tasks, which are easily and precisely monitored in the laboratory as part of electrophysiology and neuroimaging studies. Many cognitive control studies use oculomotor tasks that require rapid eye movements (saccades) toward an object or location of interest. Damage to the neural network for saccade control may help to explain the symptoms of patients with neurological or psychiatric conditions that affect cognitive control, such as their impaired ability to suppress unwanted saccades. Therefore the main objective of my dissertation was to identify the neural mechanism by which a particular component of this saccade control network facilitates the performance of a context-appropriate saccade.

Chapter 1

Literature Review: the Oculomotor System and Cognitive Control

1.1 – Outline

Following this brief outline, the second part of *Chapter 1* will provide an introduction to the oculomotor system. The main components of the oculomotor system will be presented in *Sections 1.2.1 to 1.2.3*, starting with a summary of the oculomotor plant, oculomotor neurons, and brainstem saccade generator. This will be followed by a detailed description of the superior colliculus (*Sections 1.2.4 to 1.2.7*) and subcortical structures that it sends projections to (*Sections 1.2.8 to 1.2.10*), which together form the subcortical saccade-generating circuit that is summarized in *Section 1.2.11*. The superior colliculus receives extensive input from both cortical and subcortical components of the saccade control network (*Sections 1.2.12 to 1.2.20*), including the prefrontal cortex which is proposed to implement cognitive control by influencing the activity of other brain areas that are more directly involved in the generation of a response. Cognitive control will be introduced in the third part of *Chapter 1 (Sections 1.3.1 to 1.3.*3). Well-established tests of cognitive control include working memory tasks (*Sections 1.3.4 to 1.3.6*), which require short-term maintenance of relevant information, and the anti-saccade task (*Section 1.3.7*), which requires looking away from a suddenly-appearing stimulus. *Sections 1.3.8 to 1.3.12* will discuss the contributions of the saccade control network to anti-saccade task performance, while *Section 1.3.13* will introduce the inhibitory model of prefrontal function, and *Section 1.3.14* will present the main objective and specific aims of this dissertation. Finally, *Section 1.4.1* will summarize methods for lesions and deactivation, while *Section 1.4.2* will explain cooling and the cryoloop method of reversible cryogenic deactivation.

1.2 – Neurophysiology of the Oculomotor System

The oculomotor plant, oculomotor neurons, and brainstem saccade generator, for which I provide summaries below, have been reviewed extensively elsewhere (Moschovakis et al., 1996; Leigh and Zee, 1999b; Leigh and Zee, 1999a; Goldberg, 2000; Munoz et al.,

2000; Tessier-Lavigne, 2000; Scudder et al., 2002; Sparks, 2002; Horn and Leigh, 2011).

The retina is a multi-layered extension of the central nervous system that lines the back of the eyes. There are two types of photoreceptors in the retina: rods and cones. Rods are sensitive to light and thus mediate light perception, while cones are sensitive to colour which enables high visual acuity. The ratio of rods to cones in the retina is approximately 20:1, except at the fovea which is densely packed with colour-sensitive cones and thus capable of analysing visual details with high resolution.

1.2.1 The Oculomotor Plant

The fovea is aligned with a visual target by coordinated rotation of the eyes, each of which is part of an oculomotor plant that consists of the globe, extraocular muscles, pulleys, and orbital tissue. Each globe is rotated by six extraocular muscles that are divided into three orthogonal pairs. Horizontal eye movements are primarily controlled by the medial rectus and lateral rectus muscles that rotate the globe medially and laterally, which adducts and abducts the fovea. Vertical eye movements are controlled by the superior rectus and inferior rectus muscles which rotate the globe upwards and downwards, causing elevation and depression of the fovea. Also contributing to vertical eye movements are the superior oblique and inferior oblique muscles which rotate the globe up and towards the nose, and down and away from the nose, causing intortion and extortion of the fovea.

1.2.2 Oculomotor Neurons

The extraocular muscles are innervated by the III (oculomotor), IV (trochlear), and VI (abducens) cranial nerve nuclei. The oculomotor nerve innervates the inferior oblique, medial rectus, superior rectus, and inferior rectus muscles, while the trochlear nerve innervates the superior oblique muscle, and the abducens nerve innervates the lateral rectus muscle. The discharge of motor neurons in the cranial nerve nuclei stimulates serially-connected muscle fibres in the corresponding extraocular muscle, the force of which summates to drive contraction of the agonist muscle with a “pulse-slide-step” signal. Eye movements, however, are constrained by the viscous drag and elastic

restoring forces of the orbital supporting tissues, and thus the viscoelastic properties of the oculomotor plant. The "pulse" is a phasic, high-frequency burst of action potentials that overcomes orbital viscous drag to move the eyes to a new position. The "slide" is a gradual, exponential decrease from the phasic "pulse" discharge to the tonic "step" discharge. To hold the eyes at the new position, "step" innervation of the agonist muscle must resist the elastic restoring force of the antagonist muscle, otherwise ocular drift will bring the eyes back toward their starting position. The height and width of the motor neuron "pulse" discharge corresponds with the velocity and duration of the saccade, respectively, while the height of the "step" discharge corresponds with the amplitude of the saccade.

1.2.3 Brainstem Saccade Generator

Oculomotor neuron discharge is determined by the saccade-generating circuit in the brainstem reticular formation. Horizontal saccades are generated by structures in the paramedian pontine reticular formation (PPRF), while vertical saccades are generated by structures in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). The brainstem saccade generator is composed of medium-lead burst neurons (MLBN), both excitatory (EBN) and inhibitory (IBN), a neural integrator, long-lead burst neurons (LLBN), inhibitory interneurons, and omnipause neurons (OPN). Motor neurons receive both a phasic "pulse" signal from EBNs and a tonic "step" signal from the neural integrator, the latter of which is a transformation of the phasic signal sent to the neural integrator by the EBNs. In addition to providing the premotor burst that drives contraction of the agonist muscles, the EBN signal also inhibits antagonist muscles in both eyes: EBNs innervate a) interneurons in the cranial nerve nuclei, which suppress the activity of motor neurons for the antagonist muscle of the same eye, and b) IBNs, which send decussating projections that suppress the activity of contralateral motor neurons which innervate the antagonist muscle of the opposite eye.

The brainstem saccade generator receives decussating projections from the contralateral superior colliculus (SC). A saccade command from the SC is interpreted as a target signal by the LLBNs and MLBNs, and a trigger signal by the OPNs. LLBNs in the nuclei reticularis tegmenti pontis (NRTP) relay this signal to the cerebellum, while

LLBNs in the PPRF relay this signal to EBNs. The tonic discharge of OPNs suppresses EBNs. Inhibitory interneurons are proposed to disinhibit EBNs by suppressing OPNs both before and for the duration of a saccade. 'Trigger' interneurons are innervated by the SC and suppress OPNs at about the same time that the saccade target signal arrives at the EBNs. This allows the EBNs to discharge and activate 'Latch' interneurons that suppress OPNs for the duration of the premotor burst. In addition to suppressing OPNs by way of inhibitory interneurons, the SC also sends excitatory projections directly to OPNs. These contrasting effects of the SC on OPNs can be explained by the functional organization of the SC.

1.2.4 Superior Colliculus: Retinocentric Encoding

The SC is a sensorimotor integration structure that consists of alternating fibre and cell layers. Visual neurons are located in the superficial gray layer and have contralateral visual response fields (RF) that encode stimulus locations relative to the retina on a topographic map (Cynader and Berman, 1972; Goldberg and Wurtz, 1972). This retinotopic map encodes the upper and lower visual fields in the medial and lateral SC, respectively, while locations proximal and distal to the retina are encoded in the rostral and caudal SC, respectively. Fixation neurons and saccade neurons, located in the intermediate gray layer, have motor RFs that encode contralateral saccade target locations on a retinotopic motor map (Robinson, 1972), which is aligned with the retinotopic visual map in the superficial layer (Schiller and Stryker, 1972; Wurtz and Goldberg, 1972).

1.2.5 Superior Colliculus: Superficial Gray Layer

Neurons in the superficial gray layer of the SC discharge in response to the appearance of a stimulus in their visual RF (Schiller and Koerner, 1971; Cynader and Berman, 1972; Goldberg and Wurtz, 1972). The superficial gray layer receives projections from the retina, visual cortex, and frontal eye field (FEF) (Hubel et al., 1975; Fries, 1984; Huerta et al., 1985; Stanton et al., 1988a), then sends projections to the visual thalamus (Harting et al., 1978) and intermediate gray layers of the SC (Isa and Hall, 2009). The visual response of neurons in the intermediate and deep gray layers of the SC has been

described as a priority signal, which is determined both by salience from 'lower' sensory structures and relevance from 'higher' control structures (Boehnke and Munoz, 2008). Top priority is assigned to the location at which the cumulative discharge across visual neurons is greatest, then used to coordinate visuomotor processing at cortical sensory and subcortical premotor areas.

1.2.6 Superior Colliculus: Intermediate and Deep Gray Layers

Neurons in the deeper (intermediate and deep gray) layers of the SC receive intracollicular projections from the superficial (gray and optic) layers (Isa and Hall, 2009), corticotectal projections from the FEF, supplementary eye field (SEF), posterior parietal cortex (PPC), and dorsolateral prefrontal cortex (dlPFC), and nigrotectal projections from the substantia nigra pars reticulata (SNpr) of the basal ganglia (Goldman and Nauta, 1976; Jayaraman et al., 1977; Leichnetz et al., 1981; Lynch et al., 1985; Stanton et al., 1988a; Shook et al., 1990). Munoz and colleagues have proposed that the intermediate layer consists of two distinct zones across the rostrocaudal axis: a rostral fixation zone and a caudal saccade zone (Munoz et al., 2000). Neurons in the rostral fixation zone have a parafoveal motor RF, discharge tonically when fixating on a stimulus in their motor RF, and pause for saccades in any direction (Munoz and Wurtz, 1993a). These fixation neurons (SCFNs) are proposed to inhibit saccade generation (Munoz and Wurtz, 1993b; Dorris and Munoz, 1995) by sending excitatory signals to OPNs (Gandhi and Keller, 1997; Buttner-Ennever et al., 1999; Shinoda et al., 2011), which tonically inhibit EBNs (Keller, 1974; Strassman et al., 1987), and inhibitory signals to SC saccade neurons (Munoz and Istvan, 1998). Neurons in the caudal saccade zone have a peripheral motor RF, discharge phasically for a saccade into their motor RF, and cease discharging during active fixation or for saccades out of their motor RF (Munoz and Wurtz, 1995). These saccade neurons are proposed to facilitate saccade generation (Hikosaka and Wurtz, 1985, 1986; Dorris et al., 1997) by sending inhibitory signals to brainstem OPNs (by way of inhibitory interneurons) and excitatory signals to brainstem LLBNs and MLBNs (Harting, 1977; Raybourn and Keller, 1977; Scudder et al., 1996; Shinoda et al., 2011). OPNs, therefore, receive both excitatory projections

from SCFNs, and inhibitory projections from SC saccade neurons (Shinoda et al., 2011).

1.2.7 Superior Colliculus: Types of Saccade Neurons

Motor saccade neurons discharge for only a saccade into their motor RF, while visuomotor saccade neurons discharge for both a stimulus in their visual RF and a saccade into their motor RF (Wurtz and Goldberg, 1972). The visual and motor RFs are closely aligned, but not identical, given that a motor RF is typically larger than a visual RF (Wurtz and Goldberg, 1972; Sparks et al., 1976; Marino et al., 2008). Munoz and Wurtz (1995) classified SC saccade neurons as burst neurons (SCBN), which discharge a high-frequency burst of action potentials for a saccade into their motor RF, or buildup neurons (SCBUN), which also have low-frequency discharge prior to stimulus onset that encodes saccade preparation, although SCBNs have been shown to demonstrate preparatory activity when there is a high probability of saccade direction (Dorris and Munoz, 1998). This prestimulus 'preparatory' activity correlates with the probability of saccade direction, independent of saccade generation, such that an increase of preparatory activity is not necessarily indicative of a saccade to that location (Sparks et al., 2000).

On the other hand, it has been argued that rather than being divided into a rostral fixation zone and caudal saccade zone, the SC consists of a single continuous map of goal locations instead (Hafed et al., 2008; Hafed and Krauzlis, 2008). To test this goal location hypothesis, monkeys tracked an invisible midpoint between two moving stimuli. The parafoveal movement goal, encoded by the rostral SC, was dissociated from the peripheral visual stimuli, which were encoded by the caudal SC. They found there was greater modulation in the rostral SC than the caudal SC during tracking, which suggests that the SC encodes goal location rather than stimulus location. Furthermore, localized SC deactivation did not cause any motor or fixation impairments, but rather created a biased estimate of goal location directed away from the retinotopic site of deactivation. Fixation impaired by inactivation of the rostral SC (Munoz and Wurtz, 1993b) is interpreted by the goal location hypothesis as a biased competition between goal locations in which activity at the fixation location is

weakened by deactivation. The appearance of a stimulus provides visual activation that elicits a saccade to the location with greater activation, which would be the stimulus location rather than the deactivated fixation location.

1.2.8 Tectal Efferents: Brainstem Saccade Generator

While SCFNs are proposed to provide the major excitatory input to OPNs, SCBUNs may also send excitatory signals that sustain OPN discharge and thus saccade inhibition when SCFN discharge decreases prior to saccade onset (Everling et al., 1998c). SCBNs, on the other hand, are proposed to send a high-frequency inhibitory signal to OPNs which, when greater than the combined low-frequency excitatory signals of SCFNs and SCBUNs, would inhibit OPNs (Everling et al., 1998c). Both SCBNs and SCBUNs discharge for a saccade into their motor RF (Munoz and Wurtz, 1995), and thus both contribute to the saccade target signal that the SC sends to LLBNs and MLBNs. The location encoded by the saccade target signal is determined by population coding of SC saccade neurons (Sparks et al., 1976). A neuron responds to all locations encompassed within their motor RF, graded relative to the distance from their preferred location, to which they respond with the greatest discharge. Adjacent neurons have overlapping motor RFs, and thus all neurons for which the location is contained within their motor RF will discharge, albeit to varying degrees. Vector averaging then computes the amplitude and direction of the saccade target signal by taking a weighted average of the discharge of all SC saccade neurons (Lee et al., 1988). The benefit of broadly-tuned motor RFs, combined with large populations of active saccade neurons, is that saccade metrics are not adversely affected by the variability of individual neuron discharge.

1.2.9 Tectal Efferents: Oculomotor Cerebellum

The brainstem saccade generator also receives input from the cerebellum, which is a subcortical structure that is proposed to calibrate the accuracy of saccades (Quaia et al., 1999). The SC sends projections to LLBNs at the NRTP (Harting, 1977; Scudder et al., 1996), which transmits the saccade target signal both directly to the caudal fastigial nucleus (CFN), and indirectly by way of the dorsal oculomotor vermis (Yamada and Noda, 1987; Noda et al., 1990). The CFN shares a reciprocal connection with the

contralateral brainstem saccade generator: decussating projections carry a saccade-related signal to contralateral MLBNs and LLBNs, from which an efference copy is sent back to the CFN by way of the paramedian tracts (Noda et al., 1990). The signal from the CFN is proposed to augment the premotor burst of EBNs at the onset of contralateral saccades, as demonstrated by CFN neuron discharge that is time-locked to saccade onset, and hypometric contralateral saccades caused by CFN deactivation. The CFN is also proposed to truncate the premotor burst of EBNs at the end of ipsilateral saccades, as demonstrated by CFN neuron discharge that is time-locked to saccade end, and hypermetric ipsilateral saccades caused by CFN deactivation (Ohtsuka and Noda, 1991; Fuchs et al., 1993; Robinson et al., 1993).

1.2.10 Tectal Efferents: Oculomotor Thalamus

The oculomotor thalamus contains neurons with saccade-related activity (Tanaka, 2007), has an increased rCBF for voluntary saccades (Petit et al., 1993), and thus is proposed to regulate the cortical processing of saccade control (Tanaka and Kunimatsu, 2011). In addition to the saccade command that is sent downstream to the cerebellum and brainstem saccade generator (Scudder et al., 1996; Shinoda et al., 2011), the SC also sends corollary discharge signals upstream to cortical saccade-related areas, such as the FEF, by way of the thalamus (Sommer and Wurtz, 2004). Corollary discharge conveys information about the impending saccade, which enables us to monitor our own actions and perceive a stable visual world. The subcortical cerebellum, basal ganglia, and brainstem saccade generator also send ascending projections to the oculomotor thalamus, which then relays these signals to cortical saccade-related areas (Lynch et al., 1994; Prevosto et al., 2009; Tanaka and Kunimatsu, 2011).

1.2.11 Summary of Subcortical Saccade-Generating Circuit

The contributions of subcortical saccade-related structures to oculomotor control can be summarized as follows: SCFNs maintain fixation by suppression of SC saccade neurons and enhancement of OPNs. To generate a saccade, SCFNs must pause to allow SC saccade neurons to discharge and send a saccade target signal to EBNs. When SCFNs pause, OPNs must maintain their tonic inhibition of EBNs prior to the arrival of the

saccade target signal, to prevent the premature triggering of a saccade by low-frequency non-target signals. SCBUNs are proposed to sustain the activity of OPNs until SCBNs discharge a high-frequency burst that provides both an inhibitory trigger signal to OPNs, and an excitatory target signal to EBNs. When the OPNs are suppressed, then the EBNs can discharge a premotor burst which is sent to oculomotor neurons that innervate the extraocular muscles. The SC also sends this saccade command to the oculomotor cerebellum, which controls saccade accuracy, and the oculomotor thalamus, which facilitates the internal monitoring of our own movements.

1.2.12 Tectal Afferents

The saccade command that the SC sends to the brainstem saccade generator is influenced by input from the retina and visual cortex, cortical saccade-related areas including the FEF, SEF, PPC, dlPFC, and anterior cingulate cortex (ACC), and the subcortical basal ganglia (Johnston and Everling, 2008; McDowell et al., 2008; Muri and Nyffeler, 2008). These tectal afferents follow either of two routes: the retinotectal pathway which transmits visual input directly to the superficial layers of the SC, or the retinogeniculocortical pathway which transmits visual and motor information to the deeper layers of the SC that has been modulated by cognitive processes.

1.2.13 Retinotectal Pathway

Visual information is detected by retinal photoreceptors and transmitted to retinal ganglion cells, the axons of which converge and exit the orbit as the optic nerve (Tessier-Lavigne, 2000). While the majority of axons are relayed to primary visual cortex by way of the lateral geniculate nucleus of the thalamus, a small proportion of them diverge from the optic tract to innervate the SC instead (Wurtz and Kandel, 2000). This direct retinotectal pathway transmits a visual signal that could elicit the rapid orienting of gaze towards a stimulus in the peripheral visual field. The latency of this foveation reflex (Schiller and Koerner, 1971) approaches the minimum conduction time from the retina to the extraocular muscles (Carpenter, 1981), and thus has been termed an ‘express’ saccade. In the laboratory, a visually-guided saccade task requires that subjects fixate upon a central fixation stimulus until a peripheral target stimulus

appears. Removal of the fixation stimulus 200 ms prior to target appearance creates a "gap" period which reduces saccade latency in all directions (Fischer and Boch, 1983; Fischer and Ramsperger, 1984). The disengagement of ocular fixation hypothesis attributes this "gap effect" to a reduction of the fixation signal in the gap period, which disinhibits the saccade-generating circuit (Dorris and Munoz, 1995). Consequently there is a decrease of SCFN activity and increase of SCBUN activity in the gap period (Dorris et al., 1997).

The increase of SCBUN activity by gap-related disinhibition also corresponds with the incidence of express saccades into the neuron's response field (Dorris et al., 1997), both of which increase with prior training and predictability of the response direction (Dorris and Munoz, 1998). This supports the oculomotor preparation hypothesis, which states that advanced preparation of a specific motor command facilitates the occurrence of express saccades (Pare and Munoz, 1996). A mechanism for express saccades can be explained with an accumulator model, wherein an increased level of SCBUN preparatory activity enables a stimulus-driven burst of visual activity to reach threshold, and discharge in support of an express-latency saccade toward the stimulus in the neuron's response field (Munoz et al., 2000). For normal-latency saccades, a lower level of preparatory activity prevents the stimulus-driven burst from reaching threshold, which is followed soon afterwards by a motor burst that achieves threshold (Dorris et al., 1997). SC saccade neurons, therefore, have both a visual and motor burst for normal-latency saccades, but only a single burst for express-latency saccades. A comparison by Edelman and Keller (1996) of these three types of bursts found that a) the express burst and visual burst have a similar onset latency, which suggests that express saccades are stimulus-driven, b) the express burst and motor burst are of a similar size, which suggests saccade threshold is the same for both normal and express saccades, and c) both the express burst and motor burst were larger than the visual burst, which suggests that the express burst is either an enhanced visual burst, or a physiological summation of the visual and motor bursts. Conversely, Sparks and colleagues (2000) argued that express saccades are not directly triggered by a visual response, given that saccade latency correlated with latency of the motor burst, rather than the visual burst. They showed that as the interval between the visual and motor

bursts decreased, saccade latency also decreased, such that at the latency of express saccades, the bursts were not distinct from one another. This suggests that the visual burst was supplanted by the motor burst, and thus implies that express-latency saccades share the same mode of saccade initiation as normal-latency saccades: the motor burst of SC saccade neurons.

1.2.14 Retinogeniculocortical Pathway

Of all the retinal ganglion axons that exit the orbit as the optic nerve, the majority of them continue past the optic chiasm and along the optic tract to innervate the lateral geniculate nucleus of the thalamus (Wurtz and Kandel, 2000). The visual signal then continues along the optic radiation to striate cortex (area V1) where it undergoes initial processing, followed by processing of greater complexity in extrastriate cortex (areas V2, V3, V4, V5). The lower visual areas (areas V1, V2, V3) send visual signals both to superficial layers of the SC and the higher visual areas (areas V4, V5). The ventral visual pathway from V4 to inferotemporal cortex processes nonspatial visual information for object perception, while the dorsal visual pathway from V5 to the PPC processes visuospatial information for visually-guided actions. The PPC sends projections to both the intermediate layers of the SC (Lynch et al., 1985), and saccade-related areas in the frontal lobe (FEF, SEF, dlPFC) (Cavada and Goldman-Rakic, 1989). The FEF and SEF also send direct projections to the intermediate layers of the SC (Stanton et al., 1988a; Shook et al., 1990), and saccades are evoked by microstimulation of the FEF, SEF, and PPC (Bruce et al., 1985; Schlag and Schlag-Rey, 1987; Thier and Andersen, 1996). However, PPC microstimulation required much larger currents (up to 200 microAmps) than FEF or SEF microstimulation (less than 50 microAmps), which suggests that despite having direct corticotectal projections, the PPC does not play a direct role in saccade production. Likewise, the dlPFC sends direct projections to the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981), but microstimulation of the dlPFC up to 150 microAmps did not evoke saccades (Boch and Goldberg, 1989). Therefore it appears that while all these cortical saccade-related areas have corticotectal projections, the FEF and SEF play a more direct role in the generation of saccades. Another component of the cortical saccade control network, the ACC, does not appear to have

corticotectal projections, however could still influence saccades by its connections with the other cortical saccade-related areas (Selemon and Goldman-Rakic, 1988; Huerta and Kaas, 1990; Bates and Goldman-Rakic, 1993; Wang et al., 2004), which themselves have corticotectal projections. It has been proposed that as part of this cortical saccade control network, the SEF regulates volitional saccades, the FEF triggers volitional saccades, the dlPFC inhibits unwanted reflexive saccades, the PPC manipulates visual information for the purposes of generating a saccade, and the ACC prepares these areas for their respective roles in the production of volitional saccades (Pierrot-Deseilligny et al., 2004; Nyffeler et al., 2007a).

1.2.15 Posterior Parietal Cortex

The saccade-related area of the PPC has been identified in the lateral bank of the intraparietal sulcus in monkeys, and the medial bank of the posterior intraparietal sulcus in humans (Grefkes and Fink, 2005). As part of the dorsal visual pathway, extrastriate area V5 sends visual signals to the PPC, which has strong connections with the FEF and SC (Lynch et al., 1985), and thus the PPC can be considered an important interface between the visual and oculomotor systems. Goldberg and colleagues (2002) have hypothesised that the PPC transforms a visual signal into a covert shift of attention by encoding visuospatial attention on a saliency map, which the oculomotor system interprets as a guidance signal toward which to direct an overt shift of gaze. An increase of saccade latency with lesions or transcranial magnetic stimulation (TMS) of the PPC in humans (Pierrot-Deseilligny et al., 1991a; Elkington et al., 1992), and PPC deactivation in monkeys (Li et al., 1999), has suggested that the PPC is directly involved in the production of visually-guided saccades. On the other hand, PPC deactivation was shown to affect the latency of target selection rather than saccade generation (Wardak et al., 2002), which suggests that the PPC facilitates visually-guided saccades by covert rather than direct processes.

1.2.16 Frontal Eye Field

The FEF is located at the junction of the superior frontal sulcus and precentral sulcus in humans (Paus, 1996), and in the anterior bank of the arcuate sulcus in monkeys (Bruce

and Goldberg, 1985; Bruce et al., 1985). There are generally four types of FEF cells: fixation cells that discharge when a stimulus appears in their parafoveal RF, visual cells that discharge when a stimulus appears in their peripheral RF, movement cells that discharge for a saccade into their peripheral RF, and visuomovement cells that discharge both when a stimulus appears in, and for a saccade into, their peripheral RF (Bruce and Goldberg, 1985; Segraves and Goldberg, 1987; Sommer and Wurtz, 2000). It has been proposed that visual cells mediate covert shifts of attention, while movement cells mediate overt gaze shifts, such that visuospatial processing is differentiated at the level of FEF neurons (Thompson et al., 2005). FEF corticotectal projections have a topographic organization: the lateral FEF sends projections to the anterior end of the caudal SC, while the medial FEF sends projections to the posterior end of the caudal SC (Sommer and Wurtz, 2000). Consequently, lateral FEF microstimulation evokes small-amplitude saccades, while medial FEF microstimulation evokes large-amplitude saccades (Bruce et al., 1985). A causal role of the FEF in volitional, visually-guided saccades is supported by an increased latency of visually-guided saccades in patients with FEF lesions, and with FEF deactivation in monkeys (Rivaud et al., 1994; Dias et al., 1995; Sommer and Tehovnik, 1997).

1.2.17 Supplementary Eye Field

The SEF is located on the dorsomedial surface of the frontal lobe, anterior to the supplementary motor area (SMA) and vertical plane of the anterior commissure (VAC), and posterior to the pre-SMA. This can be found at the upper part of the paracentral sulcus in humans (Grosbras et al., 1999), and medial to the superior limb of the arcuate sulcus in monkeys (Schlag and Schlag-Rey, 1985, 1987). There are many similarities between the SEF and FEF: they both receive visuospatial signals from the PPC (Cavada and Goldman-Rakic, 1989), have the same four types of neurons (Schlag and Schlag-Rey, 1987; Sommer and Wurtz, 2000), send direct projections to the SC and omnipause region of the brainstem saccade generator (Shook et al., 1988; Stanton et al., 1988a; Shook et al., 1990; Segraves, 1992), and evoke saccades when stimulated with low current (less than 50 microAmps) (Bruce et al., 1985; Schlag and Schlag-Rey, 1987). Differences, on the other hand, are that the FEF encodes retinocentric locations (i.e.

relative to the eyes), while the SEF encodes craniocentric locations (i.e. relative to the head) (Tehovnik et al., 2000), although Russo and Bruce (1993, 1996) contend that the SEF, like the FEF, encodes retinocentric locations as well. It has also been shown that SEF deactivation has much less of an effect on visually-guided saccades than FEF deactivation (Sommer and Tehovnik, 1997, 1999; Tehovnik et al., 2000). Therefore it has been proposed that rather than play a role in saccade initiation, the SEF facilitates the learning of new tasks (Tehovnik et al., 2000), and regulates complex intentional saccades (Pierrot-Deseilligny et al., 2004) such as sequential saccades (Gaymard et al., 1990; Gaymard et al., 1993; Sommer and Tehovnik, 1999; Isoda and Tanji, 2002).

1.2.18 Anterior Cingulate Cortex

The ACC is a supracallosal, dorsomedial prefrontal area that extends from the genu of the corpus callosum to between the vertical planes of the anterior and posterior commissures (Paus, 2001). In monkeys this area is restricted to the banks of the cingulate sulcus, while in humans this area extends into the paracingulate gyrus (Cole et al., 2009). Investigations of the ACC with regards to visually-guided saccades have been restricted to early neuroimaging studies (Paus et al., 1993; Petit et al., 1993), and thus not much is known about the saccade-related properties of the ACC. Instead, it has been proposed that the ACC regulates saccade generation by monitoring task performance, given that the ACC is interconnected with cortical saccade-related areas (Selemon and Goldman-Rakic, 1988; Huerta and Kaas, 1990; Bates and Goldman-Rakic, 1993; Wang et al., 2004), and receives a dopaminergic training signal from the midbrain that is elicited by errors (Holroyd and Coles, 2002). Time-locked to the onset of an incorrect response is the generation of a negative-polarity event-related potential (i.e. an error-related negativity) that has been attributed to the ACC (Holroyd and Coles, 2002). In addition to errors, ACC neurons also signal reward (Niki and Watanabe, 1979; Ito et al., 2003). Alternatively, the ACC has been proposed to monitor task conditions for conflict (Carter and van Veen, 2007) and error-likelihood (Brown and Braver, 2005). According to the conflict-monitoring hypothesis, the ACC detects conflict while the dlPFC resolves conflict by implementing control, such that the ACC signals the dlPFC to increase top-down control in support of the desired response (Ridderinkhof et

al., 2004; Carter and van Veen, 2007). In support of this, a human neuroimaging study found there was increased dlPFC activation and enhanced performance on trials following conflict-related ACC activation (Kerns et al., 2004), however this was also found following error-related ACC activation, and thus also supports the performance-monitoring hypothesis. On the other hand, encoding of error-likelihood (Brown and Braver, 2005) and implementation of control (Johnston et al., 2007) by the ACC supports a regulatory hypothesis by which the ACC plays a more direct role in conflict resolution by sending top-down bias signals to other brain areas. Finally, the ACC has also been proposed to facilitate reward-guided behaviour, as demonstrated by ACC lesions which impaired both corrective behaviour following errors, and sustained performance of rewarded behaviours (Rushworth et al., 2003; Kennerley et al., 2006; Buckley et al., 2009). It appears, therefore, that the ACC plays a role in monitoring for conflict, errors, and reward, the detection of which enhances preparation of cortical saccade-related areas for the generation of an intentional saccade.

1.2.19 Dorsolateral Prefrontal Cortex

In humans, the dlPFC is located anterior to the precentral sulcus and dorsal to the inferior frontal sulcus, consisting of both the superior frontal gyrus and middle frontal gyrus. In monkeys, the dlPFC consists of both the superior convexity, which corresponds with the superior frontal gyrus, and the cortical tissue lining the principal sulcus, which corresponds with the middle frontal gyrus (Petrides and Pandya, 1999). The dlPFC is an association area which integrates inputs from a variety of sources, including multimodal sensory input from auditory, visual, and somatosensory areas (Miller and Cohen, 2001). The dlPFC has neurons with stimulus-related and saccade-related activity (Boch and Goldberg, 1989; Funahashi et al., 1990, 1991; Funahashi et al., 1993b), and direct corticotectal projections to the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981), however microstimulation of the dlPFC at currents of up to 150 microAmps did not evoke a saccade (Boch and Goldberg, 1989). While this may suggest that the dlPFC does not play a direct role in saccade generation, alternatively Tehovnik and colleagues (1999) demonstrated that the current required to evoke saccades with microstimulation was greater when stimulation was applied during

fixation than when not fixating, and dlPFC microstimulation was applied while the animal was fixating. However Boch and Goldberg (1989) were able to evoke saccades with FEF microstimulation at currents less than 10 microAmps, and thus the state of fixation does not appear to explain why dlPFC microstimulation at currents up to 150 microAmps was unable to evoke a saccade. The dlPFC is also interconnected with cortical saccade-related areas (ACC, FEF, SEF) that are involved in the triggering of intentional saccades (Selemon and Goldman-Rakic, 1988; Bates and Goldman-Rakic, 1993), and sends projections to the basal ganglia (Selemon and Goldman-Rakic, 1985) which facilitates the triggering of intentional saccades by a direct pathway, and the inhibition of reflexive saccades by indirect and hyperdirect pathways (Hikosaka et al., 2000).

1.2.20 Basal Ganglia

The basal ganglia is a subcortical structure that consists of many interconnected nuclei, including the striatum, globus pallidus, subthalamic nucleus (STN), and substantia nigra (Hikosaka et al., 2000). The striatum contains the main input nuclei of the basal ganglia, while the substantia nigra pars reticulata (SNpr) is an output nucleus of the basal ganglia that suppresses SC saccade neurons with tonic inhibition signals. Cortical input to the basal ganglia (Selemon and Goldman-Rakic, 1985; Stanton et al., 1988b) influences the SNpr by direct, indirect, and hyperdirect pathways. The direct pathway consists of an inhibitory projection from the caudate to the SNpr, which disinhibits the SC and thus allows saccade neurons to send a saccade command to the brainstem saccade generator. The indirect pathway, on the other hand, consists of inhibitory projections from both the caudate to the external segment of the globus pallidus (GPe), and the GPe to the STN. There is also a hyperdirect pathway by which the cortex sends an excitatory projection directly to the STN. The STN then sends an excitatory projection to the SNpr, and thus both the indirect and hyperdirect pathways enhance saccade inhibition. The direct pathway, on the other hand, facilitates saccade initiation. Therefore the basal ganglia, like the dlPFC, may facilitate both the inhibition of reflexive saccades, and the triggering of intentional saccades.

1.3 – Cognitive Control

While the aforementioned cortical and subcortical saccade-related areas are proposed to play a role in the generation of visually-guided saccades, human neuroimaging studies have shown that these areas are involved to an even greater extent in the generation of more cognitively-demanding saccades, such as memory-guided saccades and anti-saccades (O'Driscoll et al., 1995; O'Sullivan et al., 1995; Sweeney et al., 1996; Brown et al., 2004; Brown et al., 2007).

1.3.1 Cognitively-Demanding Saccades

Visually-guided saccades are directed toward an externally-generated (visible) target, whereas memory-guided saccades and anti-saccades are directed toward an internally-generated (invisible) target; memory-guided saccades to the location where a stimulus had been presented prior to a delay (Funahashi et al., 1989), anti-saccades away from a suddenly-appearing stimulus and toward the mirror position in the opposite visual field (Hallett, 1978). Memory-guided saccades also require that the stimulus location be held in working memory, while anti-saccades require suppression of a prepotent saccade toward the stimulus, and thus both memory-guided saccades and anti-saccades have greater cognitive demands than visually-guided saccades. In support of this, positron emission tomography (PET) studies have shown greater regional cerebral blood flow (rCBF), and functional magnetic resonance imaging (fMRI) studies have found higher blood oxygenation level dependent (BOLD) signals, for memory-guided saccades than visually-guided saccades (Anderson et al., 1994; O'Sullivan et al., 1995; Sweeney et al., 1996; Brown et al., 2004), and for anti-saccades than pro-saccades (O'Driscoll et al., 1995; Sweeney et al., 1996; Doricchi et al., 1997; DeSouza et al., 2003; Matsuda et al., 2004; Ford et al., 2005; Brown et al., 2006; Brown et al., 2007), at the FEF, SEF, PPC, dlPFC, ACC, thalamus, and striatum. A higher BOLD signal for anti-saccades than pro-saccades has also been found at most of these saccade-related areas in non-human primates (Ford et al., 2009). This demonstrates that there is greater activation of the saccade control network for the more cognitively-demanding tasks, which require high-level cognitive processing to guide behaviour in the appropriate manner. Consequently, the reaction times of memory-guided saccades and anti-saccades are greater than for

visually-guided saccades (Funahashi et al., 1989; Amador et al., 1998; Bell et al., 2000; Dafoe et al., 2007).

1.3.2 Cognitive Control of Behaviour

Cognitive control plays an important role both when performing saccade tasks in the laboratory, and in our daily lives as well. For example, most of us have an established routine, such that we tend to do the same things every day of the week: we wake up early in the morning, drive the kids to school, go to work, etc. Weekends, however, are different: we wake up later in the morning, do chores around the house, run errands, etc. Our behaviour during the week, being different than on the weekend, is thus determined by context (i.e. which day it is). Cognitive control plays a role in the detection of these contextual cues, without which we could find ourselves driving the kids to school on the weekend, or waking up later in the morning on a weekday. Further context-dependence, and thus greater cognitive control, is required when a weekday is a holiday, such that we must be able to swap our usual weekday routine with one that is more similar to the weekend routine. In addition to contextual cues, cognitive control also detects mistakes, such that by remembering the context in which a mistake was made, we are less likely to make the same mistake again. Cognitive control plays a role in all aspects of context-dependent memory, including the preparation of information for storage in long-term memory, retrieval of information from long-term memory, then the maintenance, monitoring, and manipulation of information held in working memory. Also supported by cognitive control are the integration and organization of large amounts of information, which facilitate complex tasks such as coordinating our busy schedule with those of our colleagues, wife/husband, and children. In addition, cognitive control helps focus our attention on relevant information, which reduces the amount of information that we must process, and furthermore prevents distraction by information that is not currently relevant. Ultimately, cognitive control enables us to guide our behaviour in an appropriate, context-dependent manner.

1.3.3 Prefrontal Bias Signals

The prefrontal cortex mediates cognitive control by processing and encoding task-relevant information in a context-dependent manner (Miller, 2000). When the context changes, so does the context-dependent rule which differentiates between the information that should be attended to, and that which can be ignored (Miller and Cohen, 2001). The prefrontal cortex is highly versatile and adapts its coding to the rule (Duncan, 2001). For example, while prefrontal encoding may distinguish between different colours under one set of circumstances, such as identifying whether the traffic light is red, yellow, or green; under different circumstances the prefrontal cortex may encode the shape of an object instead, such as whether a screwdriver is Phillips, Robertson, or slotted. With an adaptive coding mechanism, the prefrontal cortex can encode any type of relevant information. This includes encoding the rule that determines which 'top-down' signals the prefrontal cortex sends to other brain areas, to influence the processing of sensory and motor information. Processing only relevant information is important because the processing capacity of the brain is inherently limited, which creates competition for neural processing. A biased competition model for the visual system was proposed in which selective visual attention enhances processing of the relevant stimulus feature (e.g. colour or shape) (Desimone and Duncan, 1995). The visual areas then send this 'bottom-up' signal to motor areas in support of a stimulus-driven response. The motor areas also receive a top-down signal from the prefrontal cortex in support of a context-driven response, which either enhances or suppresses the bottom-up signal, depending on the rule. Stimulus-dependent rules associate a stimulus with a response, such that if you have a Phillips screw, then you must use a Phillips screwdriver, and thus the response is determined by the bottom-up signal. Context-dependent rules, on the other hand, can associate multiple responses with the same stimulus, such that while it is illegal to drive through an intersection when the traffic light is red, an exception is made if you are driving an emergency vehicle. The prefrontal cortex is proposed to implement cognitive control by sending top-down bias signals to guide the flow of activity along the appropriate neural pathways, and thus dictate the outcome of sensorimotor processing (Miller and Cohen,

2001). This top-down control plays an even more important role when a weak but task-relevant behaviour is in competition with a stronger, more habitual response.

1.3.4 Working Memory

Working memory temporarily maintains and manipulates information that is currently relevant to our behaviour. A model of working memory (Baddeley, 2003) has been proposed in which auditory and visual information are held in the phonological loop and visuospatial sketchpad, respectively, while related bits of information are compiled in the episodic buffer. This information can then be used by the central executive to control behaviour. Working memory is typically probed by delayed alternation and delayed response tasks in which a delay separates the presentation of a stimulus from the performance of a response. Task-related information must be maintained "on-line" for the duration of the delay. In the delayed alternation task, an object at one of two locations must be selected, the correct choice being the location that was not selected on the preceding trial. In the manual delayed response task, an object at one of multiple locations must be selected, the correct choice being the location at which the object had been presented prior to the delay. The memory-guided saccade task is an oculomotor version of the delayed response task in which the location is selected with an eye movement rather than a manual response (Funahashi et al., 1989).

1.3.5 Prefrontal Contributions to Working Memory

In humans, evidence for a role of the dlPFC in working memory has been provided by patients with dlPFC lesions that are impaired on the memory-guided saccade task, as demonstrated by increased latency and reduced accuracy (Pierrot-Deseilligny et al., 1991b; Ploner et al., 1999), and neuroimaging studies which have shown an increased rCBF or BOLD signal at the dlPFC on tasks that probe working memory (Owen et al., 1996; Cohen et al., 1997; Courtney et al., 1997; Courtney et al., 1998). In monkeys, the principal sulcus region of the dlPFC has been implicated in working memory by ablation and deactivation studies that impaired performance on delayed response tasks, which require selection of the location that was indicated before a delay, and delayed alternation tasks, which require selection of the location that was not chosen on the

previous trial (Butters and Pandya, 1969; Fuster and Alexander, 1970; Goldman and Rosvold, 1970; Bauer and Fuster, 1976; Passingham, 1985; Funahashi et al., 1993a). On delayed response and delayed alternation tasks, the sustained delay activity of neurons in the principal sulcus region is a proposed neural correlate of the task-related information held in working memory (Fuster and Alexander, 1971; Kubota and Niki, 1971; Fuster, 1973; Funahashi et al., 1989; Funahashi et al., 1993b; Goldman-Rakic, 1995; Rao et al., 1997). In addition to encoding the stimulus and/or response, neurons in the principal sulcus region also encode the task rules which guide stimulus-response associations. Rule-selective activity has been found when comparing a) shape-match and location-match rules (Hoshi et al., 1998), b) spatial and conditional rules (White and Wise, 1999), c) object, spatial, and association rules (Asaad et al., 2000), d) match and nonmatch rules (Wallis et al., 2001), e) pro-saccade and anti-saccade rules (Everling and DeSouza, 2005), f) shape-match and colour-match rules (Mansouri et al., 2006).

1.3.6 Working Memory for Rules

To test the hypothesis that the principal sulcus region maintains rules in working memory, monkeys with lesions of the principal sulcus region performed an analogue of the Wisconsin Card Sorting Test (WCST) (Buckley et al., 2009). The WCST is a rule-matching task that requires sorting cards based on either the colour, shape, or quantity of objects on the cards. The correct matching rule is not indicated, but rather must be determined based on feedback from the experimenter, and is switched by the experimenter without notice. While the WCST is typically used with patients that have neurological or psychiatric disorders, an analogue of the WCST was created that can be used with monkeys instead (e.g. Mansouri et al., 2006). This WCST analogue requires selecting a choice object that matches either the colour or shape of the sample object, depending on which is the correct matching rule. Buckley and colleagues (2009) found that performance of the WCST analogue was impaired by lesions of the principal sulcus region, as demonstrated by responses that were selected based on an incorrect rule. These errors were attributed to impaired working memory for the rule, given that the impairment was greater when the rule had to be maintained for a longer period of time

between trials. They also found that lesions of the anterior cingulate sulcus region impaired performance of the WCST analogue, which was demonstrated by an impaired ability to maintain an extended sequence of correct responses within a block of trials. They concluded that the anterior cingulate sulcus region plays a critical role in mediating reinforcement-guided behaviour, such as determining the extent to which recent outcomes should influence future decisions.

1.3.7 The Anti-Saccade Task

The anti-saccade task is a well-established test of cognitive control that requires subjects to look-away from a suddenly-appearing stimulus (Hallett, 1978). This requires the inhibition of a prepotent pro-saccade toward the stimulus, inversion of the saccade vector from toward the stimulus to away from the stimulus, and generation of a voluntary anti-saccade away from the stimulus and toward the mirror position in the opposite visual field (Munoz and Everling, 2004). The latency of anti-saccades is greater than pro-saccades for both humans (Fischer and Weber, 1992; Evdokimidis et al., 1996; Dafoe et al., 2007) and monkeys (Amador et al., 1998; Everling et al., 1999; Bell et al., 2000; Everling and Munoz, 2000), which can be explained by the additional task requirements. Anti-saccades also have a slower peak velocity than pro-saccades (Fischer and Weber, 1992; Amador et al., 1998; Bell et al., 2000). On an anti/pro-saccade task, the task instruction can be provided by the colour or shape of the central fixation point. In the overlap condition, the central fixation point remains visible for the entire duration of the trial, whereas in the gap condition, the central fixation point is removed 200 ms prior to stimulus appearance. Both pro-saccade and anti-saccade latencies are shorter in the gap condition than the overlap condition (Fischer and Weber, 1997; Everling et al., 1999; Bell et al., 2000; Everling and Munoz, 2000), a phenomenon known as the “gap effect” (Saslow, 1967). An error occurs on the anti-saccade task when a saccade is directed toward the stimulus rather than away from it. The anti-saccade error rate in humans is typically found to be around 20% (Hutton and Ettinger, 2006), for example 23 +/- 17% from a large population of healthy young males (Evdokimidis et al., 2002), however this varies considerably across studies, with mean anti-saccade errors ranging from 0 to 30% (Everling and Fischer, 1998). Some of this

variation may be attributed to the task condition, given that there is a greater incidence of anti-saccade errors in the gap condition than the overlap condition (Fischer and Weber, 1997; Everling et al., 1999; Bell et al., 2000). The gap condition also elicits pro-saccades with latencies that approach the minimum conduction time in the oculomotor system (Carpenter, 1981). The latency of these express saccades is typically 70-100 ms for monkeys (Fischer and Boch, 1983), and 100-130 ms for humans (Fischer and Ramsperger, 1984), as compared to 180-220 ms for regular-latency pro-saccades in the overlap condition. There is a greater incidence of express saccades with prior training at a particular stimulus location, and increased predictability of the stimulus location (Fischer et al., 1984; Boch and Fischer, 1986). This suggests that express saccades are spatially-selective and thus can occur when there is advanced motor preparation at a specific location on the retinotopic SC saccade map (Pare and Munoz, 1996). Advanced motor preparation, however, does not affect saccade velocity, which is the same for both express-latency and regular-latency saccades (Edelman and Keller, 1996). Anti-saccade errors have been reported with latencies in the range of express saccades (Fischer and Weber, 1997; Everling et al., 1999), which suggests that anti-saccade errors may occur by the same mechanism as express saccades (see *Section 1.2.13*). In support of this, SC saccade neurons have a higher level of preparatory activity for express-latency than regular-latency saccades (Dorris et al., 1997), and for anti-saccade errors than correct anti-saccades (Everling et al., 1998a). This suggests that when a stimulus appears in the cell's response field, a stimulus-driven burst of visual activity combines with the increased level of preparatory activity to reach saccade threshold and discharge in support of a saccade toward the stimulus, which is an error on the anti-saccade task. A reduced level of preparatory activity on correct anti-saccade trials would prevent the stimulus-driven burst from reaching saccade threshold, which would allow a saccade to be directed away from the stimulus instead. It was proposed, therefore, that anti-saccade errors occur as the result of insufficient suppression of the oculomotor system, such that a prepotent saccade toward the stimulus is triggered before a voluntary saccade away from the stimulus (Munoz and Everling, 2004).

1.3.8 Saccade Control Network for Anti-Saccades

Anti-saccade task performance is dependent upon a widely-distributed network of oculomotor structures to a) inhibit a saccade toward the stimulus, which requires suppression of saccade-generating structures in the instruction period prior to stimulus onset, and b) generate an anti-saccade away from the stimulus, in the response period that follows stimulus onset (Munoz and Everling, 2004). Human neuroimaging studies have shown a greater instruction-related BOLD signal at the dlPFC and ACC for anti-saccades than pro-saccades, and for correct anti-saccades than error anti-saccades, which suggests that they play a preparatory role related to the task instruction (DeSouza et al., 2003; Ford et al., 2005; Brown et al., 2007). The PPC, FEF, and SEF, on the other hand, had a greater response-related BOLD signal for anti-saccades than pro-saccades, and for correct anti-saccades than error anti-saccades, which suggests that they play a more direct role in generating the response (Curtis and D'Esposito, 2003; Brown et al., 2007).

1.3.9 Posterior Parietal Cortex

Proposed to play a covert rather than direct role in anti-saccade task performance (Pare and Dorris, 2011), the PPC had a higher response-related BOLD signal, and PPC neurons had greater stimulus-related activity, for anti-saccades than pro-saccades, and correct anti-saccades than anti-saccade errors (Gottlieb and Goldberg, 1999; Curtis and D'Esposito, 2003; Brown et al., 2007). Furthermore, ipsilesional anti-saccade accuracy was impaired for a human patient with a PPC lesion, while visual and motor processing were otherwise normal, which suggests that the PPC plays a critical role in the vector inversion process for anti-saccades (Nyffeler et al., 2007a). In support of this, it has been shown that there is a shift of activity from the PPC contralateral to the stimulus, to the PPC contralateral to the saccade, on anti-saccade trials (Everling et al., 1998b; Medendorp et al., 2005; Moon et al., 2007). A switch from stimulus encoding to response encoding has also been found in PPC visual neurons, wherein a visual response in the PPC contralateral to the stimulus was followed 50 ms later by a "paradoxical" visual response in the PPC contralateral to the saccade, which they proposed triggers the sensorimotor transformation for anti-saccades (Zhang and Barash,

2000). The PPC, therefore, appears to play a role in the inversion of a saccade vector from toward the stimulus to away from the stimulus on anti-saccade trials.

1.3.10 Frontal Eye Field

Anti-saccade latency increased both for human patients with FEF lesions (Rivaud et al., 1994; Gaymard et al., 1999), and healthy humans when TMS was applied to the FEF (Muri et al., 1991; Olk et al., 2006), which suggests that the FEF plays a role in the triggering of an intentional saccade away from the stimulus (Pierrot-Deseilligny et al., 2004). The FEF sends direct projections to the SC (Stanton et al., 1988a), and saccade neurons in both the FEF and SC have lower preparatory, stimulus-related, and saccade-related activity on anti-saccade trials than pro-saccade trials (Everling et al., 1999; Everling and Munoz, 2000), which demonstrates greater suppression of the oculomotor system on anti-saccade trials. Human neuroimaging studies, on the other hand, have consistently shown a higher rCBF or BOLD signal at the FEF for anti-saccades than pro-saccades (O'Driscoll et al., 1995; Sweeney et al., 1996; Connolly et al., 2002; Curtis and D'Esposito, 2003; DeSouza et al., 2003; Ford et al., 2005; Brown et al., 2007). These contradictory findings could be explained by either the difference in species (humans vs. monkeys) or recording technique (single neuron recordings vs. fMRI). Single neuron recordings appear to be biased toward large pyramidal neurons and thus the output of an area, while the BOLD signal is proposed to reflect both dendritic synaptic processes and the activity of interneurons, and thus the input to and local processing at an area (Logothetis et al., 2001). To address this discrepency between monkey electrophysiology and human neuroimaging studies, a monkey neuroimaging study found a higher BOLD signal at the FEF for anti-saccades than pro-saccades, which suggests the discrepancy was the result of different recording techniques (Ford et al., 2009). This greater input to the FEF on anti-saccade than pro-saccade trials could be attributed to stronger suppression of the oculomotor system when a saccade toward the stimulus must be inhibited.

1.3.11 Supplementary Eye Field

There is evidence to suggest that on anti-saccade trials, the SEF may facilitate either the inhibition of a saccade toward the stimulus, the generation of a saccade away from the stimulus, or both. With regards to saccade generation, both the response-related BOLD signal at the SEF and the saccade-related activity of SEF movement neurons was greater for anti-saccades than pro-saccades, and correct anti-saccades than anti-saccade errors (Schlag-Rey et al., 1997; Curtis and D'Esposito, 2003; Amador et al., 2004; Brown et al., 2007). SEF movement neurons were task-selective starting 40 ms before saccade onset (Amador et al., 2004), and thus early enough to influence the generation of an anti-saccade. The SEF sends direct projections to the FEF and SC (Huerta and Kaas, 1990; Shook et al., 1990), which suggests that the SEF could facilitate the generation of an anti-saccade. However, the SEF also sends direct projections to the omnipause region of the brainstem saccade generator (Shook et al., 1988), which suggests that the SEF may play a role in saccade inhibition instead. In support of this, human EEG found a negative potential over dorsomedial frontal cortex at around the time of stimulus onset, that was greater for anti-saccades than pro-saccades (Everling et al., 1997), and correct anti-saccades than anti-saccade errors (Everling et al., 1998b). Also greater for anti-saccades than pro-saccades was the stimulus-related activity of SEF visual neurons (Schlag-Rey et al., 1997), and the tonic discharge of SEF fixation neurons (Amador et al., 2004). Anti-saccade errors, however, were not greater in human patients with SEF lesions (Gaymard et al., 1990; Pierrot-Deseilligny et al., 1991a), and thus the role of the SEF in anti-saccade task performance remains unclear.

1.3.12 Basal Ganglia and Thalamus

Human neuroimaging and monkey electrophysiology studies support a role of the basal ganglia and thalamus in anti-saccade task performance: greater rCBF or BOLD signals have been found at the striatum, globus pallidus, and thalamus (O'Driscoll et al., 1995; Matsuda et al., 2004; Ettinger et al., 2008), and greater single neuron activity in the caudate (Ford and Everling, 2009; Watanabe and Munoz, 2009), globus pallidus (Yoshida and Tanaka, 2009), and thalamus (Kunimatsu and Tanaka, 2010), for anti-saccades than pro-saccades. Studies of patients with neurological or psychiatric

disorders, however, have provided mixed results. Patients with Huntington's disease, which affects the caudate and SNpr, have increased anti-saccade latency and errors (Lasker et al., 1987; Peltsch et al., 2008), while patients with Parkinson's disease, which affects the substantia nigra pars compacta (SNpc), were found to be impaired in some studies (Briand et al., 1999; Chan et al., 2005), but not others (Lueck et al., 1990; Fukushima et al., 1994; Vidailhet et al., 1994). Furthermore, anti-saccade task performance was unaffected in patients with striatonigral degeneration (Vidailhet et al., 1994), and lesions of the lentiform nucleus (GP and putamen) (Vermersch et al., 1996), striatum (Condy et al., 2004), or thalamus (Condy et al., 2004). On the other hand, deactivation of the GPe (basal ganglia) or VA/VL nuclei (thalamus) was found to increase anti-saccade errors made by monkeys on a randomly-interleaved anti/pro-saccade task (Yoshida and Tanaka, 2009; Kunimatsu and Tanaka, 2010). Patients with Parkinson's disease or corticobasal degeneration also had increased anti-saccade errors when pro-saccade and anti-saccade trials were randomly-interleaved, however pro-saccade errors increased as well, and thus these effects were attributed to "mixing costs", rather than impaired saccade inhibition (Rivaud-Pechoux et al., 2007). Anti-saccade task performance, therefore, is not always impaired in patients with neurological or psychiatric disorders that affect the basal ganglia and thalamus, despite the results of neuroimaging, electrophysiology, and deactivation studies that support a role of these subcortical structures in saccade inhibition.

1.3.13 Dorsolateral Prefrontal Cortex and Anterior Cingulate Cortex

Also implicated in saccade inhibition are two highly-interconnected prefrontal areas: the dlPFC and ACC (Bates and Goldman-Rakic, 1993; Morecraft and Van Hoesen, 1993; Paus et al., 2001). One of the earliest clinical applications of the anti-saccade task found increased anti-saccade errors in patients with frontal lobe lesions (Guitton et al., 1985). Patients with schizophrenia, which is thought to be caused by frontal lobe dysfunction, were also found to have increased anti-saccade errors (Fukushima et al., 1988; Fukushima et al., 1990; Rosse et al., 1993; Clementz et al., 1994; Fukushima et al., 1994; Sereno and Holzman, 1995). While the FEF was initially proposed to be the area of the frontal lobe that was responsible for saccade inhibition (Guitton et al., 1985), it

was later found that lesions restricted to the FEF did not increase anti-saccade errors (Pierrot-Deseilligny et al., 1991a; Rivaud et al., 1994; Gaymard et al., 1999). Instead, anti-saccade errors increased with lesions of dorsolateral (dlPFC) and medial (ACC) prefrontal structures (Pierrot-Deseilligny et al., 1991a; Gaymard et al., 1998; Milea et al., 2003; Pierrot-Deseilligny et al., 2003; Ploner et al., 2005). In support of a role of the dlPFC and ACC in anti-saccade task performance, monkey electrophysiology studies have identified task-selective dlPFC and ACC neurons with higher levels of prestimulus activity for anti-saccades than pro-saccades (Everling and DeSouza, 2005; Johnston and Everling, 2006; Johnston et al., 2007). Furthermore, human neuroimaging studies have shown that in the preparatory period prior to stimulus onset, there is a larger BOLD signal at the dlPFC and ACC for anti-saccades than pro-saccades (Ford et al., 2005; Brown et al., 2007), and for correct anti-saccades than anti-saccade errors (Ford et al., 2005). On the other hand, monkey electrophysiology studies have identified SC saccade neurons which have less prestimulus activity for correct anti-saccades than anti-saccade errors (Everling et al., 1998a). Together these findings suggest that the dlPFC and ACC must be engaged, and SC saccade neurons suppressed, on anti-saccade trials. From this an inhibitory model of prefrontal function was proposed by which the prefrontal cortex suppresses the activity of SC saccade neurons, to inhibit an unwanted saccade toward the stimulus (Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009).

1.3.14 Main Objective and Specific Aims

In summary, human neuroimaging studies have identified a correlation between the prefrontal BOLD signal and anti-saccade task performance, while monkey electrophysiology studies have identified a correlation between the activity of SC saccade neurons and anti-saccade task performance. Stimulation and deactivation studies, on the other hand, are able to establish a causal relationship between a brain area and behaviour. Electrical microstimulation of the dlPFC or ACC (Wegener et al., 2008; Phillips et al., 2011), and both transcranial magnetic stimulation and reversible muscimol deactivation of the dlPFC (Condy et al., 2007; Nyffeler et al., 2007b), have

previously identified a causal role of the these prefrontal areas in anti-saccade task performance. A causal relationship between the prefrontal cortex and the activity of SC saccade neurons, however, has yet to be identified. Therefore the **Main Objective** of my dissertation was to investigate the neural mechanism by which the prefrontal cortex facilitates anti-saccade task performance, by deactivating the dorsolateral (dlPFC) and medial (ACC) prefrontal cortex with the cryoloop method of reversible cryogenic deactivation (Lomber et al., 1999).

The **first Specific Aim** was to assess the roles of the dlPFC and ACC in saccade control, by directly comparing the behavioural effects of unilateral dlPFC and ACC deactivation on pro-saccades and anti-saccades. Unilateral dlPFC deactivation impaired contralateral saccades, both pro-saccades and anti-saccades, which implies that the dlPFC has an excitatory influence on the oculomotor system, and thus does not agree with the proposed inhibitory model of prefrontal function. The **second Specific Aim** was to address this discrepancy by performing a direct test of the inhibitory model. For this we deactivated the dlPFC unilaterally, and recorded the activity of SC saccade neurons, while the monkey performed the same anti/pro-saccade task. Unilateral dlPFC deactivation caused a neural imbalance at the SC, such that there was a decrease of saccade neuron activity at the SC ipsilateral to deactivation, and an increase of saccade neuron activity at the SC contralateral to deactivation. This suggests that the dlPFC has an excitatory influence on the oculomotor system by enhancing the activity of ipsilateral SC saccade neurons. While unilateral dlPFC deactivation allowed me to identify the excitatory nature of this influence, the neural imbalance potentially confounds the effects that were related to impairments of cognitive control. Bilateral dlPFC deactivation, on the other hand, was designed to not cause a neural imbalance, and thus was used to identify the effects of dlPFC deactivation that were related to cognitive control impairments, which was the **third Specific Aim**. Bilateral dlPFC deactivation increased the stimulus-related activity, and decreased the saccade-related activity, of SC saccade neurons. Given an excitatory influence of the dlPFC on SC saccade neurons, this suggests that the dlPFC enhances the saccade-generating signal at the SC. Furthermore, an increase of anti-saccade errors was more substantial in a “rule memorized” condition, in which the task instruction was not available at the time of the

response. This suggests that the dlPFC plays an important role in rule maintenance. Therefore I propose that the dlPFC facilitates anti-saccade task performance by first maintaining the relevant rule in working memory, then implementing the rule by enhancing the saccade-generating signal at the SC.

1.4 – Methods

1.4.1 Lesions and Reversible Deactivations

While all methods of lesions and reversible deactivation can help to establish a causal relationship between a brain area and behaviour, there are many reasons why the cryoloop method of reversible cryogenic deactivation was the technique that we chose (Lomber, 1999). Experimental lesion methods include ablations, electrolysis, and excitotoxins. While experimental lesions are the most comparable to human lesions (i.e. "the natural un-natural condition"), there are also many limitations. These include damaging fibres of passage, interrupting blood flow to adjacent cortical areas, and allowing for recovery of function by other brain areas which compensates for the loss of the lesioned area, and thus negates the effects of the lesion.

Electrolytic and radiofrequency lesions are small and suitable for deep brain structures (Winn, 1991). Electrolytic lesions are made with direct current ion flow from an anode to a cathode. The size of the lesion is determined by the intensity and duration of current. Tissue is damaged by diffusion of metallic ions (anode) or formation of gas bubbles (cathode) at the electrode tip. Collateral damage is caused by stimulation of adjacent tissue by metallic ions, and scar tissue from hemorrhaged blood vessels or glial reaction to metallic ions. Radiofrequency lesions are made with a high-frequency alternating current that generates heat in the tissue. Temperature is raised slowly, maintained above 43 Celsius for 60 seconds, then stopped. Damaged blood vessels are cauterized, which reduces the risk of hemorrhage and scar tissue, however tissue will not be damaged when current is shunted through the vasculature, and there is no way of knowing this at the time of the procedure.

All neurons are sensitive to glutamate, however there is a high rate of glutamate metabolism and reuptake, and thus only minimal damage is caused by the presence of

excess glutamate. Excitotoxins are structurally and physiologically related to glutamate, but with greater excitatory effects (Coyle and Schwarcz, 1983; Winn, 1991). A subtoxic dose of excitotoxins will depolarize cells. Excitotoxin-induced neuronal degeneration has a rapid onset of effects: glucose metabolism and cell discharge is reduced within 30 minutes, proximal degeneration and gliosis within 72 hours, followed by axonal retraction, distal degeneration, and atrophy. Remote lesions may be caused by overstimulation of pathways or anoxic damage from seizures. Two types of excitotoxins are kainate and ibotenate. Kainate has an ionotropic (ligand-gated) receptor, whereas ibotenate uses a metabotropic (G-protein coupled) receptor, and thus they could potentially lesion different subsets of neurons. Kainate is highly potent and makes quick lesions (within 24 hours), however convulsions have an adverse effect on the animal's well-being and thus kainate is not feasible for behavioral studies, nor particularly ethical by today`s standards. The precise mechanism of kainate is unknown, however excitotoxicity is reduced by anaesthesia and anticonvulsants, and thus the excitatory and neurotoxic effects may be mediated by the same receptor, which is not affected by glutamate antagonists. Ibotenate, on the other hand, provides the benefits of kainate without convulsions, however cell death is slower (up to 1 week). Lesions are more discrete and homogenous with no distal degeneration. Ibotenate is unaffected by anaesthesia but induces sedation, and thus acts by a different mechanism than kainate.

Reversible chemical deactivation circumvents most of these experimental lesion issues, however gamma-aminobutyric acid (GABA) recovers from deactivation within minutes (i.e. too quickly), muscimol recovers from deactivation within hours (i.e. too slowly), and lidocaine deactivates fibres of passage (i.e. a potentially confounding effect). GABA has a short onset of deactivation and binds both GABA-A and GABA-B receptors (Hupe et al., 1999). Due to a high rate of metabolism and reuptake, GABA deactivation is highly localized and of short duration. Muscimol is a selective GABA agonist that binds to GABA-A receptors with greater affinity than GABA and is not recognized by GABA reuptake mechanisms, however GABA also binds to GABA-B receptors and thus twice as many receptors as muscimol (Segraves, 2002). Other types of GABA agonists either stimulate GABA release or potentiate the effect of GABA by inhibiting GABA metabolism or reuptake. Lidocaine is a sodium channel blocker with a

short onset and duration of deactivation that can be applied with great precision, however axons are also affected and thus the effects of lidocaine may not be restricted to the site of deactivation (Tehovnik and Sommer, 1997). This can be verified by applying a technique that affects only synaptic receptors, such as muscimol, which has a longer onset and duration of deactivation than lidocaine, but does not affect fibres of passage. Sommer and Tehovnik (1997) found that lidocaine had a high level of deactivation between 5 and 30 minutes after injection, a low level of deactivation up to an hour, with complete recovery by 2 hours. Muscimol, on the other hand, had a low level of deactivation starting an hour after deactivation and a high level of deactivation starting at an hour and a half and continuing for many hours. These differences may be attributed to the fact that lidocaine binds to sodium channels with low affinity, whereas muscimol binds to GABA receptors with high affinity.

The diffusion of reversible chemical deactivation, however, is uncontrolled, and can be highly variable within a small range. The spread of reversible cryogenic deactivation, on the other hand, can be controlled by adjusting the temperature of the cooling probe (Lomber, 1999). Cooling disrupts synaptic transmission by slowing synaptic mechanisms. This only affects axons at temperatures well below experimental conditions, permits controlled length of deactivation, and allows quick recovery from deactivation such that normal behaviour and cell activity can be observed both before and immediately following deactivation. A disadvantage of cooling, however, is that while the spread of cooling typically conforms to a symmetric slope, vascularization can cause asymmetric deviations. Cooling effects spread actively by small blood vessels can cause minor distortion of an otherwise symmetrical diffusion, while large blood vessels adjacent to the cooling probe can restrict diffusion such that deactivation extends further in the opposite direction. Another disadvantage is that cooling disrupts transmission at all synapses, whereas chemical deactivation methods can target specific receptors and cortical layers, and thus provide a more highly circumscribed and targeted deactivation than cooling. Cooling though can be adjusted to deactivate only the more superficial cortical layers, leaving the deeper cortical layers unaffected (Lomber et al., 1999). Furthermore, cooling does not cause any structural, metabolic, or functional damage and thus can be repeated an unlimited number of times, whereas cumulative damage

from either needle penetrations or the chemical itself can limit the number of injections made at a particular location with reversible chemical deactivation.

There is also a substantial difference in the size of the deactivated area: reversible chemical deactivation typically deactivates an area less than 5 mm^3, while reversible cryogenic deactivation can deactivate an area of up to 100 mm^3, depending on the size and intensity of the cooling device (Lomber, 1999). Orthodromic effects of deactivation can be identified by recording the activity of cells in an area that receives projections from the deactivated area (Sandell and Schiller, 1982). Matching response fields between the two areas is facilitated by a larger area of deactivation. While the size of the deactivated area can be increased with multiple chemical injections, cooling is still the more practical and thus preferred method for combined deactivation and recording studies. A disclaimer though is that despite the presence of direct connections between one area and another, the effects of deactivation could also be mediated by indirect pathways, depending on the connectivity of the areas investigated.

Cooling plates have previously been used for the purpose of combined deactivation and recording studies (Chafee and Goldman-Rakic, 2000). Cooling plates operate by the Peltier principle: heat is transferred from one side of the plate to the other when a direct current is run through it, such that one side of the plate is made cold by the removal of heat. A typical Peltier cooling device consists of a gold-plated copper cylinder inside a chamber, connected to a gold-plated copper plate positioned between two Peltier plates, with a copper heat sink on the outside side to remove heat from the Peltier plates. An advantage of cooling plates is that they require only simple electrical connections, and furthermore are applied to the surface of the dura in an acute manner, which does not require an invasive surgical procedure. On the other hand, the disadvantage of cooling plates is that they a) are applied to the dura which provides a layer of insulation and thus must impede cooling of the cortical tissue below, and b) do not conform to the shape of the targeted cortical area which results in the deactivation of an area that includes but is not limited to the targeted area.

Cryoloops, on the other hand, are custom-designed to match the size and shape of the targeted cortical area (Lomber et al., 1999), and can be implanted chronically inside a sulcus (Lomber and Payne, 1996). For these reasons, cryoloops were ideal for

the purposes of our study. Cryoloops are constructed from 23-gauge hypodermic stainless steel tubing, and implanted adjacent to cortical tissue which is deactivated by the effect of chilled methanol pumped through the cryoloop. This is accomplished by pumping methanol through teflon tubing which passes through a methanol ice bath that is reduced to subzero temperatures by the addition of dry ice. Chilled methanol pumped through a cryoloop is then returned to the same reservoir from whence it came. Cryoloop temperature is monitored with an attached thermocouple, controlled by adjusting the flow rate of the peristaltic pump, and maintained in the range of 1-3°C for approximately 10-15 minutes. Alternatively, cryotips are the best type of cooling probe for the purposes of highly localized deactivations in deep brain structures (Zhang et al., 1986; Campeau and Davis, 1990). The cooling probe, however, is thicker than the needle used for chemical injections, and thus causes greater damage to overlying structures. Therefore reversible chemical deactivation may be the preferred method for deactivating deep brain structures, unless the benefits of cooling are thought to outweigh the collateral cortical damage.

1.4.2 Cooling

The spontaneous firing and evoked responses of postsynaptic neurons is impaired by reversible cryogenic deactivation (cooling), which slows synaptic mechanisms by disrupting synaptic transmission (Jasper et al., 1970; Moseley et al., 1972). This could be attributed to temperature-sensitive membrane properties such as passive membrane permeability and active ion transport, which influence the resting membrane potential and thus spike generation (Adey, 1974; Schiff and Somjen, 1985; Volgushev et al., 2000a; Volgushev et al., 2000b). The efficacy of cooling has been demonstrated by cell recordings, thermal recordings, and radiolabeling of cortical tissue, which map the deactivated area adjacent to the cooling device (Lomber et al., 1999).

Cell recordings provide a direct measure of neural activity by insertion of an electrode into the cortical tissue, and have shown that cortical tissue and brainstem nuclei are deactivated below 20°C (Jasper et al., 1970; Benita and Conde, 1972; Lomber et al., 1994; Lomber and Payne, 1996). At this temperature, action potentials decrease in amplitude and increase in width (Jasper et al., 1970; Gahwiler et al., 1972; Moseley et

al., 1972; Ferster et al., 1996). There is also an increased latency of evoked potentials, and decreased frequency of spontaneous firing, followed by cessation of neuronal discharge somewhere between 10°C and 20°C. It has been proposed that cooling slows synaptic mechanisms by interfering with the opening of voltage-gated Ca^{2+} channels in the presynaptic axon terminal (Llinas, 1979). This delays the release of neurotransmitter into the synaptic cleft, and thus disrupts synaptic transmission. Cooling neural tissue above 0°C does not cause any structural, metabolic, or functional damage (Lomber et al., 1999; Yang et al., 2006), however cooling below -10°C in the brainstem, or 0°C in cortical tissue, has been shown to cause irreversible physiological damage by cryocoagulation or haemorrhage (Miyazaki et al., 1963; Benita and Conde, 1972). The temperature of the cryoloops was always maintained above 0°C for the duration of the cooling period, and furthermore the consistency of behavioral effects across all sessions suggests that cortical tissue adjacent to the cryoloops was not damaged by the effects of cooling.

Thermal recordings provide an accurate estimate of neural activity by insertion of a temperature probe into the cortical tissue, and have shown that the distance of the 20°C thermocline (i.e. the temperature at which cortical tissue is deactivated) from the cortical surface is dependent on the type of cooling device. The lateral thermocline for cryoloops is 1.0 – 2.0 mm (Carrasco and Lomber, 2009; Lomber et al., 2010), while thermocline depth is 1.5 – 2.5 mm for cryoloops and cryotips (Zhang et al., 1986; Campeau and Davis, 1990; Lomber et al., 1996a; Lomber et al., 1996b), and 5.0 mm for cooling plates (Chafee and Goldman-Rakic, 2000). It has previously been shown that cooling deactivates all cortical layers (Lomber and Payne, 1996; Payne et al., 1996; Lomber et al., 1999). In some instances the thermocline may extend through gray matter and into the white matter (Lomber et al., 1996b), however the deactivation temperature for fibre conduction is approximately 0°C (Benita and Conde, 1972; Campeau and Davis, 1990), and thus would not be affected by temperatures that approach the 20°C thermocline.

While cell and thermal recordings provide a reasonable estimate of the deactivated area, they are limited to providing a measure from only the recorded locations, and by the cumulative damage of tissue penetrations. The uptake of

radiolabeled 2-deoxyglucose (2DG), on the other hand, identifies the entire area that is deactivated, including sites that the deactivated area sends projections to, relative to their functional impact (Vanduffel et al., 1997). 2DG is a direct measure of metabolic activity: an area of the brain that is more metabolically active will consume more energy and thus take up more glucose, whereas deactivation of an area will decrease metabolic activity and thus glucose uptake (Payne and Lomber, 1999). Active cells require greater amounts of glucose, and thus 2DG provides an indirect but highly accurate measure of neuronal activity. On the downside, the use of 2DG is expensive, radioactive, and technically-demanding. The deactivated area identified by reduced 2DG uptake is similar to that of the 20°C thermocline, such that all layers of cortical tissue are deactivated within a lateral range of approximately 1.5 mm (Lomber et al., 1999). Prior studies, therefore, have demonstrated the efficacy of cooling. While we would have liked to provide this evidence ourselves, three of our animals succumbed to neural infections prior to completion of the experiments, while the fourth animal was considered vital to future experiments. Therefore based on prior studies, the estimated volume of cortical tissue deactivated in my study was 72 - 96 mm^3, given that the cryoloops were 4 x 6 mm in dimension and thus deactivated an area of 24 mm^2, on both sides of the cryoloop (48 mm^2), with a thermocline range of 1.5 - 2.0 mm.

1.5 – Bibliography

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Chapter 2

Prefrontal Contributions to Saccade Control Revealed by Reversible Deactivation of the Dorsolateral Prefrontal Cortex and Anterior Cingulate Cortex

2.1 – Introduction

The dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) are prefrontal components of a cortical saccade control network that includes the posterior parietal cortex (PPC), frontal eye field (FEF), and supplementary eye field (SEF) (Johnston and Everling, 2008; McDowell et al., 2008; Muri and Nyffeler, 2008). These cortical saccade-related areas are highly-interconnected (Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; Huerta and Kaas, 1990; Bates and Goldman-Rakic, 1993), and all but the ACC send direct projections to the superior colliculus (Goldman and Nauta, 1976; Leichnetz et al., 1981; Fries, 1984; Stanton et al., 1988; Shook et al., 1990), which is a critical midbrain structure for saccade initiation (Wurtz and Goldberg, 1972; Pierrot-Deseilligny et al., 1991b). Human neuroimaging studies have implicated these cortical saccade-related areas in the performance of cognitively-demanding saccades, such as anti-saccades and memory-guided saccades (Sweeney et al., 1996; Brown et al., 2004; Brown et al., 2007). Furthermore, lesions of the ACC, PPC, and FEF in human patients (Pierrot-Deseilligny et al., 1991a; Henik et al., 1994; Gaymard et al., 1998), and transcranial magnetic stimulation (TMS) of the dlPFC, FEF, and SEF in healthy subjects (Nagel et al., 2008), increased contralateral saccadic reaction times, which suggests that contralateral saccades are facilitated by the cortical saccade control network. In support of this, studies with monkeys have shown that a) dlPFC, PPC, and FEF neurons have predominantly contralateral response fields (Bruce and Goldberg, 1985; Segraves and Goldberg, 1987; Boch and Goldberg, 1989; Funahashi et al., 1989, 1990; Barash et al., 1991; Funahashi et al., 1991), b) microstimulation of the PPC, FEF, and SEF evokes contralateral saccades (Bruce et al., 1985; Schlag and Schlag-Rey, 1987; Thier and Andersen, 1996), and c) deactivation of

the dlPFC, PPC, and FEF impairs contralateral saccades (Dias et al., 1995; Sommer and Tehovnik, 1997; Li et al., 1999; Condy et al., 2007).

The saccade-related properties of the ACC, on the other hand, have not been extensively examined, and thus the purpose of this study was to assess the contributions of the ACC to saccade control. The ACC has been proposed to prepare cortical saccade-related areas for the performance of intentional saccades (Pierrot-Deseilligny et al., 2004), and thus we hypothesized that the ACC, like the rest of the cortical saccade control network, also facilitates contralateral saccades. To test this hypothesis, we used the cryoloop method of reversible cryogenic deactivation (Lomber et al., 1999) to directly compare the effects of dlPFC and ACC deactivation on the performance of both pro-saccades toward a stimulus, and anti-saccades away from the stimulus (Hallett, 1978; Munoz and Everling, 2004). We implanted cryoloops in the posterior end of the principal sulcus to deactivate the dlPFC, and in the anterior cingulate sulcus to deactivate the same area of the ACC in which we had previously found neurons with rule-selective prestimulus activity (Johnston et al., 2007). We predicted that contralateral saccade impairments would be found with both dlPFC and ACC deactivation, and to a greater extent for the more cognitively-demanding anti-saccade task.

2.2 – Methods

All surgical, training, and experimental procedures were in accordance with the Canadian Council on Animal Care policy on the use of laboratory animals, and approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care (*Appendix 1*).

2.2.1 Surgical Procedures

Three male rhesus monkeys (*Macaca mulatta*, 9-16 kg) were prepared for chronic deactivation studies by performing two surgeries under supervision of a university veterinarian. In the first surgery a head restraint post was anchored to a dental acrylic implant, which enabled us to train the animal on the behavioral task. A preformed eye coil (3 turns of stainless steel wire, Cooner Wire, Chatsworth, California, USA) was

implanted behind the conjunctiva of the left eye of monkey D, for use with the magnetic search coil system (Fuchs and Robinson, 1966; Judge et al., 1980), whereas a video eye tracker was used with monkeys A and C (Eyelink, SR Research Ltd., Kanata, ON, Canada). Magnetic resonance imaging provided an image of the neural anatomy *in situ*, from which stainless steel cryoloops (Fig. 2.1A) were designed to fit the shape and location of the cortical areas that were targeted for deactivation. In the second surgery, cryoloops were designed and implanted according to methods that have previously been described (Lomber et al., 1999). The cortex of the posterior sulcus principalis is the likely macaque homologue of the human middle frontal gyrus (Petrides and Pandya, 1999), and thus cryoloops were implanted in the posterior end of the principal sulcus to deactivate area 46 of the dlPFC (Fig. 2.1C). Cryoloops were also implanted in the anterior cingulate sulcus to deactivate area 24c of the ACC (Fig. 2.1C). The location of the anterior cingulate sulcus cryoloops was determined by placing them at the same position on the anterior-posterior axis as the cryoloops in the posterior end of the principal sulcus. This area of the ACC was the same at which we had previously found neurons with rule-selective prestimulus activity in an uncued blocked task consisting of pro-saccades and anti-saccades (Johnston et al., 2007).

2.2.2 Cryoloop Method of Reversible Cryogenic Deactivation

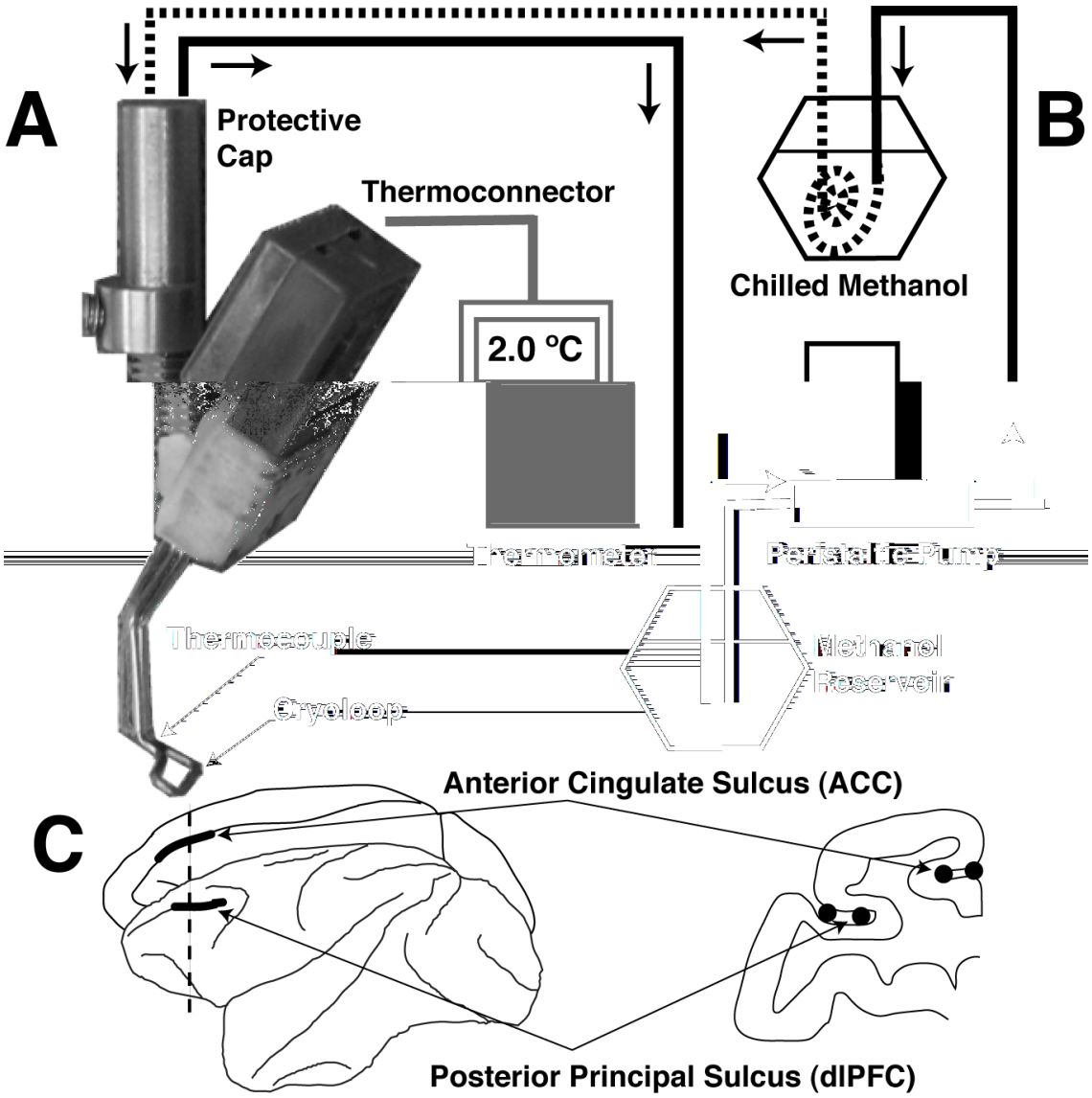
Cryoloops were constructed from 23-gauge hypodermic stainless steel tubing (Fig. 2.1A), and designed to deactivate both the upper and lower banks of the sulcus in which they were implanted (Fig. 2.1C). Cryoloops that we implanted in both the posterior principal sulcus and anterior cingulate sulcus were 4 x 6 mm in dimension. Given that 24 mm^2 of cortical tissue were deactivated on each side of the cryoloop (total 48 mm^2), with an estimated range of 1.5 - 2.0 mm, we calculate that the cryoloops deactivated 72 - 96 mm^3 of cortical tissue. Given the identical design of the implanted cryoloops, we assume that the same area of cortical tissue was deactivated in both the dlPFC and ACC. Methanol pumped through teflon tubing was chilled when passed through a methanol ice bath that was reduced to subzero temperatures by the addition of dry ice (Fig. 2.1B). Chilled methanol pumped through a cryoloop inactivates adjacent cortical tissue by disrupting synaptic activity therein. Each cooling session consisted of precool,

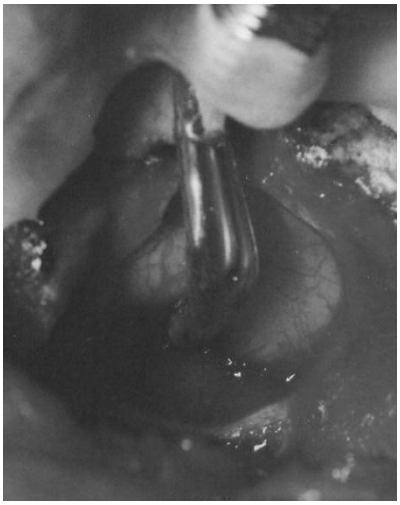
Figure 2.1 – Cryoloop Method of Reversible Cryogenic Deactivation

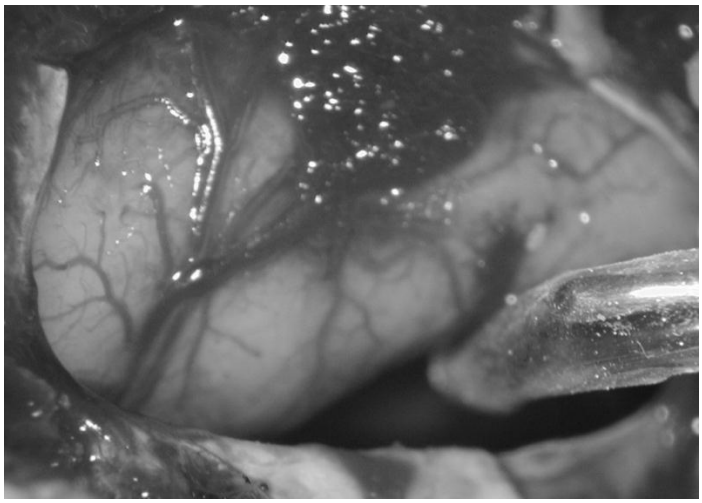
A: The cryoloop is constructed from 23-gauge stainless steel tubing, and designed to match the shape of the targeted cortical structure. Adhered to the union of the cryoloop is a thermocouple, which attaches to an external thermometer by way of a thermoconnector. At the opposite end of the cryoloop there are both an input and output (not shown), which are covered by a protective cap when not in use.

B: Room-temperature methanol (*solid line*) is pumped through teflon tubing that passes through a methanol ice bath which is reduced to subzero temperatures by the addition of dry ice. Chilled methanol (*dashed line*) is pumped through the cryoloop, then back to the reservoir from whence it came. Cryoloop temperature is monitored with the attached thermocouple, and maintained in the range of 1-3°C by adjusting the flow rate of the peristaltic pump.

C: Cryoloops were implanted in the anterior cingulate sulcus to deactivate the ACC, and the posterior principal sulcus to deactivate the dlPFC. The location of the coronal section is indicated by the *dashed line*. Photographs taken during surgery show a cryoloop implanted in the posterior principal sulcus (left), which was used to deactivate the dlPFC, and in the anterior cingulate sulcus (right), which was used to deactivate the ACC. Both the dlPFC and ACC cryoloops were 4 x 6 mm in dimension, and thus deactivated an estimated 72 – 96 mm^3 of cortical tissue.


A
Protective Cap
Thermoconnector
2.0 °C
B
Chilled Methanol
Thermometer
Peristaltic Pump
Thermocouple
Methanol Reservoir
Cryoloop
C
Anterior Cingulate Sulcus (ACC)
Posterior Principal Sulcus (dlPFC)





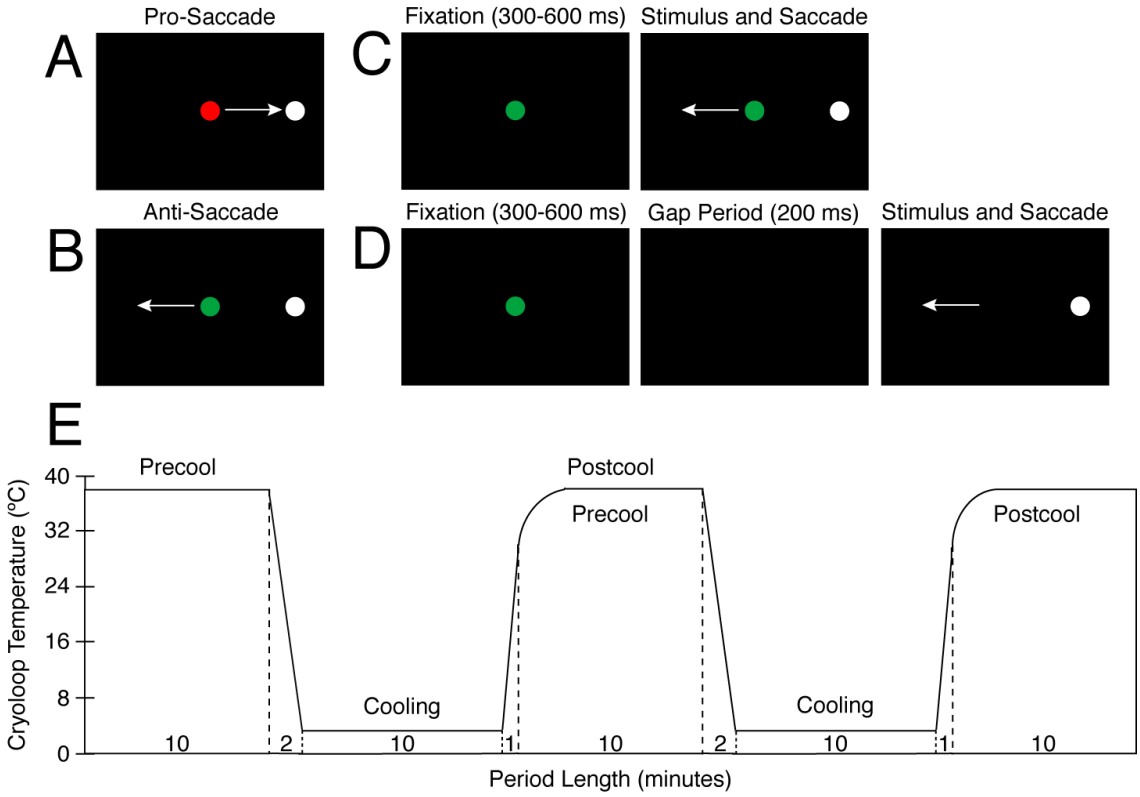


Figure 2.2 – Anti/Pro-saccade Task and Cooling Timeline

The task instruction was provided by the colour of the central fixation point: either a pro-saccade toward the peripheral stimulus (*A*), or an anti-saccade away from the stimulus (*B*). Stimulus appearance was the signal to perform the instructed saccade. The arrow indicates the correct saccade direction, but only for the purposes of this figure, and thus was not included as part of the task display. In the overlap condition, the central fixation point was visible for the duration of the trial (*C*), whereas in the gap condition, the central fixation point was removed 200 ms prior to stimulus appearance (*D*).

E: This schematic illustrates the timeline of a typical cooling session, in which the precool, cooling, and postcool periods were approximately ten minutes in duration. Cryoloop temperature achieved the desired range of 1-3°C within two minutes of starting the pump, reached 30°C within one minute of stopping the pump, and returned to normal body temperature within a few minutes. A postcool period could also serve as a precool period for comparison to the cooling period that followed.

cooling, and postcool periods that were between 10 and 15 minutes in duration (Fig. 2.2E). We chose this length of time to avoid frustrating an animal that was impaired on the task in the cooling period, while still allowing the animal enough time with which to perform a sufficient number of trials for analysis. A cooling session started with a precool period, after which the pump was turned on. Cryoloop temperature was monitored with an attached thermocouple and maintained in the desired range of 1-3ºC by adjusting flow rate of the peristaltic pump. At the end of the cooling period, the pump was turned off and cryoloop temperature returned rapidly to normal, such that normal behaviour could be observed both before and almost immediately following deactivation. A postcool period could also serve as a control period for comparison to the cooling period that followed, and this cycle was continued for as long as the animal was willing to work. Further details with regards to the cryoloop method have been published previously (Lomber et al., 1999).

2.2.3 Behavioral Task

Three monkeys were trained to perform a randomly-interleaved anti/pro-saccade task in which they were required to look either toward (pro-saccade; Fig. 2.2A) or away from (anti-saccade; Fig. 2.2B) a peripheral visual stimulus. Eye movements were monitored at 500 Hz with high-speed infrared video eye tracking for monkeys A and C, and at 1000 Hz with a magnetic search coil system for monkey D. The task instruction was provided on each trial by the colour of the central fixation point, either red or green, which the monkey was required to fixate on for between 300 and 600 ms. This relatively short fixation period was necessitated by the tendency of the first animal used in this study, monkey D, to break fixation soon after having initiated fixation. With this 300-600 ms fixation period, the animal was able to follow the majority of trials through to completion. Subsequently the same fixation period had to be used with the other two animals as well, even though they did not have the same tendency to break fixation as the first animal. In the overlap condition the central fixation point remained visible for the duration of the trial (Fig. 2.2C), whereas in the gap condition the central fixation point was removed 200 ms prior to stimulus appearance (Fig. 2.2D). At the end of the fixation period, a white dot stimulus was presented at 8º on the horizontal axis, to either

the left or right of fixation. For sessions in which the activity of a superior colliculus (SC) saccade neuron was recorded, the stimulus appeared within a range of 4-12º on the horizontal axis. This was because in the cell recording sessions, the stimulus was presented either in the cell's response field, or at the mirror location in the opposite visual field. The activity of these SC saccade neurons has been analysed in *Chapter 3*. Stimulus appearance was the signal for the monkey to perform the instructed saccade, and a correct response was followed immediately by a water reward.

2.2.4 Behavioral Analysis

Data analysis was performed using custom-designed software programmed in Matlab (Mathworks, Natick, MA). Saccade onset was identified as the time at which, following stimulus onset, saccade velocity exceeded 30º/s (Everling and DeSouza, 2005), while saccade end was identified as the time at which saccade velocity fell below 30º/s for at least 5 ms. All trials were visually inspected and excluded from analysis if there were blinks, broken fixation, or saccade latencies either below 80 ms (anticipations) or above 500 ms (no response). Also excluded from analysis were a) trials within the first three minutes of the cooling and postcool periods, to allow cortical tissue sufficient time with which to reach the desired state of deactivation or reactivation (Horel, 1991), b) trials with saccade latency, velocity, or duration that were more than two standard deviations either greater than or less than the session mean, and c) sessions with fewer than five trials (correct and error combined) per condition in any of the precool, cooling, or postcool periods.

Task performance was identified as the percentage of correct trials per session, which was calculated as the number of correct trials divided by the number of correct and error trials combined. Session means were calculated for the saccade latency and metrics of correct responses. Saccade latency was calculated as the time between stimulus onset and saccade onset. Saccade velocity was calculated as the difference in eye position sampled at either 500 Hz with the video eye tracker or 1000 Hz with the magnetic search coil. Saccade duration was calculated as the time between saccade onset and saccade end. Analyses of task performance, saccade latency, peak saccade velocity, saccade duration, and saccade gain were performed with one-way repeated

measures ANOVAs. These analyses were performed for each monkey individually, and all monkeys combined. There were 8 conditions for dlPFC and ACC deactivations: ipsilateral pro, ipsilateral anti, contralateral pro, and contralateral anti, in both the overlap and gap conditions. All ANOVAs were evaluated at $p < 0.05$.

To conclude that there was an effect of deactivation, the mean value in the cooling period had to be either significantly less than or greater than both the mean value in the precool period, and the mean value in the postcool period, evaluated with paired t-tests at $p < 0.05$. An exception was made for the effects of ACC deactivation on contralateral anti-saccade task performance, for which the mean value in the cooling period was significantly less than in the precool period, but not the postcool period. To determine whether this reduced performance in the postcool period was found only for contralateral anti-saccades, or for both contralateral and ipsilateral anti-saccades, we used a paired t-test to compare contralateral anti-saccade task performance with ipsilateral anti-saccade task performance in the postcool period, evaluated at $p < 0.05$. Sham sessions were performed in which either the pump remained off during the cooling period, or room temperature methanol was pumped through the cryoloops, which reduced their temperature to approximately 27°C. There were no effects of sham deactivation on the performance, kinematics, or metrics of saccades.

To address the longer saccade latencies and durations in the control (precool and postcool) periods of dlPFC sessions than ACC sessions, we calculated mean saccade latencies and durations by combining all conditions from all monkeys, in the precool and postcool periods combined. We then compared the dlPFC and ACC sessions with a nonpaired t-test, evaluated at $p < 0.05$. To assess the individual differences between monkeys, we then calculated mean saccade latencies and durations in the control periods for each monkey, across all conditions and including both the dlPFC and ACC sessions.

2.3 – Results

We performed 59 dlPFC deactivation sessions (23 with monkey A, 35 with monkey C, 1 with monkey D), and 43 ACC deactivation sessions (21 with monkey A, 12 with monkey C, and 10 with monkey D). We were only able to collect one dlPFC

deactivation session from monkey D before the cryoloop in the posterior principal sulcus became clogged, and thus could no longer be used. The results presented below are for all monkeys combined. The results for each monkey individually are also provided for dlPFC deactivation in the overlap condition (*Appendix 2*) and gap condition (*Appendix 3*), and for ACC deactivation in the overlap condition (*Appendix 4*) and gap condition (*Appendix 5*). Briefly, with dlPFC deactivation monkeys A and C demonstrated an impairment of contralateral saccades, both pro-saccades and anti-saccades, and a facilitation of ipsilateral pro-saccades. With monkey D there were effects of ACC deactivation on pro-saccades and anti-saccades, whereas there were no effects of ACC deactivation with monkeys A and C.

2.3.1 dlPFC and ACC Deactivation Increased the Incidence of Ipsilateral Saccades

Analysis of pro-saccade task performance revealed an increase of ipsilateral saccades with unilateral deactivation of the dlPFC or ACC, for all monkeys combined. With unilateral dlPFC deactivation there was improved performance of ipsilateral pro-saccades ($F(2,116) = 26.50$, $p < 0.001$; pre = 94.1 ± 0.7 %, cool = 98.5 ± 0.5 %, post = 95.0 ± 0.6 %, $p < 0.001$; Fig. 2.3B), and impaired performance of contralateral pro-saccades ($F(2,116) = 10.20$, $p < 0.001$; pre = 93.2 ± 0.8 %, cool = 85.2 ± 2.3 %, post = 92.0 ± 1.3 %, $p < 0.005$; Fig. 2.3D), in the gap condition. With unilateral ACC deactivation there was improved performance of ipsilateral pro-saccades in both the overlap condition ($F(2,84) = 11.70$, $p < 0.001$; pre = 92.3 ± 1.5 %, cool = 97.5 ± 0.6 %, post = 91.2 ± 1.7 %, $p < 0.001$; Fig. 2.3A) and gap condition ($F(2,84) = 7.94$, $p < 0.001$; pre = 91.7 ± 1.4 %, cool = 96.7 ± 0.8 %, post = 92.1 ± 1.4 %, $p < 0.005$; Fig. 2.3B). There was also, with unilateral deactivation of the dlPFC or ACC, impaired performance of contralateral anti-saccades in both the overlap condition (dlPFC: $F(2,116) = 24.01$, $p < 0.001$; ACC: $F(2,84) = 18.00$, $p < 0.001$; Fig. 2.3G) and gap condition (dlPFC: $F(2,116) = 22.24$, $p < 0.001$; ACC: $F(2,84) = 13.27$, $p < 0.001$; Fig. 2.3H).

With dlPFC deactivation, contralateral anti-saccade performance decreased from the precool period to the cooling period in both the overlap condition (pre = 93.7 ± 0.7 %, cool = 83.2 ± 1.7 %; $p < 0.01$; Fig. 2.3G) and gap condition (pre = 75.5 ± 1.9 %,

Figure 2.3 – Effects of dlPFC or ACC Deactivation on Task Performance

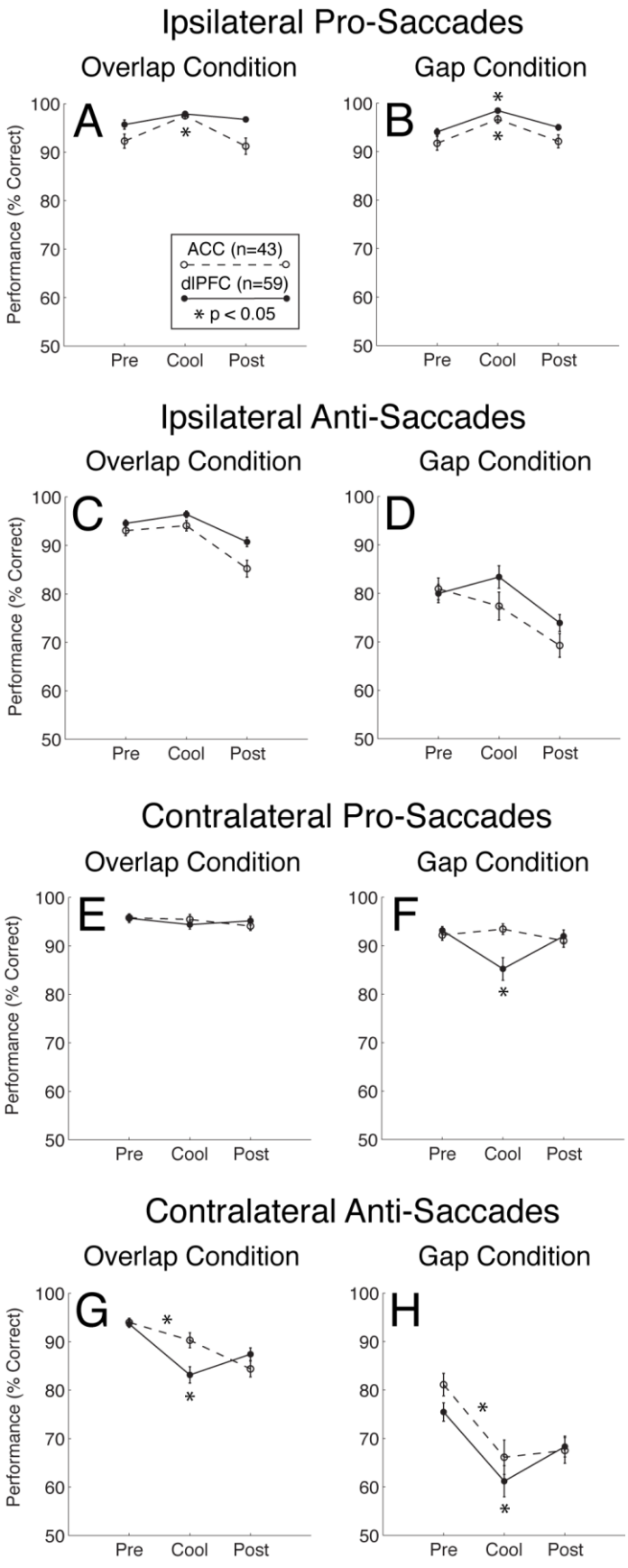
The percentage of correct trials in the precool (Pre), cooling (Cool), and postcool (Post) periods is indicated for dlPFC deactivation (*solid line*) and ACC deactivation (*dashed line*) sessions. A significant difference ($p < 0.05$) between the cooling period and both the precool and postcool periods is indicated with an *asterisk* adjacent to the cooling marker. A significant difference ($p < 0.05$) between only the cooling period and the precool period is indicated with an *asterisk* adjacent to the line that connects the precool and cooling markers.

A: Task performance for ipsilateral pro-saccades in the overlap condition.

B: Same as in *A*, but in the gap condition.

C, *D*: Same as in *A* and *B*, but for ipsilateral anti-saccades.

E, *F*, *G*, *H*: Same as in *A*, *B*, *C*, *D*, but for contralateral saccades.


Ipsilateral Pro-Saccades
Overlap Condition
Gap Condition
A
B
Performance (% Correct)
ACC (n=43)
dlPFC (n=59)
* p < 0.05
Pre
Cool
Post
Ipsilateral Anti-Saccades
C
D
Contralateral Pro-Saccades
E
F
Contralateral Anti-Saccades
G
H

cool = 61.2 ± 3.2 %; $p < 0.005$; Fig. 2.3H). ACC deactivation also reduced contralateral anti-saccade performance in both the overlap condition (pre = 94.0 ± 0.9 %, cool = 90.3 ± 1.5 %; $p < 0.05$; Fig. 2.3G) and gap condition (pre = 81.1 ± 2.3 %, cool = 66.1 ± 3.5 %; $p < 0.001$; Fig. 2.3H). Following dlPFC deactivation, contralateral anti-saccade performance increased from the cooling period to the postcool period in both the overlap condition (cool = 83.2 ± 1.7 %, post = 87.4 ± 1.3 %; $p < 0.01$; Fig. 2.3G) and gap condition (cool = 61.2 ± 3.2 %, post = 68.3 ± 2.2 %; $p < 0.005$; Fig. 2.3H), which demonstrates a slight but significant recovery from the effects of dlPFC deactivation. On the other hand, contralateral anti-saccade performance did not improve following ACC deactivation in the gap condition (cool = 66.1 ± 3.5 %, post = 67.5 ± 2.7 %; $p = 0.72$; Fig. 2.3H), and in the overlap condition performance actually became significantly worse (cool = 90.3 ± 1.5 %, post = 84.4 ± 1.6 %; $p < 0.01$; Fig. 2.3G). This suggests that either the effects of ACC deactivation extended beyond the cooling period, or the animal's behaviour simply deteriorated over time.

Consistent with a general degradation of the more effortful (anti-saccade) task, ipsilateral anti-saccade performance was not affected by ACC deactivation in the overlap condition (pre = 93.1 ± 1.1 %, cool = 94.1 ± 1.1 %; $p = 0.41$; Fig. 2.3C) or gap condition (pre = 80.9 ± 2.3 %, cool = 77.4 ± 2.9 %; $p = 0.13$; Fig. 2.3D), but in the postcool period was reduced to the same level as contralateral anti-saccade performance in both the overlap condition (ipsilateral = 85.2 % ± 1.7 %, contralateral = 84.4 ± 1.6 %; $p = 0.70$; Figs. 2.3E, G) and gap condition (ipsilateral = 69.2 % ± 2.5 %, contralateral = 67.5 ± 2.7 %; $p = 0.56$; Figs. 2.3F, H). Therefore both ipsilateral and contralateral anti-saccades were impaired in the postcool period following ACC deactivation, which suggests that this impairment may have been caused by fatigue. There also appears to have been an effect of fatigue with dlPFC deactivation, given that contralateral anti-saccade performance in the postcool period was significantly less than in the precool period for both the overlap condition (pre = 93.7 ± 0.7 %, post = 87.4 ± 1.3 %; $p < 0.001$; Fig. 2.3G) and gap condition (pre = 75.5 ± 1.9 %, cool = 68.3 ± 2.2 %; $p < 0.001$; Fig. 2.3H). These findings suggest that the lack of recovery of contralateral anti-saccade performance in the postcool period was likely the result of fatigue or decreased

motivation, given that ipsilateral anti-saccade performance was also impaired in the postcool period, such that in the postcool period all anti-saccades were affected, whereas only contralateral anti-saccades were impaired by cooling. In summary, both dlPFC and ACC deactivation increased the incidence of ipsilateral saccades, as demonstrated by the impaired performance of contralateral anti-saccades and improved performance of ipsilateral pro-saccades, while dlPFC deactivation also impaired the performance of contralateral pro-saccades.

2.3.2 dlPFC Deactivation Increased the Latency of Contralateral Saccades

Analysis of saccadic reaction times (latency) revealed an increase of contralateral saccade latency with deactivation of the dlPFC but not the ACC, for all monkeys combined. With unilateral dlPFC deactivation there was increased latency of contralateral pro-saccades in both the overlap condition ($F(2,116) = 68.95$, $p < 0.001$; pre = 240.4 ± 8.4 ms, cool = 282.7 ± 10.2 ms, post = 246.1 ± 8.4 ms; $p < 0.001$; Fig. 2.4C) and gap condition ($F(2,116) = 44.11$, $p > 0.001$; pre = 192.8 ± 7.1 ms, cool = 236.4 ± 10.4 ms, post = 203.7 ± 8.1 ms; $p < 0.001$; Fig. 2.4D). There was also an increased latency of contralateral anti-saccades in both the overlap condition ($F(2,116) = 21.6$, $p < 0.001$; pre = 241.2 ± 8.5 ms, cool = 266.3 ± 8.5 ms, post = 258.8 ± 8.8 ms; $p < 0.05$; Fig. 2.4G) and gap condition ($F(2,116) = 26.04$, $p < 0.001$; pre = 212.4 ± 7.6 ms, cool = 243.0 ± 9.3 ms, post = 225.3 ± 8.1 ms; $p < 0.001$; Fig. 2.4H).

Mean saccade latency in the control periods (precool and postcool combined) of the dlPFC sessions was slower than in the ACC sessions. This can be explained by individual differences among monkeys, and the different number of sessions contributed by each monkey to the dlPFC and ACC analyses, that together bias mean saccade latency. We found that in the control periods, monkey C had longer saccade latencies (269.8 ± 1.6 ms) than monkey A (162.6 ± 1.4 ms) and monkey D (184.3 ± 2.7 ms), and contributed more dlPFC sessions (35) than ACC sessions (12), which produced a longer mean saccade latency for the dlPFC sessions than ACC sessions across all conditions (dlPFC = 224.8 ± 2.1 ms, ACC = 200.0 ± 2.5 ms; $p < 0.001$; Fig. 2.4).

Figure 2.4 – Effects of dlPFC or ACC Deactivation on Saccade Latency

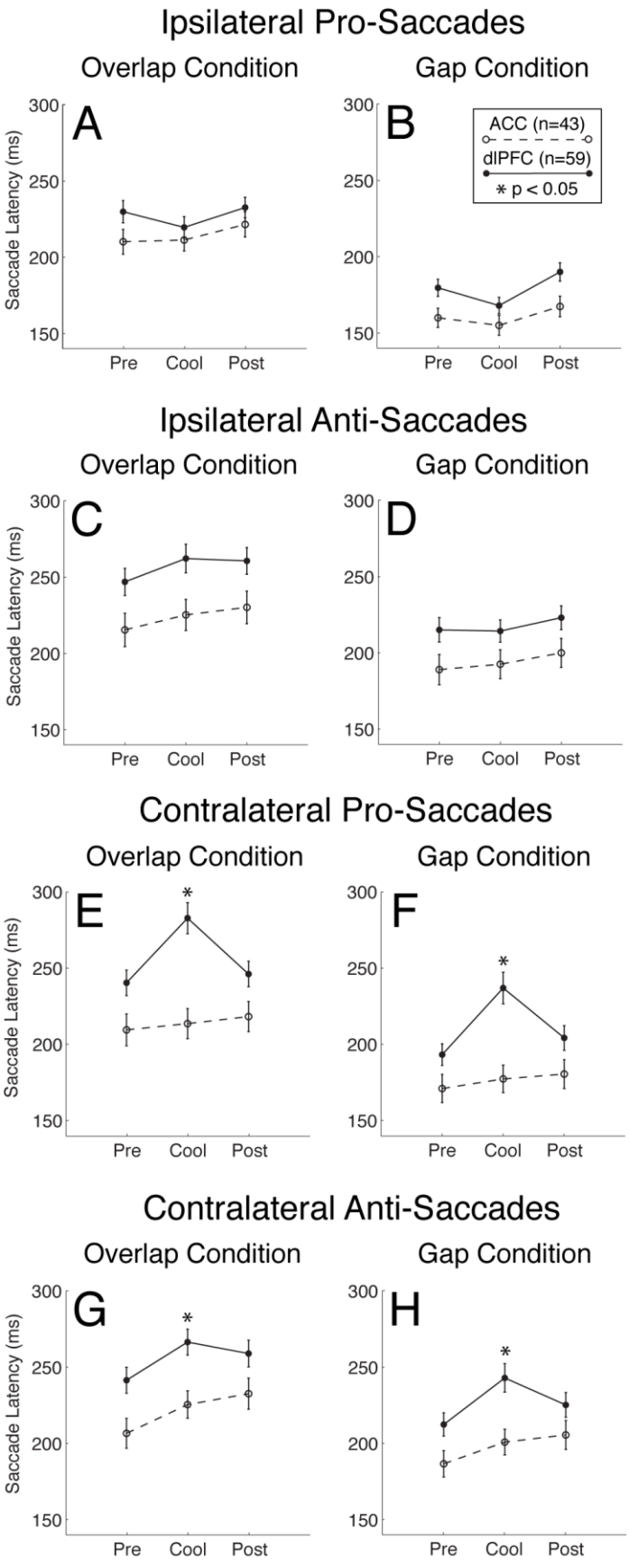
The latency of saccade onset for correct trials in the precool (Pre), cooling (Cool), and postcool (Post) periods is indicated for dlPFC deactivation (*solid line*) and ACC deactivation (*dashed line*) sessions. A significant difference ($p < 0.05$) between the cooling period and both the precool and postcool periods is indicated with an *asterisk* adjacent to the cooling marker. A significant difference ($p < 0.05$) between only the cooling period and the precool period is indicated with an *asterisk* adjacent to the line that connects the precool and cooling markers.

A: Latency for ipsilateral pro-saccades in the overlap condition.

B: Same as in *A*, but in the gap condition.

C, *D*: Same as in *A* and *B*, but for ipsilateral anti-saccades.

E, *F*, *G*, *H*: Same as in *A*, *B*, *C*, *D*, but for contralateral saccades.


Ipsilateral Pro-Saccades
Overlap Condition
Gap Condition
A
B
ACC (n=43)
dlPFC (n=59)
* p < 0.05
Saccade Latency (ms)
300
250
200
150
Pre
Cool
Post
Ipsilateral Anti-Saccades
Overlap Condition
Gap Condition
C
D
Contralateral Pro-Saccades
Overlap Condition
Gap Condition
E
F
*
Contralateral Anti-Saccades
Overlap Condition
Gap Condition
G
H
*

2.3.3 dlPFC Deactivation Decreased the Velocity of Contralateral Saccades

Prefrontal lesion and deactivation studies tend to report effects on task performance and saccade latency, but not other saccade parameters. An exception is Fukushima and colleagues, who have reported increased anti-saccade velocity in human patients with schizophrenia (1988), a psychiatric disorder which is thought to disrupt prefrontal function, and with lesions that include both the dlPFC and FEF (1994). Neither pro-saccade nor anti-saccade velocity, however, were affected by dlPFC microstimulation with monkeys that began prior to saccade initiation (Wegener et al., 2008). Therefore, it remains unclear as to whether the prefrontal cortex has an influence on saccade kinematics. Analysis of saccade velocity revealed a decrease of contralateral saccade velocity with deactivation of the dlPFC but not the ACC, for all monkeys combined. With unilateral dlPFC deactivation there was a decreased velocity of contralateral pro-saccades in both the overlap condition ($F(2,116) = 16.36$, $p < 0.001$; pre = 299.1 ± 7.6 ◦/s, cool = 286.8 ± 7.6 ◦/s, post = 295.7 ± 7.4 ◦/s; $p < 0.001$; Fig. 2.5C) and gap condition ($F(2,116) = 15.18$, $p < 0.001$; pre = 291.1 ± 7.8 ◦/s, cool = 276.5 ± 8.0 ◦/s, post = 287.7 ± 7.5 ◦/s; $p < 0.001$; Fig. 2.5D). There was also a decreased velocity of contralateral anti-saccades in both the overlap condition ($F(2,116) = 60.94$, $p < 0.001$; pre = 254.2 ± 5.1 ◦/s, cool = 219.1 ± 6.9 ◦/s, post = 256.3 ± 6.0 ◦/s; $p < 0.001$; Fig. 2.5G) and gap condition ($F(2,116) = 52.59$, $p < 0.001$; pre = 253.9 ± 5.3 ◦/s, cool = 222.2 ± 6.3 ◦/s, post = 256.3 ± 5.6 ◦/s; $p < 0.001$; Fig. 2.5H).

2.3.4 dlPFC Deactivation Increased the Duration of Contralateral Anti-saccades

Analysis of saccade duration revealed an increase of contralateral anti-saccade duration with deactivation of the dlPFC but not the ACC, for all monkeys combined. With unilateral dlPFC deactivation there was an increased duration of contralateral anti-saccades in both the overlap condition ($F(2,116) = 84.70$, $p < 0.001$; pre = 49.2 ± 0.5 ms, cool = 57.8 ± 1.1 ms, post = 51.0 ± 0.7 ms; $p < 0.001$; Fig. 2.6G) and gap condition ($F(2,116) = 69.24$, $p < 0.001$; pre = 47.9 ± 0.6 ms, cool = 56.2 ± 1.0 ms, post = 49.7 ± 0.6 ms; $p < 0.001$; Fig. 2.6H). Mean saccade duration in the control (precool and postcool) periods of the dlPFC sessions was longer than for the ACC sessions, the reasons for which have already been explained (see *Section 2.3.2*). We found that in the

Figure 2.5 – Effects of dlPFC and ACC Deactivation on Saccade Peak Velocity

The peak velocity of saccades on correct trials in the precool (Pre), cooling (Cool), and postcool (Post) periods is indicated for dlPFC deactivation (*solid line*) and ACC deactivation (*dashed line*) sessions. A significant difference ($p < 0.05$) between the cooling period and both the precool and postcool periods is indicated with an *asterisk* adjacent to the cooling marker.

A: Peak velocity for ipsilateral pro-saccades in the overlap condition.

B: Same as in *A*, but in the gap condition.

C, *D*: Same as in *A* and *B*, but for ipsilateral anti-saccades.

E, *F*, *G*, *H*: Same as in *A*, *B*, *C*, *D*, but for contralateral saccades.

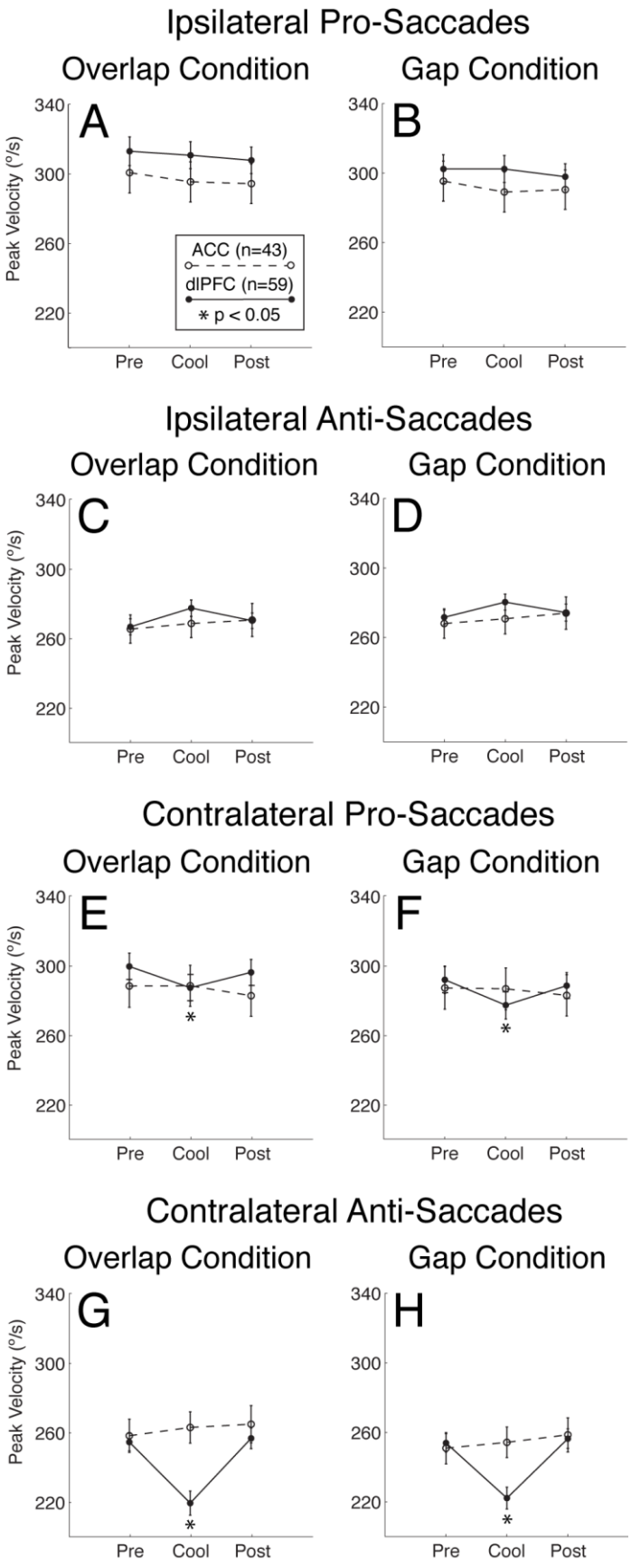

Ipsilateral Pro-Saccades
Overlap Condition
Gap Condition
A
B
Peak Velocity (°/s)
340
300
260
220
ACC (n=43)
dlPFC (n=59)
* p < 0.05
Pre
Cool
Post
Ipsilateral Anti-Saccades
Overlap Condition
Gap Condition
C
D
Contralateral Pro-Saccades
Overlap Condition
Gap Condition
E
F
*
Contralateral Anti-Saccades
Overlap Condition
Gap Condition
G
H
*

Figure 2.6 – Effects of dlPFC and ACC Deactivation on Saccade Duration

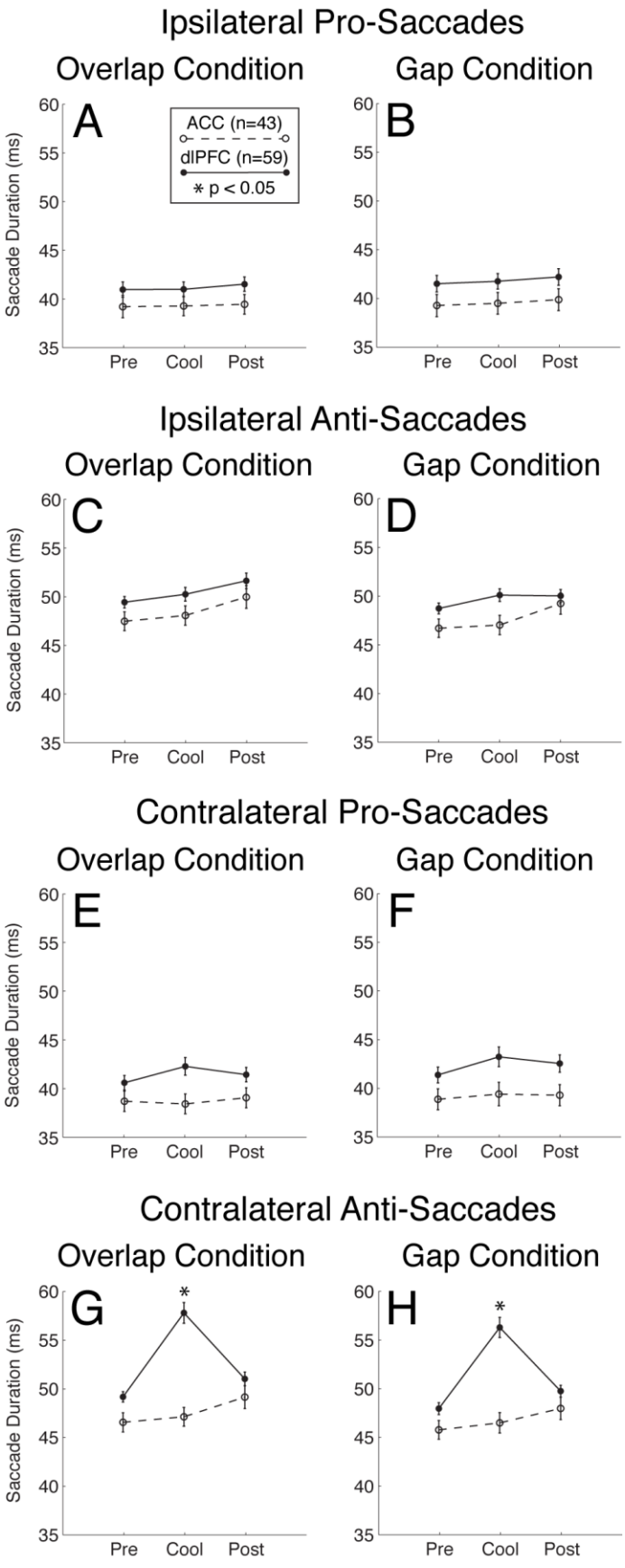
The duration of saccades for correct trials in the precool (Pre), cooling (Cool), and postcool (Post) periods is indicated for dlPFC deactivation (*solid line*) and ACC deactivation (*dashed line*) sessions. A significant difference ($p < 0.05$) between the cooling period and both the precool and postcool periods is indicated with an *asterisk* adjacent to the cooling marker.

A: Duration for ipsilateral pro-saccades in the overlap condition.

B: Same as in *A*, but in the gap condition.

C, *D*: Same as in *A* and *B*, but for ipsilateral anti-saccades.

E, *F*, *G*, *H*: Same as in *A*, *B*, *C*, *D*, but for contralateral saccades.


Ipsilateral Pro-Saccades
Overlap Condition
Gap Condition
A
B
ACC (n=43)
dlPFC (n=59)
* p < 0.05
Saccade Duration (ms)
60
55
50
45
40
35
Pre
Cool
Post
Ipsilateral Anti-Saccades
Overlap Condition
Gap Condition
C
D
Contralateral Pro-Saccades
Overlap Condition
Gap Condition
E
F
Contralateral Anti-Saccades
Overlap Condition
Gap Condition
G
H
*

control periods, monkey C had longer saccade durations (48.7 ± 0.2 ms) than monkey A (41.9 ± 0.3 ms) and monkey D (39.1 ± 0.5 ms), and contributed more dlPFC sessions (35) than ACC sessions (12), to produce a longer mean saccade duration for the dlPFC sessions than ACC sessions across all conditions (dlPFC = 45.6 ± 0.2 ms, ACC = 43.5 ± 0.3 ms; $p < 0.001$; Fig. 2.6).

2.3.5 dlPFC Deactivation Increased the Latency of Contralateral Errors

In addition to an increased incidence of ipsilateral errors on contralateral anti-saccade trials, unilateral dlPFC deactivation also increased the latency of contralateral errors on ipsilateral anti-saccade trials in the gap condition (pre = 177.5 ± 6.4 ms, cool = 232.5 ± 9.1 ms, post = 203.9 ± 7.6 ms; $p < 0.005$; Fig. 2.9A), whereas ACC deactivation did not (pre = 164.9 ± 8.0 ms, cool = 185.1 ± 9.8 ms, post = 190.6 ± 9.3 ms; $p > 0.05$; Fig. 2.9B). Neither dlPFC nor ACC deactivation affected the latency of ipsilateral errors on contralateral anti-saccade trials (Figs. 2.9B).

2.3.6 Summary of dlPFC and ACC Deactivation Effects

We found that both dlPFC and ACC deactivation improved ipsilateral pro-saccade performance and impaired contralateral anti-saccade performance, while only dlPFC deactivation impaired contralateral pro-saccade performance, increased contralateral saccade latency, decreased contralateral saccade velocity, increased contralateral anti-saccade duration, and increased the latency of contralateral errors. Neither dlPFC nor ACC deactivation had an effect on saccade accuracy (gain). Therefore both dlPFC and ACC deactivation increased the incidence of ipsilateral saccades, while only dlPFC deactivation delayed the initiation and impaired the kinematics of contralateral saccades.

2.4 – Discussion

With both dlPFC and ACC deactivation there was an increased incidence of ipsilateral saccades toward a stimulus, on both pro-saccade and anti-saccade trials. On the other hand, only dlPFC deactivation impaired the latency and kinematics of contralateral pro-saccades and anti-saccades. Contralateral saccade impairments were demonstrated by an

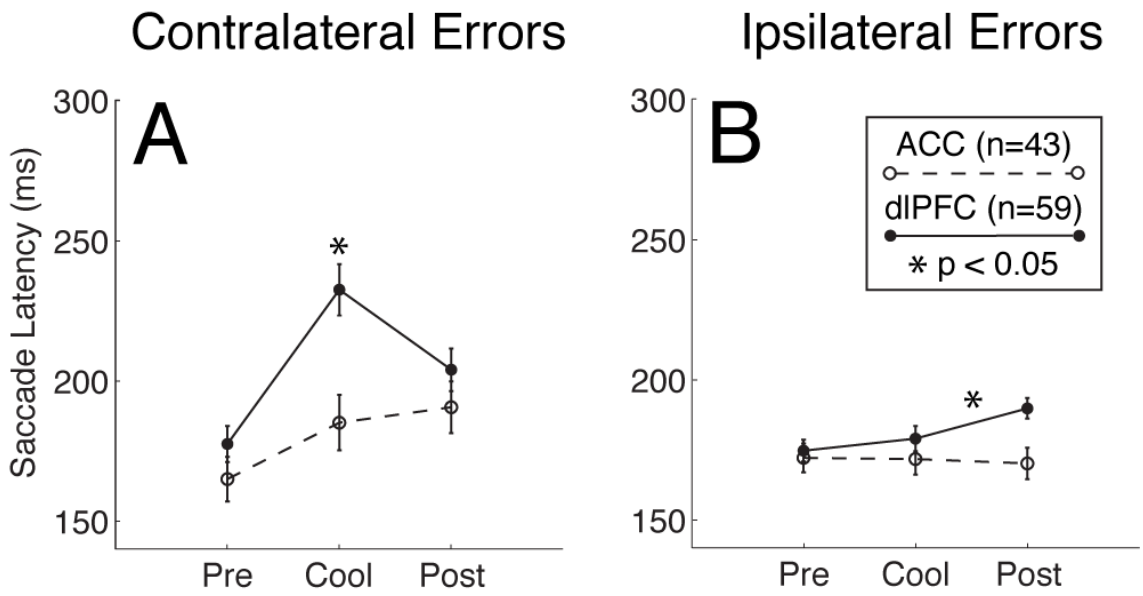


Figure 2.7 – Effects of dlPFC or ACC Deactivation on Error Latency

The latency of saccade onset for error trials in the precool (Pre), cooling (Cool), and postcool (Post) periods is indicated for dlPFC deactivation (*solid line*) and ACC deactivation (*dashed line*) sessions in the gap condition. A significant difference ($p < 0.05$) between the cooling period and both the precool and postcool periods is indicated with an *asterisk* adjacent to the cooling marker. A significant difference ($p < 0.05$) between only the cooling period and the postcool period is indicated with an *asterisk* adjacent to the line that connects the cooling and postcool markers.

A: Saccade latency of contralateral errors on ipsilateral anti-saccade trials.

B: Saccade latency of ipsilateral errors on contralateral anti-saccade trials.

increase of contralateral saccade latency, a decrease of contralateral saccade velocity, an increase of contralateral anti-saccade duration, and an increased latency of contralateral errors on ipsilateral anti-saccade trials. As predicted, contralateral saccade impairments were more substantial for anti-saccades than pro-saccades, which supports a role of the dlPFC in supporting tasks that have greater cognitive demands (Miller and Cohen, 2001).

2.4.1 Contralateral Saccades

The dlPFC sends direct projections to the superior colliculus (SC), which is a midbrain oculomotor structure that is critical for saccade initiation (Wurtz and Goldberg, 1972; Pierrot-Deseilligny et al., 1991b), and sends saccade commands to the brainstem saccade generator (Munoz et al., 2000; Scudder et al., 2002; Sparks, 2002; Gandhi and Katnani, 2011). SC saccade neurons discharge a high-frequency burst of action potentials for a saccade into their response field, while some also have a buildup of low-frequency prestimulus discharge that reflects saccade preparation (Munoz and Wurtz, 1995). This prestimulus activity is inversely related to saccade latency (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1999), and is greater for anti-saccade errors than correct anti-saccades (Everling et al., 1998). Here we found that dlPFC deactivation increased contralateral saccade latency, which suggests there was a decrease of prestimulus activity in the SC ipsilateral to deactivation. In agreement with the behavioral effects of dlPFC microstimulation and deactivation (Condy et al., 2007; Wegener et al., 2008), these findings imply that the dlPFC has an excitatory influence on SC saccade neurons. An inhibitory model of prefrontal function, on the other hand, has proposed that the dlPFC has an inhibitory influence on SC saccade neurons (Pierrot-Deseilligny et al., 1991a; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009). This discrepancy with regards to the type of influence that the dlPFC has on SC saccade neurons is addressed in *Chapter 3*.

The dlPFC, ACC, PPC, and FEF are interconnected components of the cortical saccade control network (Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; Bates and Goldman-Rakic, 1993) that send direct projections to the SC

(Goldman and Nauta, 1976; Leichnetz et al., 1981; Fries, 1984; Stanton et al., 1988), with the exception of the ACC. What appear to be corticotectal neurons in the ACC may actually have been labeled by a retrograde tracer injection that spread into the periaqueductal gray (Leichnetz et al., 1981), and furthermore another retrograde tracing study did not find corticotectal neurons in the ACC either (Fries, 1984). In support of this, deactivations of the dlPFC, PPC and FEF have been shown to increase the latency and decrease the velocity of contralateral saccades (Sommer and Tehovnik, 1997; Li et al., 1999; Condy et al., 2007), whereas we found that contralateral saccades were not affected by ACC deactivation. This supports the idea that greater contributions to saccade control are made by cortical areas which are more directly involved with the oculomotor system.

2.4.2 Ipsilateral Saccades

dlPFC deactivation also increased the incidence of ipsilateral saccades both toward and away from the stimulus on pro-saccade trials, and toward the stimulus on anti-saccade trials. Similarly, FEF deactivation has previously been shown to increase the incidence of premature ipsilateral saccades on memory-guided saccade trials (Dias et al., 1995). This suggests that both dlPFC and FEF deactivation increased the activity of saccade neurons in the SC contralateral to deactivation. dlPFC and FEF deactivation also increased the latency and decreased the velocity of contralateral saccades (Sommer and Tehovnik, 1997; Condy et al., 2007), which suggests that both dlPFC and FEF deactivation decreased the activity of saccade neurons in the SC ipsilateral to deactivation. Together these findings suggest that unilateral deactivation of the dlPFC or FEF caused a neural imbalance at the SC that was mediated by interhemispheric inhibition, such that a decrease of activity on the ipsilateral side would reduce interhemispheric inhibition and thus allow an increase of activity on the side contralateral to deactivation. This could occur at the level of either cortical areas (Schlag et al., 1998; Palmer et al., 2012) or collicular structures (Munoz and Istvan, 1998; Takahashi et al., 2005). With regards to the effects of dlPFC deactivation, reduced suppression of the contralateral SC, by way of either the contralateral dlPFC or ipsilateral SC, would allow the activity of contralateral SC saccade neurons to increase.

The route by which this occurs could be determined by simultaneously deactivating the dlPFC and recording the activity of saccade-related neurons in the ipsilateral SC, contralateral SC, and contralateral dlPFC. Additionally, the dlPFC and FEF are highly interconnected, and the FEF also sends direct projections to the SC, such that these effects of dlPFC deactivation could be mediated indirectly by way of the FEF, rather than directly to the SC. This idea could be tested by recording the activity of saccade-related neurons in the FEF while simultaneously deactivating the dlPFC. Together these behavioral effects of dlPFC deactivation suggest that the dlPFC has an excitatory influence on the ipsilateral SC that facilitates contralateral saccades, which by interhemispheric inhibition has an inhibitory influence on the contralateral SC that suppresses ipsilateral saccades.

2.4.3 Cingulate Eye Fields

ACC deactivation also increased the incidence of ipsilateral saccades, which suggests there was an increase of saccade neuron activity at the contralateral SC. This cannot be explained by interhemispheric inhibition, given that there was no effect of ACC deactivation on contralateral saccades, and thus the ipsilateral SC. Alternatively, this may be explained by a lack of direct corticotectal projections from the ACC, such that the ACC must influence the oculomotor system indirectly. This has previously been proposed as part of a cortical saccade control model in which the ACC prepares the dlPFC, PPC, FEF, and SEF for their respective roles in the performance of intentional saccades (Pierrot-Deseilligny et al., 2004; Muri and Nyffeler, 2008), and thus suggests that the ACC plays a preparatory rather than direct role in saccade control. A preparatory role implies that the detection of conflict or errors will influence preparation for the following trial. In support of this, a conflict-monitoring hypothesis of ACC function states that the dlPFC is recruited by the ACC to increase top-down control, such that the ACC detects conflict or errors, while the dlPFC resolves conflict by implementing control (Ridderinkhof et al., 2004; Carter and van Veen, 2007). Human neuroimaging has demonstrated exactly that: increased dlPFC activation and enhanced performance on trials following conflict-related or error-related ACC activation (Kerns et al., 2004). Enhanced performance is typically demonstrated as conflict adaptation or

posterror slowing effects on task performance and reaction times (Carter and van Veen, 2007). While support for this has been provided by patients with ACC lesions that do not show conflict adaptation or posterror slowing effects (di Pellegrino et al., 2007), there are other such patients that have still shown these effects (Fellows and Farah, 2005). In agreement with the latter, the analysis in this study found that these "previous trial" effects were unaffected by unilateral ACC deactivation, and thus a preparatory role of the ACC in saccade control cannot explain the overall weak effect of unilateral ACC deactivation that was observed.

The ACC is a heterogeneous area that extends from the genu of the corpus callosum to between the vertical planes of the anterior and posterior commissures (Paus, 2001). We chose to deactivate the same area of the ACC in which we had previously found neurons with rule-selective prestimulus activity (Johnston et al., 2007), however deactivation of this area had rather weak effects. This may be explained by differences between tasks: the randomly-interleaved task used for this deactivation study provided a rule cue at the beginning of each trial, whereas the recording study used an uncued blocked task in which the relevant rule was determined based on either the delivery or omission of reward. The ACC has been implicated in reinforcement-guided behaviour (Kennerley et al., 2006; Buckley et al., 2009), which may explain why this area of the ACC appeared to play a role in performance of the uncued task for the recording study, but not the cued task for the deactivation study.

Alternatively, the weak effect of ACC deactivation could have been the result of deactivating an area of the ACC that has only a weak influence on the oculomotor system. Two cingulate eye fields (CEF) have been identified in the ACC: a rostral CEF and caudal CEF (Wang et al., 2004). Both the rostral CEF for one of their monkeys, and the cryoloop that we implanted in the cingulate sulcus, were aligned with the posterior end of the principal sulcus. This suggests that we deactivated the rostral CEF, and thus implies that the rostral CEF has a weak effect on the oculomotor system. In support of this, two studies have identified the anti-saccade impairments of human patients with ACC lesions: the patients with the caudal set of lesions, centered on the vertical plane of the anterior commissure (VAC), had greater impairments than the patients with the rostral set of lesions, which were anterior to the VAC (Gaymard et al., 1998; Milea et

al., 2003). Using the VAC as a landmark (Paus, 2001), the location of these lesions roughly corresponds with the caudal CEF and rostral CEF, respectively. Therefore, both lesions and deactivation of an area corresponding to the rostral CEF had weak effects on anti-saccade task performance, which suggests that the rostral CEF has a weak influence on the oculomotor system. On the other hand, the weak effect of ACC deactivation could have been the result of deactivating an area of the ACC that was neither the rostral nor caudal CEF. While Wang and colleagues (2004) found that the rostral CEF was aligned with the posterior end of the principal sulcus in one monkey, in the other monkey there appears to have been either an anterior shift of the CEF locations, or a posterior shift of the FEF location, such that the posterior end of the principal sulcus, and thus the cryoloop in the anterior cingulate sulcus, was aligned with a gap between the rostral and caudal CEFs.

2.4.4 Neural Basis of Anti-saccade Errors

While both dlPFC and ACC deactivation increased the incidence of ipsilateral errors on contralateral anti-saccade trials, only dlPFC deactivation increased the latency of contralateral errors on ipsilateral anti-saccade trials, which suggests that unique neural processes may underlie the anti-saccade errors caused by dlPFC and ACC deactivation. These effects of dlPFC deactivation on anti-saccade errors could have occured in either of two ways. First, dlPFC deactivation could have reduced excitatory input to, and thus the activity of, saccade neurons in the ipsilateral SC. This would increase the latency of contralateral errors, assuming that the inverse relation between SC prestimulus activity and saccade latency (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1999) applies to both correct and error trials. This decreased activity of ipsilateral SC saccade neurons could then, by intercollicular inhibition (Munoz and Istvan, 1998; Takahashi et al., 2005), allow an increase of activity in the contralateral SC, which would increase the incidence of ipsilateral errors (Everling et al., 1998). Alternatively, interhemispheric cortical inhibition (Schlag et al., 1998; Palmer et al., 2012) could allow an increase of activity at the contralateral dlPFC, which would increase excitatory input to saccade neurons in the contralateral SC, and increase the incidence of ipsilateral errors.

Intercollicular inhibition could then decrease activity in the ipsilateral SC, and thus increase the latency of contralateral errors.

2.4.5 Conclusion

We hypothesised that the ACC, like the rest of the cortical saccade control network, facilitates contralateral saccades. ACC deactivation, however, did not affect the latency or kinematics of contralateral saccades. Alternatively, this lack of contralateral saccade impairments may have been the result of either deactivating an area of the ACC that did not influence contralateral saccades, or using a task that did not sufficiently probe ACC function. These issues remain to be addressed by future studies. As predicted, contralateral saccade impairments with dlPFC deactivation were greater for anti-saccades than pro-saccades, which supports a greater role of the dlPFC in more cognitively-demanding tasks. Furthermore, an impairment of contralateral saccades suggests that dlPFC deactivation decreased the activity of saccade neurons at the ipsilateral SC, which implies that the dlPFC has an excitatory influence on the oculomotor system. An inhibitory model of prefrontal function, on the other hand, has proposed that the dlPFC suppresses the activity of SC saccade neurons, and thus has an inhibitory influence on the oculomotor system. This discrepancy is addressed in the following chapter.

2.5 – Bibliography

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Chapter 3

Macaque Dorsolateral Prefrontal Cortex does not Suppress Superior Colliculus Saccade Neurons

The material in *Chapter 3* has been accepted for publication by Cerebral Cortex as Johnston K, Koval MJ, Lomber SG, Everling S (2013). Macaque dorsolateral prefrontal cortex does not suppress saccade-related activity in the superior colliculus. *Cerebral Cortex* (in press).

3.1 – Introduction

Primates possess an exceptional ability to control their behaviour on the basis of internal goals rather than the stimuli in their surrounding environment. This cognitive control is recruited when an unwanted stimulus-driven response must be suppressed in favour of a less potent but more advantageous behaviour. A well-established test of cognitive control is the anti-saccade task, which instructs subjects to look away from a suddenly-appearing stimulus (Hallett, 1978; Munoz and Everling, 2004). This requires both the inhibition of a prepotent pro-saccade toward the stimulus, and generation of a voluntary anti-saccade away from the stimulus. Studies of patients with lesions of the dorsolateral prefrontal cortex (dlPFC) (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Ploner et al., 2005), in addition to human neuroimaging (Sweeney et al., 1996; Doricchi et al., 1997; DeSouza et al., 2003; Matsuda et al., 2004; Ford et al., 2005; Brown et al., 2006; Brown et al., 2007) and transcranial magnetic stimulation (Nyffeler et al., 2007) studies, have thus far provided convergent evidence in support of what could be conceptualized as the "classic" model of anti-saccade performance, wherein the dlPFC is engaged to inhibit a prepotent saccade toward the stimulus (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003).

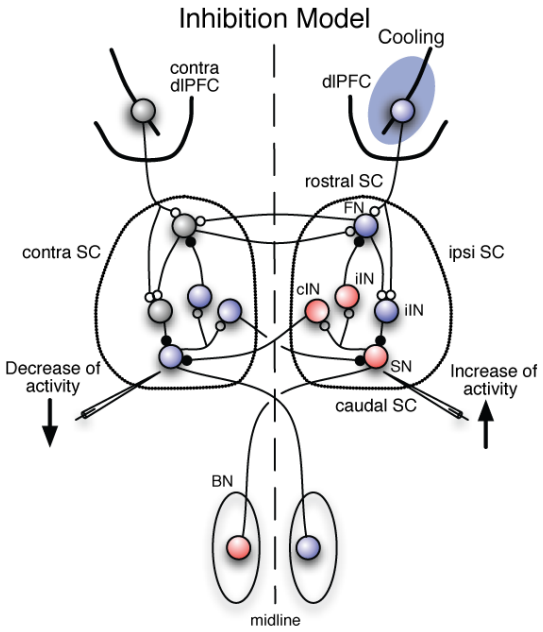
In apparent consistency with this inhibitory model of prefrontal function, neurophysiological studies in nonhuman primates have shown that dlPFC neurons exhibit task-selective activity for pro-saccades and anti-saccades (Everling and DeSouza, 2005; Johnston and Everling, 2006; Johnston et al., 2009), while

neuroanatomical studies have shown that the dlPFC sends projections directly to the superior colliculus (SC), a midbrain oculomotor structure that is critical for saccade initiation (Wurtz and Goldberg, 1972; Pierrot-Deseilligny et al., 1991). Based on the task-selective activity of antidromically-identified dlPFC corticotectal neurons, and the fact that cortical projections are excitatory (Jones, 2004), Johnston and Everling (2006) hypothesised that the dlPFC has an excitatory influence on either fixation neurons in the rostral SC, or inhibitory interneurons in the caudal SC, which then suppress the activity of SC saccade neurons (Munoz and Istvan, 1998) (Fig. 3.1).

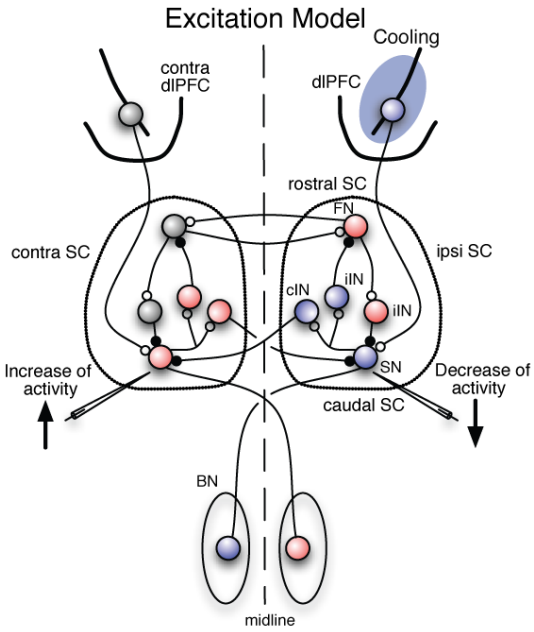
In contrast, two studies using different methods to manipulate dlPFC activity in nonhuman primates have produced results that are inconsistent with this inhibition model (Condy et al., 2007; Wegener et al., 2008). Unilateral dlPFC deactivation increased contralateral saccade latency and decreased the incidence of contralateral errors, while dlPFC microstimulation decreased contralateral saccade latency and increased the incidence of contralateral errors. These results suggest that in the SC ipsilateral to the manipulation, saccade neuron activity was decreased by dlPFC deactivation, and increased by dlPFC microstimulation, which implies that the dlPFC has an excitatory rather than inhibitory influence on the oculomotor system. We performed a direct test of the inhibition model by recording the activity of SC saccade neurons and deactivating the banks of the posterior principal sulcus in the dlPFC, while monkeys performed a randomly-interleaved anti/pro-saccade task. We found that unilateral dlPFC deactivation delayed the onset of saccade-related activity in the SC ipsilateral to deactivation, which corresponded with an increase of contralateral saccade reaction times, and furthermore supports the findings of Pouget and colleagues (2011). Unilateral dlPFC deactivation also increased the prestimulus and stimulus-related activity of saccade neurons in the SC contralateral to deactivation, which corresponded with an increased incidence of ipsilateral saccades. Together these findings suggest that the dlPFC has an excitatory influence on SC saccade neurons, and thus supports an excitatory rather than inhibitory model of prefrontal function (Fig. 3.1).

Figure 3.1 – Inhibition and Excitation Models of Prefrontal Function

The inhibition model predicts that, in the SC ipsilateral to dlPFC deactivation, there would be decreased activity of fixation neurons (FN) in the rostral SC and inhibitory interneurons (iIN) in the caudal SC, which would allow the activity of saccade neurons (SN) in the caudal SC to increase. This would increase reciprocal inhibition of FN by iIN, and intercollicular inhibition of SN in the contralateral SC by commissural intratectal neurons (cIN). This neural imbalance at the SC would enhance saccade commands sent to burst neurons (BN) in the contralateral brainstem saccade generator, and suppress saccade commands sent to ipsilateral BN, which would translate into a bias for contralateral saccades. The excitation model, on the other hand, predicts that in the ipsilateral SC, dlPFC deactivation would decrease the activity of SN, allowing the activity of FN and contralateral SN to increase. This would enhance saccade commands sent to ipsilateral BN, and suppress saccade commands sent to contralateral BN, creating a bias for ipsilateral saccades. Effect of deactivation on neural activity: decrease (*blue*), increase (*red*), none (*grey*). Axon terminals: inhibitory (*black dot*), excitatory (*white dot*). Midline indicated by *vertical dashed line*.



Bias towards contraversive saccades



Bias towards ipsiversive saccades

3.2 – Methods

All procedures were carried out in accordance with the guidelines of the Canadian Council of Animal Care Policy on the Use of Laboratory Animals and a protocol approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care (*Appendix 1*).

3.2.1 Surgical Procedures

Three male macaque monkeys (*Macaca mulatta*, 9-16 kg) were prepared for chronic dlPFC deactivation experiments and single neuron recordings in the SC using previously described techniques (Johnston and Everling, 2006). Briefly, monkeys underwent two aseptic surgical procedures. Animals received analgesics and antibiotics postoperatively and were closely monitored by a university veterinarian. In the first surgery, a plastic head restraint and a recording chamber were implanted. The recording chamber was centered on the midline and tilted 38° posterior of vertical to allow recordings from neurons in the SC. Monkeys were trained on the behavioral task. Once the animals achieved stable task performance, anatomical MR images were obtained to visualize the location of the implanted recording chambers and the shape of the principal sulci. Animals underwent a second surgery in which stainless steel cryoloops (4 mm x 6 mm) were implanted according to methods that have previously been described (Lomber et al., 1999). For each animal, cryoloops were implanted bilaterally into the posterior end of the principal sulcus (Fig. 3.2A).

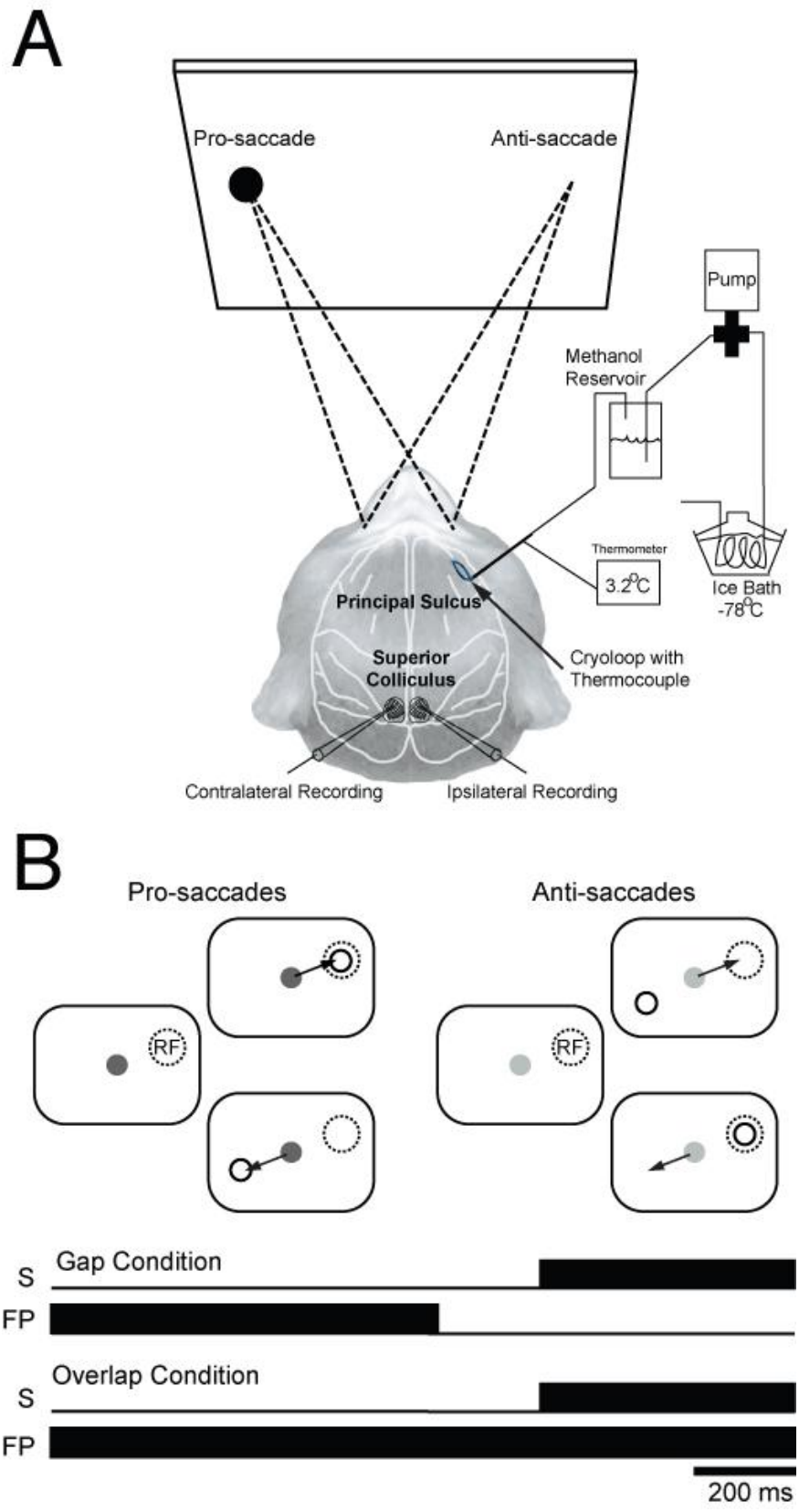
3.2.2 Cytoarchitecture and Connectivity of dlPFC Area 46

In the monkey brain, Petrides and Pandya (1999) have designated the cortex in the banks of the entire length of the principal sulcus and surrounding the anterior end of the principal sulcus as area 46. This is because they share the same cytoarchitectonic characteristics as area 46 in the middle frontal gyrus of the human brain: a layer III that is densely packed with small-to-medium sized pyramidal cells, and a well-developed layer IV. By comparison, the cortex above the posterior end of the principal sulcus also has a well-developed layer IV, but similar to area 9 has a layer III which contains large and darkly stained pyramidal neurons, and thus was designated dorsal area 9/46 (9/46d).

Figure 3.2 – Experimental Setup and Behavioral Task

A: The banks of the posterior principal sulcus were deactivated by pumping chilled methanol through an implanted cryoloop, while single neuron activity was recorded in the intermediate layers of the SC either ipsilateral or contralateral to the side of dlPFC deactivation. The monkey performed an oculomotor task that consisted of pro-saccades toward a stimulus, and anti-saccades away from the stimulus.

B: Each trial began with a fixation point (FP) that indicated, by its colour, a pro-saccade or anti-saccade trial. A visual stimulus (S) then appeared either in the neuron's response field (RF) or at the mirror location in the opposite hemifield. In this figure, the visual stimulus is indicated by a solid circle, while the response field is indicated by a dashed circle. In the gap condition, the fixation point disappeared 200 ms prior to the presentation of the peripheral stimulus, whereas the fixation remained illuminated in the overlap condition.


A
Pro-saccade
Anti-saccade
Pump
Methanol
Reservoir
Thermometer
3.2°C
Ice Bath
-78°C
Principal Sulcus
Superior
Colliculus
Cryoloop with
Thermocouple
Contralateral Recording
Ipsilateral Recording
B
Pro-saccades
Anti-saccades
RF
RF
S
Gap Condition
FP
S
Overlap Condition
FP
200 ms

Areas 46 and 9/46d have similar connectivity, such that together they have been referred to as the mid-dlPFC. Within the frontal lobe, the mid-dlPFC is connected with dorsomedial areas 32, 10, 9, 8B and 24, dorsolateral areas 6, 9/46d and 8Ad, ventrolateral areas 46v, 45, and 47/12, and to a limited extent orbital area 11 (Yeterian et al., 2012). Outside the frontal lobe, the mid-dlPFC is connected with auditory-related association areas of the superior temporal gyrus, multimodal areas of the superior temporal sulcus cortex, areas 31, PG, Opt and PGm of the parietal lobe, paralimbic, perirhinal, entorhinal, parahippocampal, and retrosplenial regions. The mid-dlPFC receives afferent projections from the caudal portion of the inferior parietal lobule, including the middle and caudal parts of the lateral bank of the intraparietal sulcus, via the superior longitudinal fasciculus (SLF) II fibre pathway, and sends efferent projections to areas 24, 23, 29, 30, and CMA via the cingulate fasciculus, areas 31, PGm, PEc, and PEci via the SLF I fibre pathway, and parietal areas POa, IPd, PG and PGop via the SLF II fibre pathway (Petrides and Pandya, 2006). The mid-dlPFC has been implicated in the on-line monitoring and manipulation of information in working memory, as demonstrated by monkeys with mid-dlPFC lesions that are impaired on visual working memory tasks (Petrides, 1991, 1995), and human neuroimaging studies in which there was an increase of regional cerebral blood flow at the mid-dlPFC on tasks that required monitoring information in working memory (Owen, 1997). This facilitates organization and planning, among many other executive functions, whereby the mid-dlPFC monitors and manipulates multiple representations in working memory.

3.2.3 Behavioral Task

Three monkeys were trained to perform a randomly-interleaved anti/pro-saccade task in which they were required to look either toward (pro-saccade) or away from (anti-saccade) a peripheral visual stimulus (Fig. 3.2B). The task instruction was provided on each trial by the colour of the central fixation point, either red or green, which the monkey was required to fixate for between 300 and 600 ms. This relatively short fixation period was necessitated by the tendency of one of the animals to break fixation soon after having initiated fixation. With this 300-600 ms fixation period, all animals was able to follow the majority of trials through to completion. For two monkeys (A

and C), a green fixation point signaled a pro-saccade trial, and a red fixation point signaled an anti-saccade trial. These colour instructions were reversed for monkey B so that we could be sure that the animal's behaviour was based on the rule represented by the colour, rather than the colour itself. In the overlap condition, the central fixation point remained visible for the duration of the trial, whereas in the gap condition, the central fixation point was removed 200 ms prior to stimulus appearance. At the end of the fixation period, a white dot stimulus (0.15°) was presented either into the neuron's response field, or at the mirror location on the opposite side of the vertical and horizontal meridian. The animals were required to maintain fixation throughout the fixation and gap periods, then perform the instructed saccade within 500 ms of stimulus appearance. The saccade endpoint was required to fall within a 5° x 5° window that surrounded either the stimulus, on pro-saccade trials, or the mirror location in the opposite visual field, on anti-saccade trials. A correct response was followed immediately by a water reward. The task, behaviour monitoring, and reward delivery were controlled using CORTEX (NIMH, Bethesda, MA) running on two Pentium PCs. Monkeys received water until satiation, after which they were returned to their home cages. Daily records were kept of the weight and health status of the monkeys, and additional water and fruit were provided as needed.

3.2.4 Cryoloop Method of Reversible Cryogenic Deactivation

Cryoloops were constructed from 23-gauge hypodermic stainless steel tubing, designed to deactivate both the upper and lower banks of a sulcus, and implanted bilaterally in the posterior principal sulci. Cryoloops were 4 x 6 mm in dimension, and thus 24 mm^2 of cortical tissue was deactivated on each side of the cryoloop (total 48 mm^2). With an estimated range of 1.5 - 2.0 mm, we calculate that the cryoloops deactivated 72 - 96 mm^3 of cortical tissue. Given the identical design and location of the implanted cryoloops, we assume that the same area of cortical tissue was deactivated in the dlPFC of both the left and right hemispheres. The dlPFC was deactivated by pumping room temperature methanol through teflon tubing that was connected to the cryoloops. This teflon tubing passed through a methanol ice bath that was reduced to subzero temperatures by the addition of dry ice (Fig. 3.2A), then returned the methanol to the

same reservoir from which it came. Chilled methanol pumped through a cryoloop deactivates adjacent cortical tissue by disrupting synaptic activity therein. Given that cortical temperature increases rapidly with distance from a cryoloop (10°C/mm), and evoked neural activity is absent in cortical tissue cooled below 20°C, we chose to maintain the cryoloop temperature at 1-3°C to inactivate as large an area of cortical tissue as possible, while avoiding potentially harmful sub-zero temperatures at the cortical surface (Lomber et al., 1999). The effective spread of cooling, therefore, was restricted to less than 2 mm, and thus each of our cryoloops, 4 mm by 6 mm in dimension, deactivated an estimated volume of 96 mm^3.

Each cooling session consisted of precool, cooling, and postcool periods that ranged from 10 to 15 minutes in duration. A cooling session started with a precool period, after which the pump was turned on. It took an average of 85 seconds for the cryoloop temperature to reach the desired range of 1-3°C. This temperature was monitored with an attached thermocouple and maintained by adjusting flow rate of the peristaltic pump. We excluded the first 3 minutes after the pump was turned on to ensure that the cortical tissue adjacent to the cryoloop was cooled below 20°C, which is the temperature below which neurons are deactivated (Jasper et al., 1970). At the end of the cooling period, the pump was turned off and cryoloop temperature reached 30°C within about 40 seconds. Data collected during a rewarming period, consisting of the first 3 minutes after the pump was turned off, were excluded from all data analysis.

3.2.5 Neuron Recordings

Extracellular single unit activity was recorded in the intermediate layers of the caudal SC (Fig. 3.2A) with a hydraulic microdrive (Narishige International USA Inc., East Meadow, New York, USA), which guided a tungsten microelectrode (UEWLFELMNN1E, FHC Inc., Bowdoin, Maine, USA) through a 23 gauge stainless steel guide tube, that was positioned inside a Delrin grid with 1 mm spacing between adjacent locations (Crist Instrument Inc., Hagerstown, Maryland, USA). The intermediate layers of the SC were identified with previously-described techniques (Everling et al., 1999). Briefly, we listened carefully to the extracellular activity that was detected by a recording electrode which descended slowly through a guide tube.

The end of the guide tube was positioned approximately 5 mm from the surface of the SC, such that there was typically very little activity detected when the electrode first exited the guide tube. As the electrode continued to descend, the surface of the SC was identified by a sudden rush of activity, which was the visual response of cells in the superficial layers of the SC. 1-3 mm below the surface of the SC is where the intermediate layers are found, within which we isolated the activity of a single saccade neuron. The response field of the neuron was determined by presenting a visual stimulus on a screen in front of the animal, and rewarding the animal for making a saccade toward the stimulus. SC saccade neurons were identified as those that discharged a motor burst for a saccade into their response field. To determine the precise location of their response field, electrical microstimulation was applied through the electrode, which evoked a saccade of a particular amplitude and direction. This was then used as the stimulus location for the anti/saccade-pro task, such that the stimulus was presented either in the neuron's response field, or at the mirror-opposite location. Neural activity was amplified, filtered, and stored by a Plexon multichannel acquisition processor (MAP) system (Plexon Inc., Dallas, TX, USA). Offline cluster separation was performed using principal component analysis, which is a statistical technique that identifies patterns (i.e. groups of related activity) in continuously recorded neural data.

3.2.6 Neuron Classification

We examined the effects of unilateral dlPFC deactivation on the activity of SC saccade neurons. The majority of SC saccade neurons that we recorded (66 of 81, 82%) had a 10° horizontal response field. This biased selection was meant to facilitate anti-saccade task performance, given that anti-saccade latency is shortest, and accuracy greatest, for anti-saccades with a horizontal amplitude of 8-10°. Furthermore, the monkeys used in this study had been extensively trained on horizontal saccades for previous studies, such that they had difficulty performing diagonal anti-saccades. For these reasons, we were unable to analyse whether the location of the SC neurons on the motor map was a factor in the changes of neural activity with unilateral dlPFC deactivation, and thus cannot comment on whether the dlPFC has a uniform effect on the SC.

To be classified as a saccade neuron, an isolated cell had to be located 1–3 mm below the dorsal surface of the SC, which was determined as the electrode depth where visual background activity was first noticed. The isolated cell also had to discharge above 100 spikes/s in the saccade epoch (10 ms before to 10 ms after saccade onset) for pro-saccades into the neuron's response field (RF), in both the gap and overlap conditions. Saccade neurons were classified as buildup neurons if they also exhibited low-frequency prestimulus activity in the gap epoch (50 ms before to 50 ms after stimulus presentation), that was significantly greater than during the fixation epoch (100 ms period starting 100 ms before FP disappearance; paired t-test, $p < 0.01$) (Munoz and Wurtz, 1995; Dorris et al., 1997), on pro-saccade trials in the gap condition. Neurons were classified as having a visual response if their activity increased by more than 20 spikes/s during the visual epoch (50 ms to 150 ms after stimulus presentation) as compared with a baseline epoch (50 ms before to 50 ms after stimulus presentation), on correct anti-saccade trials in the overlap condition, when the stimulus was presented in the neuron's response field.

3.2.7 Eye Movements

Eye movements were monitored at 500 Hz with high-speed infrared video eye tracking (Eyelink, SR Research, Kanata, Canada). All analyses were performed offline using custom-written software in Matlab (Mathworks, Natick, MA). The start and end of a saccade were defined in CORTEX as the time at which radial eye velocity exceeded, then returned below, 30°/s. Trials were labelled as either correct, incorrect, or invalid by CORTEX, then verified by visual examination of the eye traces from each session.

3.2.8 Spike Density Function

To evaluate the relationship between neural activity and onset of both the stimulus and saccade, continuous spike density functions were constructed with a resolution of 1 ms. The activation waveform was obtained by convolving each spike with an asymmetric function that resembled a postsynaptic potential (Hanes and Schall, 1996; Thompson et al., 1996). The advantage of this function over a standard Gaussian function (Richmond

and Optican, 1987) is that it accounts for the fact that spikes exert an effect forward but not backward in time.

3.2.9 Time Course of dlPFC Deactivation

To determine the time course of the effects of dlPFC deactivation on the population activity of SC neurons, we performed sliding receiver operating characteristics (ROC) analyses. For analysis of the time course relative to stimulus onset, an ROC value was calculated for a 10 ms epoch (centered on the time point) beginning 200 ms before stimulus presentation, using the convolved spike trains. This analysis was repeated in 1 ms increments until 300 ms after stimulus presentation. A single ROC time course was calculated for each neuron separately, then averaged across all SC neurons. Statistical significance of ROC values was tested using a bootstrap analysis. For this analysis, the following procedure was repeated 1,000 times: for each neuron, a random decision was made to either exchange the two activation conditions (dlPFC+ and dlPFC-) (50% probability) or leave them unchanged (50% probability). Each of the 1,000 repetitions of the analysis, performed on all SC neurons, yielded a single average time-course. The 95^{th} and 5^{th} percentile values of the distribution of 1,000 average ROC values for each time point were used to determine the 5% significance criterion. Both were plotted together with the average ROC time course of the non-randomized data.

3.2.10 Onset of Motor Activity

The onset of the motor burst was determined using a Poisson spike train analysis (Hanes et al., 1995), implemented using Matlab code developed by the Schall laboratory (http://www.psy.vanderbilt.edu/faculty/schall/scientific-tools/). On a trial-by-trial basis, the Poisson spike train analysis identifies the time at which there was a significant change in neuronal activity. A random Poisson distribution, which approximates the inter-spike interval of a neuron, was derived from the mean discharge rate of the neuron. Moving forward in time along the neuron's spike train, individual spikes were added until a significantly greater rate of discharge was found than would be expected with a random Poisson distribution.

3.3 – Results

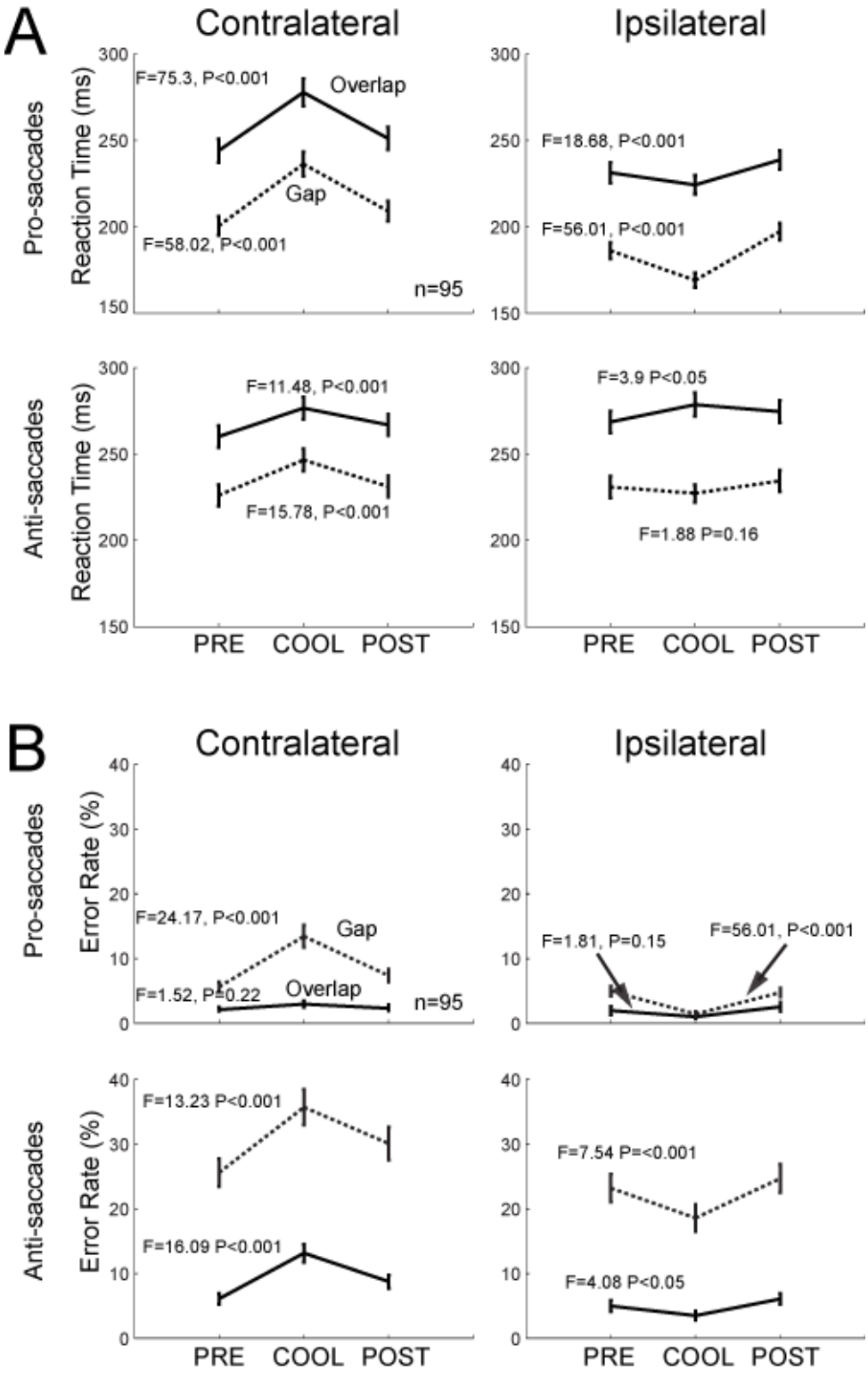
In each of the 95 experimental sessions, we recorded the activity of a single SC neuron and deactivated the dlPFC while a monkey performed a randomly-interleaved anti/pro-saccade task. With three monkeys we recorded 52 neurons from the SC ipsilateral to dlPFC deactivation, and 43 neurons from the SC contralateral to deactivation. Neural activity was recorded throughout the precool, cooling, and postcool periods, each of which were between 10 and 15 minutes in duration.

3.3.1 dlPFC Deactivation Affected Reaction Times and Error Rates

We observed effects of unilateral dlPFC deactivation on reaction times and error rates of pro-saccades and anti-saccades in both the gap and overlap conditions (Fig. 3.3). These behavioral effects were similar to those found with unilateral dlPFC deactivation in the preceding chapter (*Chapter 2*). Consistent with previous studies, we observed shorter reaction times of pro-saccades and anti-saccades in the gap compared with the overlap condition (Everling et al., 1999; Bell et al., 2000; Everling and Munoz, 2000). More importantly for the present study, there were increased reaction times for pro-saccades and anti-saccades directed contralateral to the side of dlPFC deactivation, in both the gap and overlap conditions ($p < 0.001$, ANOVA) (Fig. 3.3A). For ipsilateral saccades, dlPFC deactivation decreased pro-saccade reaction times in both the gap and overlap conditions ($p < 0.001$, ANOVA), and increased anti-saccade reaction times in the overlap condition ($p < 0.05$, ANOVA), but not the gap condition.

We also calculated the effects of dlPFC deactivation on error rates (Fig. 3.3B). For this measure, we included only trials in which the monkeys commenced central fixation, maintained fixation throughout the fixation and gap periods, and made a saccade either toward or away from the peripheral stimulus. Consistent with previous reports in monkeys and humans, we observed more direction errors on the anti-saccade than pro-saccade task, and more errors in the gap than overlap condition (Everling et al., 1998; Everling et al., 1999; Bell et al., 2000; Everling and Munoz, 2000). There was an increased incidence of errors directed toward and thus ipsilateral to the side of dlPFC deactivation, on contralateral anti-saccade trials in the overlap condition, and both

Figure 3.3 – Effects of dlPFC Deactivation on Saccadic Reaction Times and Errors
A: Saccadic reaction times for pro-saccades (*top row*) and anti-saccades (*bottom row*) in the gap (*dashed lines*) and overlap *(solid lines*) conditions, directed contralateral (*left column*) or ipsilateral (*right column*) to the side of dlPFC deactivation, in the precool (PRE), cooling, (COOL), and postcool (POST) periods. Significance was tested with a one-way repeated measures ANOVA (factors PRE, COOL, POST).
B: Same as in *A*, but for error rates (saccades in the wrong direction).


A
Contralateral
Ipsilateral
Pro-saccades
Anti-saccades
Reaction Time (ms)
F=75.3, P<0.001
Overlap
Gap
F=58.02, P<0.001
n=95
F=18.68, P<0.001
F=56.01, P<0.001
F=11.48, P<0.001
F=15.78, P<0.001
F=3.9 P<0.05
F=1.88 P=0.16
PRE COOL POST
B
Error Rate (%)
F=24.17, P<0.001
Gap
F=1.52, P=0.22
Overlap
n=95
F=1.81, P=0.15
F=56.01, P<0.001
F=13.23 P<0.001
F=16.09 P<0.001
F=7.54 P=<0.001
F=4.08 P<0.05

contralateral pro-saccade and contralateral anti-saccade trials in the gap condition ($p < 0.001$, ANOVA). There was also a decreased incidence of contralateral errors on ipsilateral anti-saccade trials in the overlap condition ($p < 0.05$, ANOVA), and both ipsilateral pro-saccade and ipsilateral anti-saccade trials in the gap condition ($p < 0.001$, ANOVA). Sham sessions were performed in which either the pump remained off during the cooling period, or room temperature methanol was pumped through the cryoloops, which reduced their temperature to approximately 27°C. There were no effects of sham deactivation on the performance, kinematics, or metrics of saccades.

While unilateral dlPFC deactivation had significant effects on reaction times and error rates (Fig. 3.3), more general aspects of task performance (percentage of skipped trials, fixation breaks, no response trials) were unaffected, indicating that the motivation and vigilance of the animals remained unimpaired (Fig. 3.4). In summary, dlPFC deactivation increased the reaction times of contralateral saccades, decreased the reaction times of ipsilateral pro-saccades, and increased the incidence of ipsilateral saccades on both pro-saccade and anti-saccade trials. These results suggest that dlPFC deactivation decreased neural activity in saccade-related areas located ipsilateral to deactivation, and increased neural activity in contralateral saccade-related areas. In the following sections we contrast data from the cooling period (dlPFC- period) with data from the precool and postcool periods combined (dlPFC+), to identify the effects of unilateral dlPFC deactivation on the activity of SC saccade neurons.

3.3.2 dlPFC Deactivation Increased Prestimulus Activity in the Contralateral SC

To investigate the effects of unilateral dlPFC deactivation on SC preparatory activity, we compared the activity of SC buildup neurons in the gap epoch (50 ms before to 50 ms after stimulus presentation) during the dlPFC+ and dlPFC- periods. Buildup neurons were analysed because this type of saccade neuron exhibits prominent prestimulus 'preparatory' activity that is negatively correlated with saccade reaction times (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1999). For this analysis, we combined trials in which the subsequent stimulus appeared either into or opposite to the neuron's RF. Figure 3.5A shows the activity of 15 SC buildup neurons, located ipsilateral to the side of dlPFC deactivation, on pro-saccade trials. Consistent with

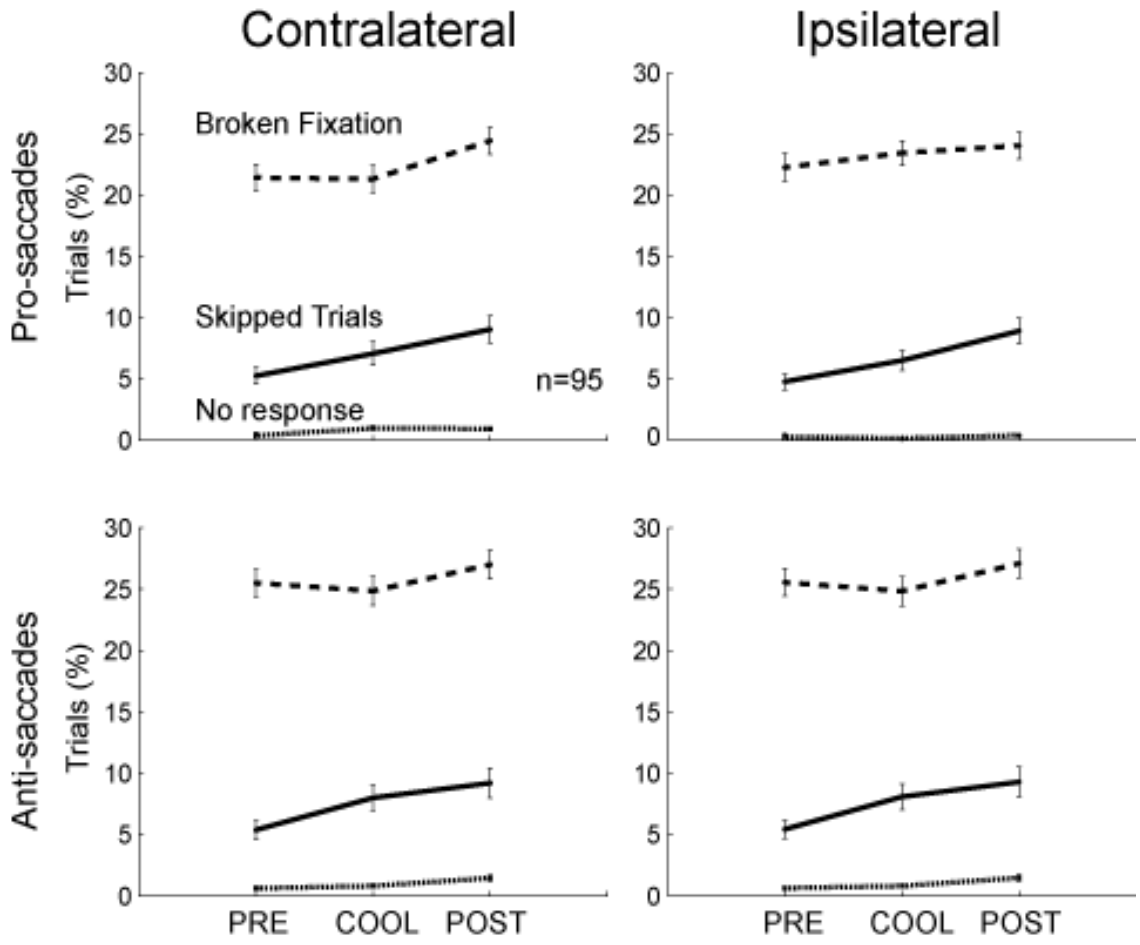


Figure 3.4 – Effects of dlPFC Deactivation on Behavioral Motivation

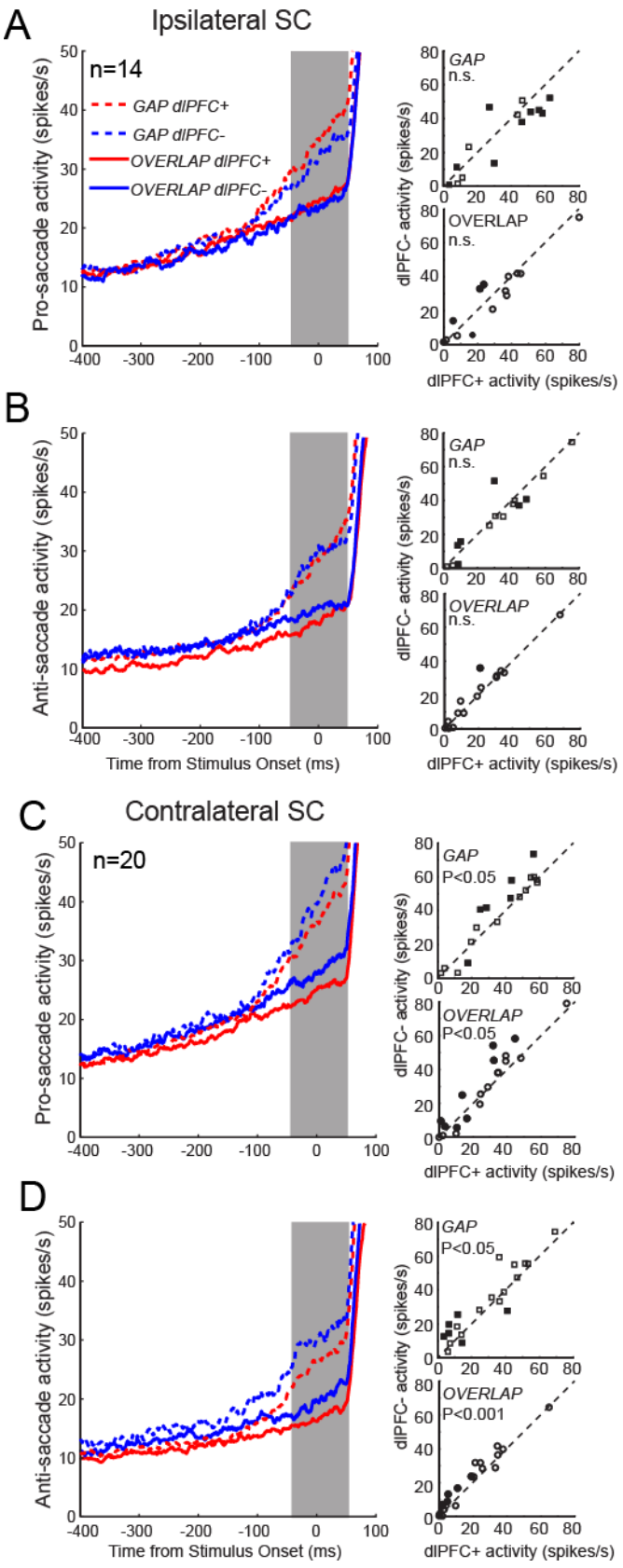
Percentages of broken fixations (*dashed lines*), skipped trials (*solid lines*), and no response trials (*dotted lines*), directed contralateral (*left column*) or ipsilateral (*right column*) to the side of dlPFC deactivation, in the precool (PRE), cooling, (COOL), and postcool (POST) periods.

Figure 3.5 – Effects of dlPFC Deactivation on Prestimulus Activity in the SC

A: Mean spike density of buildup neurons in the SC ipsilateral to dlPFC deactivation (*left plot*), on pro-saccade trials (correct and error trials combined) in the gap (*dashed lines*) and overlap (*solid lines*) conditions, in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods. The mean activity of individual neurons from the period 50 ms before to 50 ms after stimulus onset (*shaded area* in *left plot*) is plotted for the dlPFC+ period against the dlPFC- period, in the gap (*upper plot)* and overlap (*lower plot*) conditions. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $p < 0.05$). The *diagonal dashed line* represents the line of unity (slope, 1).

B: Same as in *A*, but on anti-saccade trials.

C, *D*: Same as in *A* and *B*, but with buildup neurons from the SC contralateral to dlPFC deactivation.


A
Ipsilateral SC
n=14
GAP dlPFC+
GAP dlPFC-
OVERLAP dlPFC+
OVERLAP dlPFC-
Pro-saccade activity (spikes/s)
GAP
n.s.
OVERLAP
n.s.
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
B
Anti-saccade activity (spikes/s)
Time from Stimulus Onset (ms)
C
Contralateral SC
n=20
GAP
P<0.05
OVERLAP
P<0.05
D
GAP
P<0.05
OVERLAP
P<0.001

previous reports (Dorris et al., 1997; Everling et al., 1999), there was a greater buildup of prestimulus activity in the gap condition than the overlap condition. Although dlPFC deactivation had no significant effect on the population activity of ipsilateral SC buildup neurons in the gap (37.1 ± 6.4 vs. 33.4 ± 6.0 spikes/s, $p = 0.15$; Wilcoxon signed rank test) or overlap (25.8 ± 5.7 vs. 25.2 ± 5.4 spikes/s, $p = 0.77$; Wilcoxon signed rank test) conditions, 7 of 15 (47%) ipsilateral SC buildup neurons showed significantly reduced levels of prestimulus activity with dlPFC deactivation in the gap condition ($p < 0.05$, Wilcoxon rank sum test). On anti-saccade trials (Fig. 3.5B), there were no effects of dlPFC deactivation on the prestimulus activity of ipsilateral SC buildup neurons in the gap (31.2 ± 5.5 vs. 30.5 ± 5.5 spikes/s, $p = 0.70$; Wilcoxon signed rank test) or overlap (19.9 ± 4.6 vs. 21.2 ± 4.7 spikes/s, $p = 0.29$; Wilcoxon signed rank test) conditions.

Prestimulus activity from the sample of 20 SC buildup neurons, located contralateral to the side of dlPFC deactivation, increased on pro-saccade trials in the gap (39.1 ± 4.9 vs. 43.5 ± 5.6 spikes/s, $p < 0.05$; Wilcoxon signed rank test) and overlap (25.7 ± 4.3 vs. 29.4 ± 5.0 spikes/s, $p < 0.05$; Wilcoxon signed rank test) conditions (Fig. 3.5C). There was also an increase of prestimulus activity on anti-saccade trials in the gap (27.8 ± 4.4 vs. 31.8 ± 4.5 spikes/s, $p < 0.05$; Wilcoxon signed rank test) and overlap (17.7 ± 3.8 vs. 20.8 ± 3.8 spikes/s, $p < 0.005$; Wilcoxon signed rank test) conditions (Fig. 3.5D), and thus dlPFC deactivation increased prestimulus activity in the contralateral SC on both pro-saccade and anti-saccade trials.

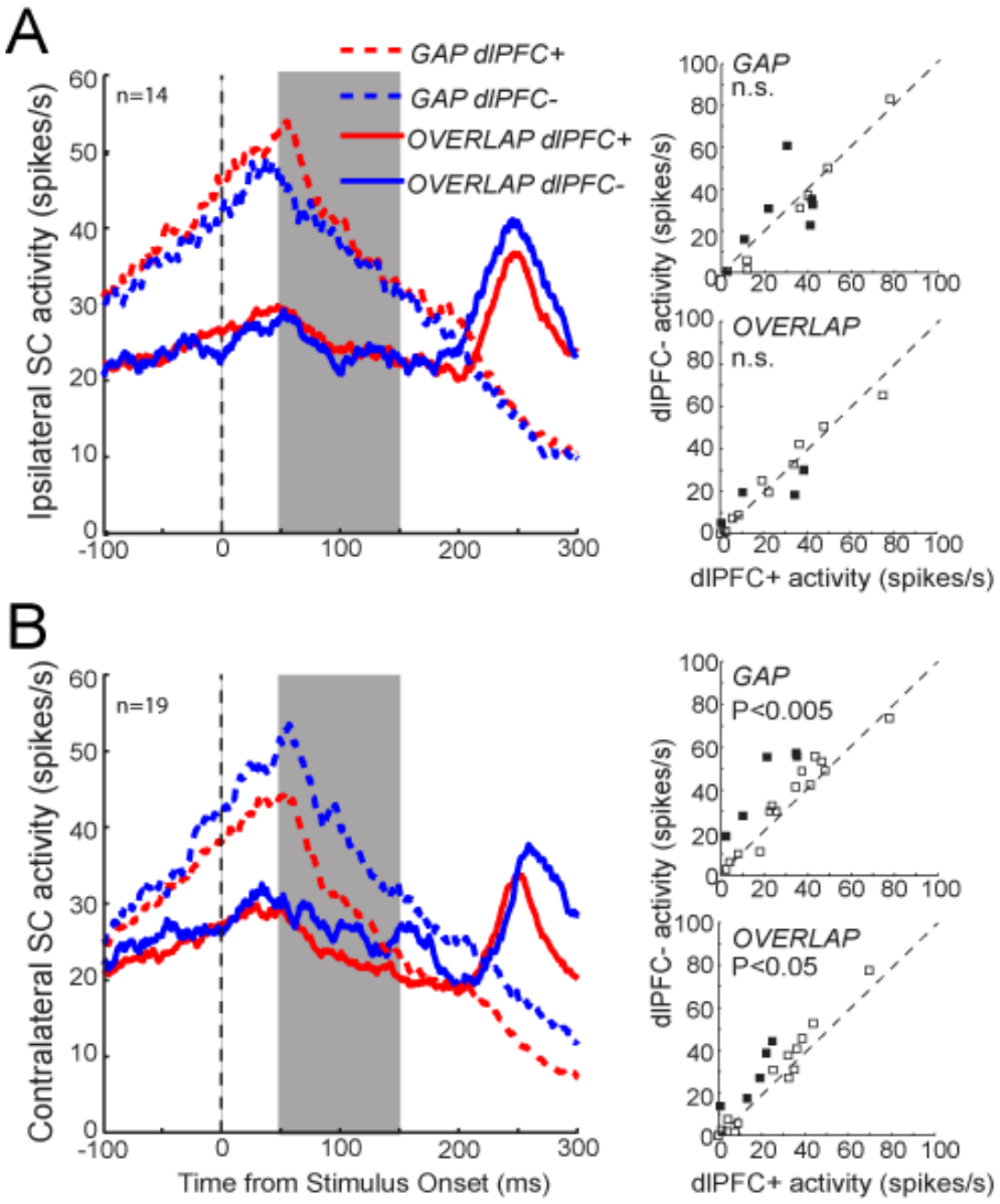
3.3.3 Prestimulus Activity of SC Buildup Neurons Persisted Beyond Stimulus Onset

The preceding section showed that unilateral dlPFC deactivation increased the prestimulus activity of buildup neurons in the SC contralateral to deactivation. To test whether these differences extended beyond the gap epoch (50 ms to 50 ms after stimulus onset), we analysed the ensuing visual epoch (50 ms to 150 ms after stimulus onset) on correct pro-saccade trials when the stimulus appeared opposite to the neuron`s RF. For the sample of 14 ipsilateral SC buildup neurons, there was a decrease of activity starting at about 50 ms after stimulus onset (Fig 3.6A), which coincides with the onset of stimulus-related activity in SC neurons (Everling et al., 1999). We found no effect of dlPFC deactivation on either gap (30.0 ± 5.6 vs. 29.0 spikes/s, $p = 0.75$; Wilcoxon

Figure 3.6 – Effects of dlPFC Deactivation on SC Activity Following Stimulus Onset

A: Mean spike density of buildup neurons in the SC ipsilateral to dlPFC deactivation (*left plot*), on correct pro-saccade trials when the stimulus appeared opposite to the neurons' response field, in the gap (*dashed lines*) and overlap (*solid lines*) conditions, of the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods. The mean activity of individual neurons from the period 50 ms to 150 ms after stimulus onset (*shaded area* in *left plot*) is plotted for the dlPFC+ period against the dlPFC- period, in the gap (*upper plot)* and overlap (*lower plot*) conditions. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $P<0.05$). The *diagonal dashed line* represents the line of unity (slope, 1).

B: Same as in *A*, but with buildup neurons from the SC contralateral to dlPFC deactivation.


A
GAP dlPFC+
GAP dlPFC-
OVERLAP dlPFC+
OVERLAP dlPFC-
n=14
Ipsilateral SC activity (spikes/s)
GAP
n.s.
OVERLAP
n.s.
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
B
n=19
Contralateral SC activity (spikes/s)
GAP
P<0.005
OVERLAP
P<0.05
Time from Stimulus Onset (ms)

signed rank test) or overlap (23.9 ± 5.8 vs. 23.2 ± 5.2 spikes/s, $p = 0.72$; Wilcoxon signed rank test) trials. For the sample of 19 contralateral SC buildup neurons (Fig. 3.6B), there was greater activity in the visual epoch with dlPFC deactivation (dlPFC-) than without (dlPFC+), in both the gap (28.1 ± 4.4 vs. 39.9 ± 4.7 spikes/s, $p < 0.005$; Wilcoxon signed rank test) and overlap (22.1 ± 4.2 vs. 26.5 ± 4.8 spikes/s, $p < 0.05$; Wilcoxon signed rank test) conditions. These findings show that the increase of prestimulus activity in the SC contralateral to dlPFC deactivation persisted beyond stimulus onset.

3.3.4 dlPFC Deactivation Increased Stimulus-related Activity in the Contralateral SC on Anti-saccade Trials

We next investigated whether unilateral dlPFC deactivation affected the activity of SC saccade neurons when a visual stimulus was presented in their RF. To determine the time course of dlPFC deactivation effects, we conducted ROC and bootstrap analyses (see *Section 3.2.8*). Figure 3.7A depicts the population activity of 27 saccade neurons from the SC ipsilateral to dlPFC deactivation on anti-saccade trials. Consistent with our analysis of prestimulus activity (see *Section 3.3.2*), dlPFC deactivation caused only a very brief reduction of stimulus-related activity in the ipsilateral SC. In contrast, when we examined the activity of saccade neurons in the SC contralateral to dlPFC deactivation (Fig. 3.7B), we observed an increase of stimulus-related activity between 80 and 200 ms after stimulus onset. These results demonstrate that on anti-saccade trials, unilateral dlPFC deactivation increased stimulus-related activity in the contralateral SC.

To directly evaluate whether the increased stimulus-related activity of contralateral SC saccade neurons could account for the increased incidence of ipsilateral errors on anti-saccade trials (see *Section 3.3.1*), we compared stimulus-related activity between correct and error trials. For this analysis we combined the data from gap and overlap trials, and included only the SC saccade neurons for which there were at least 5 correct and 5 error trials. Figure 3.8 shows the population activity of 13 contralateral SC saccade neurons on correct trials (*solid lines*) and error trials (*dashed line*) in the deactivation (dlPFC-) period. On correct trials the stimulus-related response was

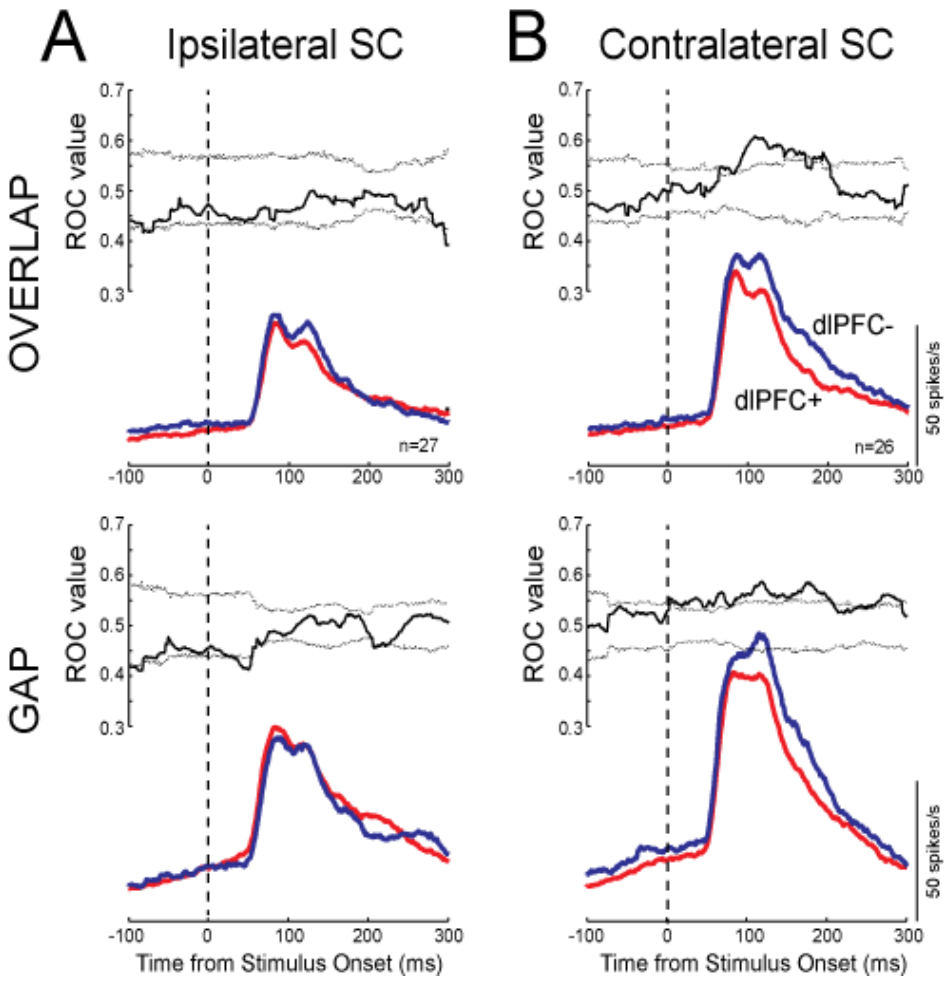


Figure 3.7 – Time Course of dlPFC Deactivation Effects on Stimulus-related Activity in the SC on Anti-saccade Trials

A: Mean spike density of saccade neurons in the SC ipsilateral to dlPFC deactivation, on anti-saccade trials (correct and error trials combined) when the stimulus was presented in the neurons' response field, in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods, of the gap (*lower plot*) and overlap (*upper plot*) conditions. Also plotted is the time course of average population ROC values for comparison of the dlPFC+ and dlPFC- periods (*solid line*). Dotted lines represent percentile values obtained from a bootstrap analysis, and thus time points with significant differences ($p < 0.05$) are found where the *solid line* is either above or below the *dotted lines*.

B: Same as in *A*, but with saccade neurons from the SC contralateral to dlPFC deactivation.

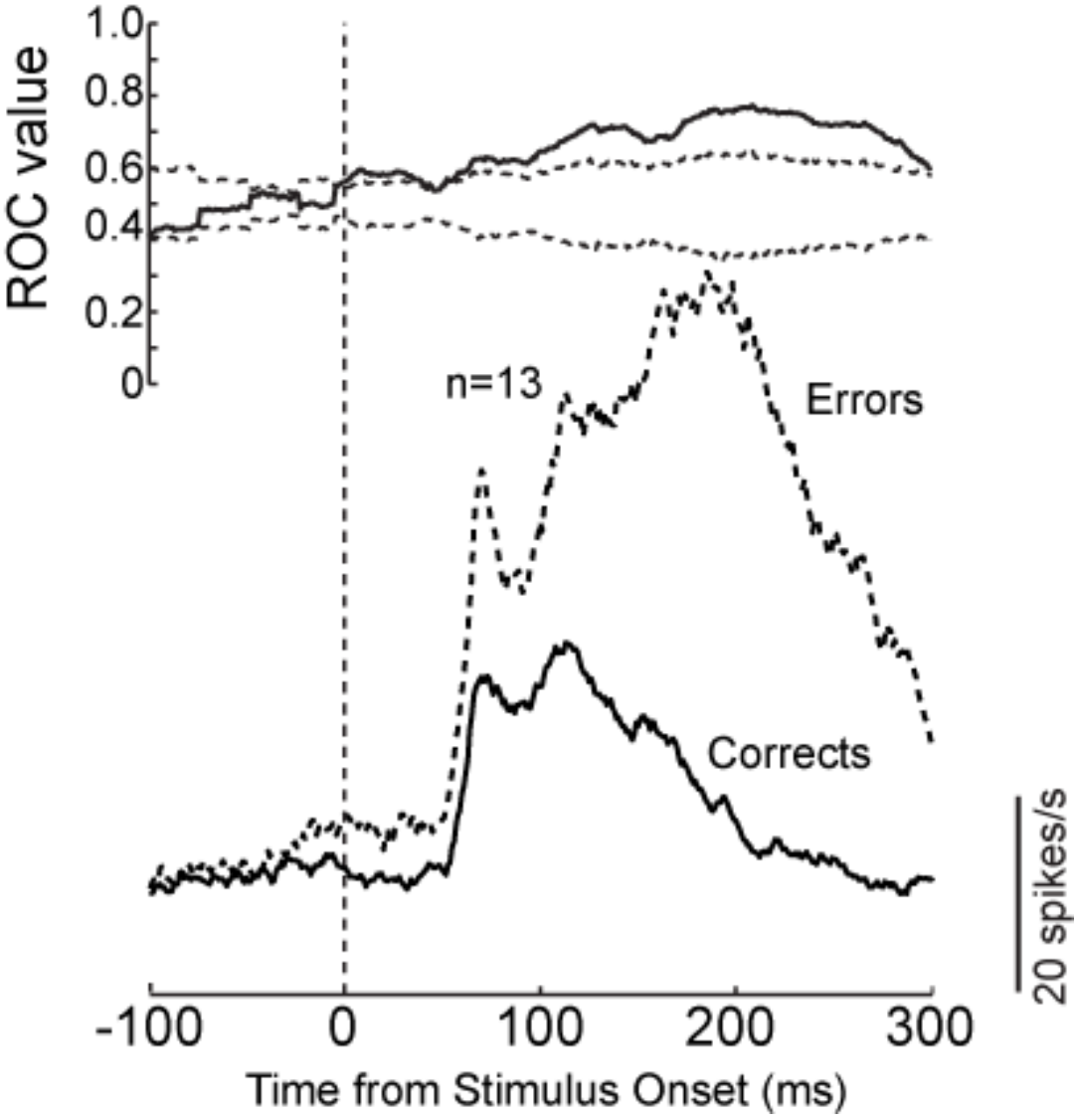


Figure 3.8 – Activity in the SC Contralateral to dlPFC Deactivation: Correct vs. Error Trials

Mean spike density of saccade neurons in the SC contralateral to dlPFC deactivation, on anti-saccade trials (gap and overlap conditions combined) when the stimulus was presented in the neurons' response field, on correct (*solid line, lower plot*) and error (*dashed line, lower plot*) trials in the dlPFC- period. Also plotted is the time course of average population ROC values for comparison of the correct and error trials (*solid line, upper plot*). Dashed lines (*upper plot*) represent percentile values obtained from a bootstrap analysis, and thus time points with significant differences ($p < 0.05$) are found where the *solid line* is either above or below the *dotted lines*.

quickly suppressed, whereas on error trials there was a larger stimulus-related response and subsequent increase in neural activity. An ROC analysis confirmed that these differences were statistically significant ($p < 0.05$), and also showed that on error trials, there were increased levels of activity immediately before the arrival of the visual signal in the SC. Together these results show that erroneous responses were associated with increased levels of prestimulus and stimulus-related activity in SC saccade neurons.

3.3.5 dlPFC Deactivation Delayed Onset of Saccade-related Activity in the Ipsilateral SC

Our behavioral analysis showed that dlPFC deactivation increased contralateral reaction times and decreased ipsilateral reaction times on pro-saccade trials (see *Section 3.3.1*). To identify the neural correlates of reaction time effects in the activity of SC saccade neurons, we used a Poisson spike train analysis (see *Section 3.2.9*) to compare the onset times of saccade-related activity between the noncool (dlPFC+) and cooling (dlPFC-) periods. We included only SC saccade neurons with no or little stimulus-related activity in this analysis, to ensure that the algorithm detected the onset of the saccadic motor burst and not stimulus-related activity. From the sample of 16 saccade neurons in the SC ipsilateral to dlPFC deactivation, we can see in Figure 3.9A that saccade-related activity increased later in the dlPFC- period (*blue lines*) than the dlPFC+ period (*red lines*), in both the gap (*thin lines*) and overlap (*thick lines*) conditions. Consistent with this, the Poisson spike train analysis showed that saccade-related activity in the ipsilateral SC began later in the dlPFC- period than the dlPFC+ period, in both the gap (Fig. 3.9B; 192.0 ± 19.2 vs. 169.3 ± 17.9 ms, $p < 0.05$; Wilcoxon signed rank test) and overlap (Fig. 3.9C; 253.5 ± 20.3 vs. 220.3 ± 13.8 ms, $p < 0.01$; Wilcoxon signed rank test) conditions. These differences reached statistical significance in 4 of 16 (25%) neurons in the gap condition, and in 7 of the 16 (44%) neurons in the overlap condition. This delayed onset of saccade-related activity in the SC ipsilateral to deactivation corresponded with the increased reaction time of contralateral saccades. Conversely, Figure 3.9D shows that saccade-related activity in the SC contralateral to dlPFC deactivation seemed to increase earlier in the dlPFC- period than the dlPFC+ period. These differences were significant in 2 of 11 (18%) neurons in the gap condition, and in

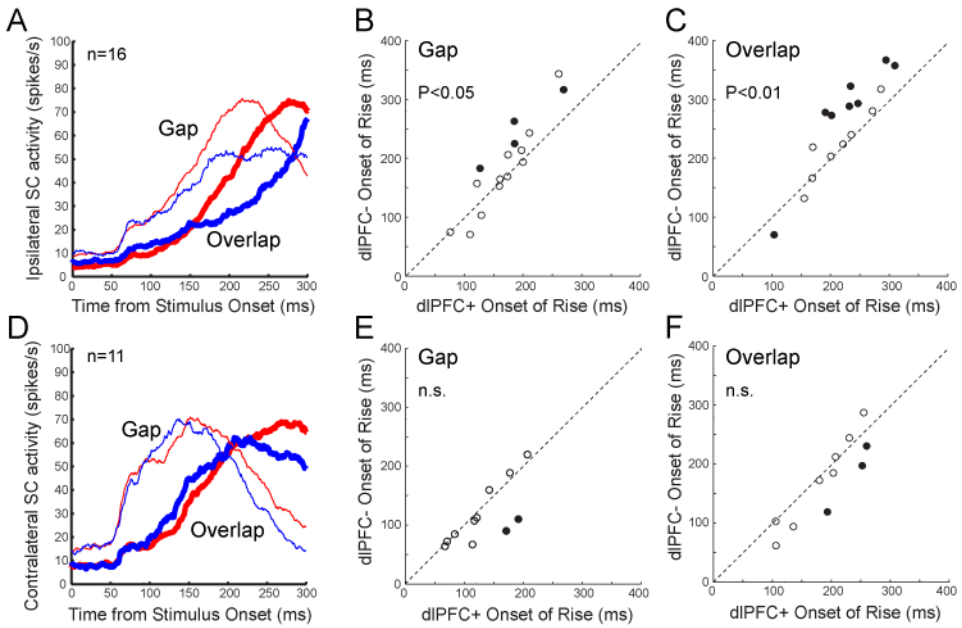


Figure 3.9 – Effects of dlPFC Deactivation on the Onset Latency of Saccade-related Activity in the SC

A: Mean spike density of saccade neurons in the SC ipsilateral to dlPFC deactivation, for pro-saccades toward a stimulus presented in the neurons' response field, in the gap (*thin lines*) and overlap (*thick lines*) conditions, of the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods.

B: Mean onset latency of the saccade-related activity from individual neurons in the SC is plotted for the dlPFC+ period against the dlPFC- period, in the gap condition. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $P<0.05$). The *diagonal dashed line* represents the line of unity (slope, 1).

C: Same as in *B*, but in the overlap condition.

D, *E*, *F*: Same as *A*, *B*, and *C*, but with saccade neurons from the SC contralateral to dlPFC deactivation.

3 of 11 (27%) neurons in the overlap condition ($P<0.05$, Wilcoxon rank sum test), but did not reach significance for the population of neurons in either the gap (Fig. 3.9E; 116.0 ± 15.6 vs. 133.2 ± 14.7 spikes/s, $p = 0.46$; Wilcoxon signed rank test) or overlap (Fig. 3.9F, 174.6 ± 21.5 vs. 193.6 ± 17.1 spikes/s, $p = 0.10$;Wilcoxon signed rank test) conditions. Therefore the onset of saccade-related activity was delayed in the SC ipsilateral to deactivation, and may have occurred earlier in the contralateral SC, however this latter finding did not reach significance.

3.3.6 Saccade Threshold was not Affected by dlPFC Deactivation

We showed earlier that the prestimulus activity of saccade neurons was increased in the contralateral SC, and decreased in the ipsilateral SC, by dlPFC deactivation (see *Section 3.3.2*). We then showed that dlPFC deactivation delayed onset of saccade-related activity in the ipsilateral SC (see *Section 3.3.5*). Here we tested whether unilateral dlPFC deactivation affected presaccadic motor activity in the SC. This analysis was performed on correct pro-saccade trials in the overlap condition, for which the saccade was directed into the neurons' RF. Based on previous physiological and anatomical studies, the latest time at which saccade initiation can be influenced by a neural signal from the SC is between 8 and 18 ms prior to saccade onset (Segraves and Goldberg, 1987; Munoz and Wurtz, 1993; Miyashita and Hikosaka, 1996; Munoz et al., 1996). Figure 3.10 shows that the activity in this time window did not differ between the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods, ($p > 0.05$, Wilcoxon-signed rank test), in the SC either ipsilateral or contralateral to dlPFC deactivation, which suggests there was no effect of dlPFC deactivation on the saccade threshold.

3.4 – Discussion

3.4.1 A Direct Test of the Inhibition Model

An inhibitory model of prefrontal function has proposed that, given the corticotectal projections of the dorsolateral prefrontal cortex (dlPFC) (Goldman and Nauta, 1976; Leichnetz et al., 1981), unwanted saccades are inhibited by directly enhancing the activity of either fixation neurons in the rostral superior colliculus (SC), or inhibitory

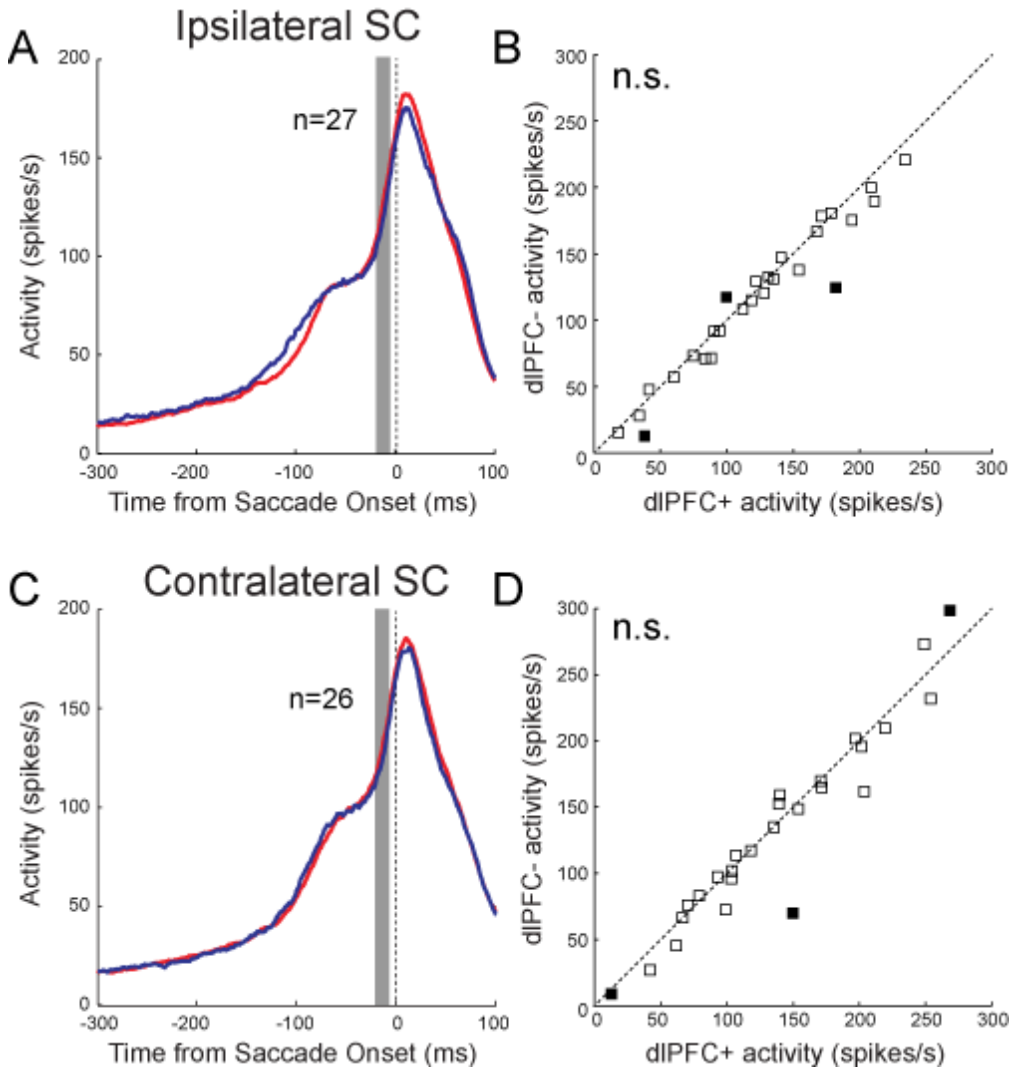


Figure 3.10 – Effects of dlPFC Deactivation on Saccade Threshold in the SC

A: Mean spike density of saccade neurons in the SC ipsilateral to dlPFC deactivation, for pro-saccades toward a stimulus presented in the neurons' response field, in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods of the overlap condition.

B: The mean activity of individual neurons from the period 18 ms to 8 ms before saccade onset (*shaded area* in *A*) is plotted for the dlPFC+ period against the dlPFC- period. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $p < 0.05$). The *diagonal dashed line* represents the line of unity (slope, 1).

C, *D*: Same as in *A* and *B*, but with saccade neurons from the SC contralateral to dlPFC deactivation.

Analysed task performance as % correct rather than % error because (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Ploner et al., 2005) that have an increased incidence of anti-saccade errors, and monkey neurophysiology studies that have investigated the response properties of identified corticotectal neurons (Johnston and Everling, 2006). The inhibition model predicts that unilateral deactivation of the dlPFC would increase the activity of saccade neurons in the ipsilateral SC, which by interhemispheric inhibition at the level of either cortical or collicular structures (Munoz and Istvan, 1998; Schlag et al., 1998; Takahashi et al., 2005; Palmer et al., 2012), would also decrease the activity of saccade neurons in the contralateral SC (Fig. 3.1). We performed a direct test of the inhibition model and found that contrary to these predictions, unilateral dlPFC deactivation increased the prestimulus activity of saccade neurons in the contralateral SC, on both pro-saccade and anti-saccade trials. There was also an increase of stimulus-related activity in the contralateral SC, on anti-saccade trials when the stimulus appeared ipsilateral to dlPFC deactivation, and delayed onset of the motor burst in the ipsilateral SC. These effects on the activity of SC saccade neurons corresponded with decreased reaction times for ipsilateral saccades, an increased incidence of ipsilateral errors on anti-saccade trials, and increased reaction times for contralateral saccades.

3.4.2 An Excitatory Influence on the Oculomotor System

Unilateral muscimol deactivation of the dlPFC has also been shown to increase contralateral anti-saccade reaction times and the incidence of ipsilateral errors on anti-saccade trials (Condy et al., 2007), but unlike unilateral cryogenic deactivation did not have any effects on pro-saccades. This difference may be attributed to the considerably larger spatial extent of cortical tissue deactivated by cryoloops as compared with single muscimol injections (Lomber, 1999). Condy and colleagues (2007) interpreted their results as being consistent with the inhibitory model of prefrontal function, by proposing that muscimol led to a paradoxical increase in the activity of dlPFC output neurons and thus suppression of SC saccade neurons. However, the fact that we obtained such similar effects rules out this explanation, given that cryogenic deactivation disrupts synaptic transmission and thus reduces dlPFC output (Jasper et al.,

1970; Moseley et al., 1972). Furthermore, the effects of unilateral dlPFC deactivation were opposite to those of dlPFC microstimulation, which had an excitatory influence on the oculomotor system (Wegener et al., 2008), suggesting that dlPFC deactivation reduced rather than increased dlPFC output. The compatibility of our current findings with the results of these pharmacological deactivation and electrical microstimulation studies provides strong evidence against the inhibition model.

In principle, dlPFC deactivation could have disinhibited saccade neurons in the contralateral SC by enhancing dlPFC output neurons that synapse with inhibitory interneurons in the rostral fixation zone of the ipsilateral SC, that send inhibitory projections to the rostrocaudal extent of the contralateral SC (Takahashi et al., 2005). While this mechanism could account for the changes in neural activity and behaviour that we observed here, it would be difficult to conceptualize the function of this mechanism with respect to the anti-saccade task. The majority of dlPFC neurons with stimulus-related activity have a strong preference for stimuli presented in their contralateral visual field (Funahashi et al., 1989, 1990; Everling et al., 2006), which under normal conditions would inhibit the activity of neurons in the contralateral SC that generate the motor command for the correct anti-saccade. This pattern of activity would not be conducive to anti-saccade task performance.

Alternatively, an explanation for the effects of cryogenic deactivation, pharmacological deactivation, and electrical microstimulation on anti-saccade task performance could be that there is an excitatory influence of dlPFC output neurons on ipsilateral SC saccade neurons that facilitates contralateral saccades. This effect could be mediated by direct projections to the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981), or indirectly by dlPFC projections to cortical saccade-related areas in the same hemisphere, which themselves have corticotectal projections (Selemon and Goldman-Rakic, 1988; Cavada and Goldman-Rakic, 1989; Bates and Goldman-Rakic, 1993). A temporary removal or reduction of dlPFC output would decrease the activity of saccade neurons in the ipsilateral SC, and as the result of reduced intercollicular inhibition (Munoz and Istvan, 1998; Takahashi et al., 2005), allow an increase of saccade neuron activity in the contralateral SC (Fig. 3.1). A concurrent reduction of interhemispheric cortical inhibition would allow an increase of dlPFC activity on the contralateral side

(Schlag et al., 1998; Palmer et al., 2012), which would further increase the activity of saccade neurons in the contralateral SC. In support of this, we found a delayed onset of saccade-related activity in the SC ipsilateral to deactivation, and an increase of both prestimulus and stimulus-related activity in the SC contralateral to deactivation.

3.4.3 Contralateral Shifts of Attention and Gaze

In addition to contralateral saccades, the dlPFC also facilitates contralateral shifts of attention, as demonstrated by single neuron recording studies with monkeys (Everling et al., 2002; Kaping et al., 2011). In one study, monkeys maintained central fixation while viewing simultaneous streams of pictures presented left and right of central fixation. Their task was to generate a saccade toward a target stimulus when it appeared in the stream of images at a previously-cued side. Many dlPFC neurons had an increased response to target stimuli that were presented at the attended side, the majority of which preferred stimuli presented in the contralateral visual field (Everling et al., 2002). More recently it has been shown that dlPFC neurons, in and around the area of the principal sulcus, respond to covert attentional shifts towards contralateral targets (Kaping et al., 2011). Monkey neurophysiology studies have shown that dlPFC neurons exhibit persistent delay activity in oculomotor delayed response tasks (Fuster and Alexander, 1971; Funahashi et al., 1989), which is thought to carry a retrospective representation of stimulus location in some neurons, and a prospective signal for the forthcoming saccade in others (Funahashi et al., 1993). Analogously, human neuroimaging studies have reported persistent activation at the middle frontal gyrus, which is the putative human homologue of the principal sulcus region in monkeys, during the delay period of oculomotor delayed response tasks (Leung et al., 2002; Brown et al., 2004). Furthermore, an increase of sustained delay activation was found at the middle frontal gyrus when covert attention was maintained on a stimulus, and for which there was a contralateral bias (Ikkai and Curtis, 2011). From this it was concluded that the dlPFC, like the posterior parietal cortex (PPC), contains a prioritized map of space that is used to guide attention allocation, spatial memory, and motor planning. This interpretation is consistent with the notion of a dorsal frontoparietal network that underlies spatial attention, stimulus salience, and saccades (Corbetta and Shulman, 2011). Together these

studies suggest that the dlPFC contributes to a spatial priority map which, in conjunction with the PPC, guides contralateral shifts of both attention and gaze.

3.4.4 Task Set: Encoding and Maintenance

The above explanation, however, does not account for the human neuroimaging studies that have found greater rCBF or BOLD activation at the dlPFC for anti-saccade trials than pro-saccade trials (Sweeney et al., 1996; Doricchi et al., 1997; DeSouza et al., 2003; Matsuda et al., 2004; Ford et al., 2005; Brown et al., 2006; Brown et al., 2007), and for correct anti-saccades than anti-saccade errors (Ford et al., 2005). Nor does it account for the increased incidence of anti-saccade errors by human patients with dlPFC lesions (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Ploner et al., 2005). To reconcile these findings, it has been proposed that the contributions of the dlPFC to cognitive control are not limited to inhibition per se, but more generally establish and maintain the currently-relevant task rule (Miller and Cohen, 2001; Munakata et al., 2011). In support of this, it has been shown that the dlPFC is recruited by a variety of cognitively-demanding tasks (Duncan and Owen, 2000), adaptively encodes task-relevant information (Duncan, 2001), including rules (White and Wise, 1999; Asaad et al., 2000; Wallis et al., 2001; Everling and DeSouza, 2005; Mansouri et al., 2006), and furthermore plays a critical role in rule maintenance (Buckley et al., 2009).

3.4.5 Conclusion

To explain an inhibitory role of the dlPFC in saccade control, it had been proposed that excitatory dlPFC projections synapse with either fixation neurons in the rostral SC, or inhibitory interneurons in the caudal SC, both of which suppress the activity of SC saccade neurons (Johnston and Everling, 2006; Johnston et al., 2009). We performed a direct test of this inhibitory model and found that unilateral dlPFC deactivation delayed the onset of saccade-related activity in the SC ipsilateral to dlPFC deactivation, which suggests there was reduced excitatory input to SC saccade neurons. This implies that the dlPFC has an excitatory influence on the oculomotor system by synapsing directly with SC saccade neurons, and thus does not agree with the inhibitory model of prefrontal

function (Fig. 3.1). While unilateral dlPFC deactivation allowed me to identify this excitatory influence, there was also an increase of prestimulus and stimulus-related activity in the SC contralateral to deactivation, and thus unilateral dlPFC deactivation caused a neural imbalance at the SC. This neural imbalance potentially confounds the effects that were related to impairments of cognitive control. Therefore the following chapter will identify the effects of dlPFC deactivation that were caused by cognitive control impairments, rather than a neural imbalance.

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Chapter 4

Prefrontal Cortex Deactivation in Macaques Alters Activity in the Superior Colliculus and Impairs Voluntary Control of Saccades

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4.1 – Introduction

We often react to sudden changes in our environment by looking towards them. While this rapid orienting response may be advantageous in certain situations, it also detracts from ongoing behavior. Therefore we can decide to ignore sensory events and instead conduct actions that are of relevance to the achievement of our current behavioral goals. The ability to suppress automatic responses and to filter-out unwanted signals is thought to depend on the dorsolateral prefrontal cortex (dlPFC) (Miller and Cohen, 2001).

The anti-saccade task is a particularly useful paradigm for testing response suppression and voluntary saccade generation in clinical populations (Everling and Fischer, 1998; Broerse et al., 2001; Hutton and Ettinger, 2006). This task requires subjects to suppress a saccade towards a flashed visual stimulus in favour of a saccade towards the opposite uncued direction (Hallett, 1978; Munoz and Everling, 2004). Patients with prefrontal lesions that involve Brodmann's area 46 (Guitton et al., 1985; Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Ploner et al., 2005) and disorders that impair prefrontal functions, like schizophrenia (Fukushima et al., 1988; Fukushima et al., 1990), have longer reaction times for anti-saccades and often fail to suppress a saccade towards the flashed stimulus. Furthermore, functional imaging studies in humans (Sweeney et al., 1996; DeSouza et al., 2003; Ford et al., 2005; Dyckman et al., 2007; Brown et al., 2008) have found higher activations at the dlPFC, in particular Brodmann's area 46, for the performance of anti-saccades compared with saccades towards visual stimuli (pro-saccades). This pattern is absent in patients with

schizophrenia (McDowell et al., 2002). A unilateral pharmacological deactivation study of sites in the ventral bank of the principal sulcus with monkeys has reported impairments in the anti-saccade task (Condy et al., 2007), and single unit recording studies in monkeys have found task-selective activity in dlPFC neurons with this task (Funahashi et al., 1993; Everling and DeSouza, 2005). A subset of dlPFC neurons sends these signals directly to the superior colliculus (SC) (Johnston and Everling, 2006), which is a vital node in the saccade network (Gandhi and Katnani, 2011).

Saccade neurons in the SC are strongly modulated by the anti-saccade task, as demonstrated by a reduction of prestimulus, stimulus-related, and saccade-related activity (Everling et al., 1999). Consequently it was proposed that a general imbalance in favour of motor preparation over inhibitory processes may account for the poor voluntary control over unwanted prepotent responses that is associated with prefrontal disorders (Everling and Fischer, 1998; Munoz and Everling, 2004). This hypothesis, however, fails to explain the long reaction times of saccades in these disorders. Alternatively, it has been proposed that response errors occur when the signal to inhibit an unwanted response is generated too late (Guitton et al., 1985; Pierrot-Deseilligny et al., 2003).

To seek the neural mechanism for increased reaction times and error rates in prefrontal disorders, we recorded single neuron activity in the SC while we deactivated area 46 of the dlPFC using cryoloops (Lomber et al., 1999a) that were chronically implanted in both the left and right principal sulcus of rhesus macaques.

4.2 – Methods

All procedures were carried out in accordance with the guidelines of the Canadian Council of Animal Care Policy on the Use of Laboratory Animals and a protocol approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care.

4.2.1 Surgical Procedures

Two male macaque monkeys (*Macaca mulatta*) were prepared for chronic dlPFC deactivation experiments and single neuron recordings in the superior colliculus (SC)

using previously described techniques (Johnston and Everling, 2006). Briefly, monkeys underwent two aseptic surgical procedures. Animals received analgesics and antibiotics postoperatively and were closely monitored by a university veterinarian. In the first surgery, a plastic head restraint and a recording chamber were implanted. The recording chamber was centered on the midline and tilted 38° posterior of vertical to allow recordings from neurons in the superior colliculus. Monkeys then underwent training on the behavioral paradigm. Once the animals were proficient on the paradigm, anatomical MR images were obtained to visualize the location of the implanted recording chambers and the shape of the principal sulci. Animals then underwent a second surgery in which stainless steel cryoloops (4 mm x 6 mm) were implanted bilaterally into the posterior portion of the principal sulcus in each animal (Fig. 4.1A). The technical details of the cryoloop technique have been described before (Lomber et al., 1999a).

4.2.2 Behavioral Task

During each experiment, the response field (RF) of an isolated SC neuron was mapped. We did not sample the SC map systematically, and only recorded one or two saccade neurons at each location on the SC map. Therefore we were unable to analyse whether the location of the SC neurons on the motor map was a factor in the changes of neural activity with bilateral dlPFC deactivation, and thus cannot comment on whether the dlPFC has a uniform effect on the SC. The animals performed an oculomotor task that consisted of randomly-interleaved pro-saccades and anti-saccades. Each trial began with the presentation of a coloured central fixation point (FP). For monkey A, a green FP signaled a pro-saccade trial and a red FP signaled an anti-saccade trial. The colour instructions were reversed for monkey B. On half the trials, the colour cue remained visible throughout the trial (‘rule visible’ condition) (Fig. 4.2A). On the other half of trials, the FP changed to yellow 500-700ms before stimulus presentation on pro-saccade and anti-saccade trials, requiring the monkeys to maintain the task rule (‘rule memorized’ condition) (Fig. 4.2B). This 1000-1200 ms fixation period in both the ‘rule visible’ and ‘rule memorized’ conditions was longer than the 300-600 ms fixation period in both the overlap and gap conditions that were used in Chapters 2 and 3. This was because the animal that had a tendency to break fixation soon after having initiated

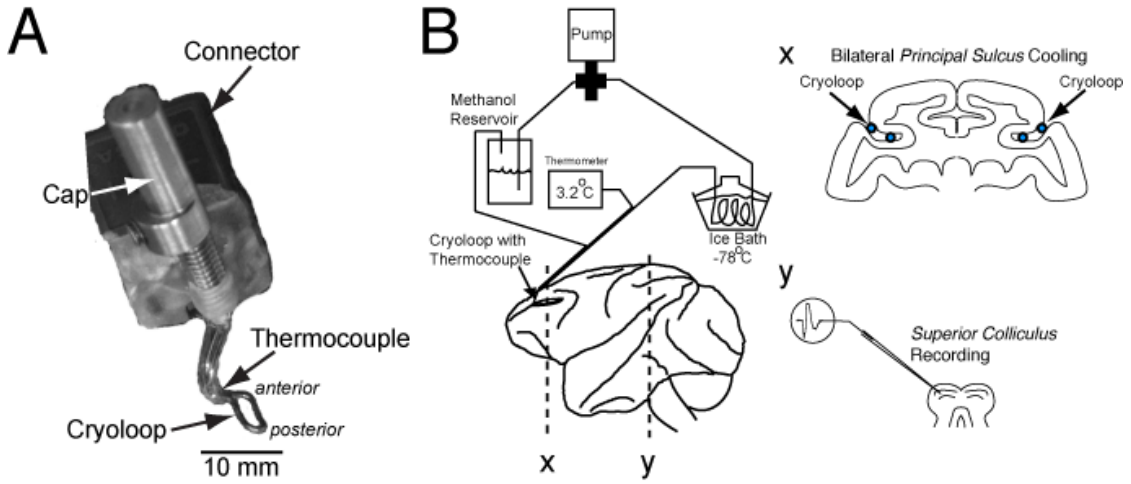


Figure 4.1 – Experimental Setup

A: Cryoloop to be implanted in left principal sulcus. Anterior and posterior refers to be orientation of the loop in the principal sulcus.

B: The dlPFC was bilaterally deactivated by pumping chilled methanol through cryoloops implanted in the left and right principal sulci (*coronal section x*), while single neuron activity was recorded in the intermediate layers of the SC (*coronal section y*).

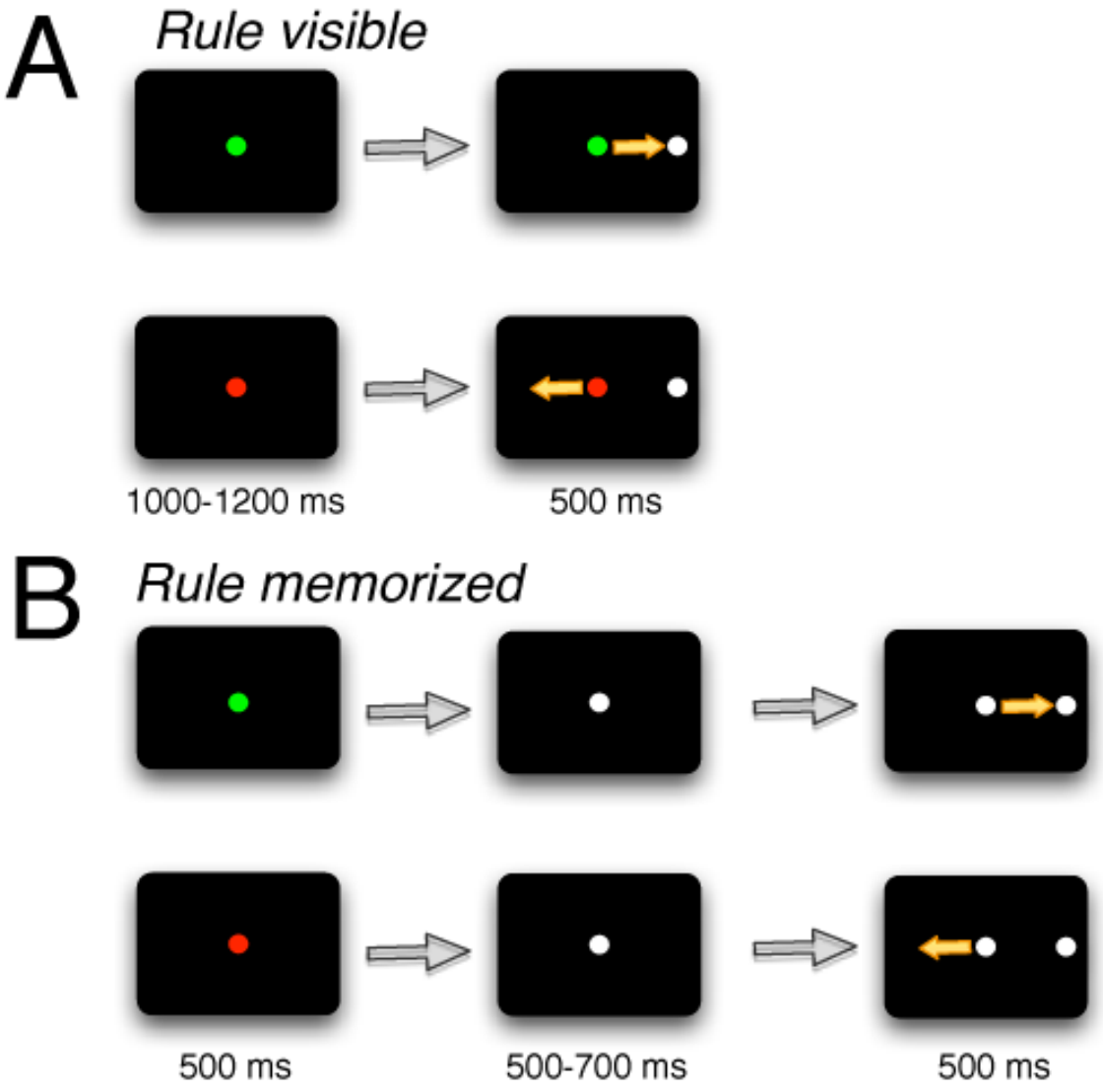


Figure 4.2 – Experimental Paradigm

A: Rule visible task: each trial began with a coloured fixation point (FP) that indicated either a pro-saccade or anti-saccade trial. A stimulus then appeared either in the neuron's response field (RF), or opposite to the RF and on the other side.

B: Rule memorized task: same as *A*, but the colour of the FP changed to a neutral colour 500-700 ms before stimulus onset. This required the monkey to briefly memorize the task rule.

fixation, was not included in this study of bilateral dlPFC deactivation. At the end of the fixation period, a white visual stimulus appeared randomly with equal probability either in the neuron's RF or at the mirror-opposite location. Monkeys received a water reward if they looked towards the stimulus on pro-saccade trials, and away from the stimulus to its mirror location on anti-saccade trials.

4.2.3 Cryoloop Method of Reversible Cryogenic Deactivation

Although reversible pharmacological deactivations are frequently used to investigate the role of cortical or subcortical areas in the control of behaviour, these techniques are less well-suited for combined deactivations and neural recordings. Lidocaine is a sodium channel blocker which also inactivates axons that pass through the area. Muscimol is a gamma-aminobutyric acid (GABA) agonist that does not inactivate passing fibres but lasts for several hours, preventing the observation of functional recovery following deactivation. The main disadvantage of pharmacological deactivations is that often multiple injections are necessary to obtain behavioral effects (Wardak et al., 2002). Even then the effects are often spatially very localized, making it extremely difficult to match a neuron's RF with the deactivated spatial region in combined deactivation and recording studies. The effects of unilateral deactivation suggest there is a shift in the balance between the two hemispheres (Schiller and Chou, 1998; Wardak et al., 2006), thereby creating neglect (Rafal, 1994), which could mask other more specific symptoms. While bilateral cortical deactivation does not have this potentially confounding effect, it is very difficult to achieve with pharmacological deactivations.

Cooling has been used in several studies to temporarily and reversibly deactivate the prefrontal cortex (Fuster and Alexander, 1970; Alexander and Fuster, 1973; Bauer and Fuster, 1976; Fuster et al., 1985; Chafee and Goldman-Rakic, 2000). Cortical cells are depolarized between 20 and 29°C, the action potentials of which become broad, small in amplitude, and less frequent; to the point where below 20°C many neurons are reduced to complete silence in extracellular recordings (Moseley et al., 1972; Lomber et al., 1999a). Previous studies with prefrontal cortical cooling used thermoelectric coolers attached to a cooling probe that rested on the dura. A disadvantage of this approach is that the deactivated area is large and that it is very difficult to fully deactivate cortical

tissue in the depths of a sulcus. Here we implanted cryoloops directly in the principal sulcus, which therefore limited prefrontal deactivation to area 46 in the dorsal and ventral banks (Fig. 4.1), given that the spread of cooling effects is limited to approximately the thickness of cortical gray matter (Lomber et al., 1999b). Chilled methanol pumped through a cryoloop deactivates adjacent cortical tissue by disrupting local synaptic activity while sparing axonal fibers of passage.

Cryoloops were constructed from 23-gauge hypodermic stainless steel tubing and custom-designed to conform to the shape of the principal sulci (Fig. 4.1A). The procedures for the manufacturing, surgery, and use of cryoloops have been described in detail (Lomber et al., 1999a). Cryoloops were 4 x 6 mm in dimension, and thus 24 mm^2 of cortical tissue was deactivated on each side of the cryoloop (total 48 mm^2). With an estimated range of 1.5 - 2.0 mm, we calculate that the cryoloops deactivated 72 - 96 mm^3 of cortical tissue. Given the identical design and location of the implanted cryoloops, we assume that the same area of cortical tissue was deactivated in both the left and right hemispheres of the dlPFC, such that bilateral dlPFC deactivation had a balanced effect on the SC. Room-temperature methanol was pumped through teflon tubing that passed through a methanol ice bath which was reduced to subzero temperatures by the addition of dry ice. Chilled methanol pumped through a cryoloop was then returned to the same reservoir from which it came. Cryoloop temperature was monitored by an attached microthermocouple. Each cooling session started with a precool period during which the pump was turned off for 10 to 15 minutes. The cooling period began when the pump was turned on. It took on average 85 s to bring the temperature of the loops down to 3°C. We excluded the first 4 minutes after the pumps were turned on to ensure that the cortical tissue adjacent to the cryoloop was cooled below 20°C, the temperature at which neurons are deactivated (Jasper et al., 1970). Cortical temperature, however, increases rapidly with distance from a cryoloop: the extent of deactivated tissue is limited to a range of 2 mm when cryoloop temperature is reduced to 1°C (Lomber et al., 1996a; Lomber et al., 1996b). Therefore we maintained cryoloop temperature in the range of 1-3°C to deactivate as large an area of cortical tissue as possible, while avoiding potentially harmful sub-zero temperatures at the cortical surface. Cryoloop temperature was controlled by adjusting flow rate of the

pump, and maintained in the range of 1-3°C for between 10 and 15 minutes. The pumps were then turned off. The temperature of the cryoloops returned to 30°C within 40 s. The first 3 minutes after the pumps were turned off were excluded from all data analysis.

4.2.4 Recording Method

Standard electrophysiological techniques were used to record single neuron activity in the intermediate layers of the SC using a Plexon MAP system (Dallas, TX) (Johnston and Everling, 2006). We included only neurons in our analysis that did not show any significant differences of activity in the 500 ms period before stimulus onset, between the precool and postcool periods (t-test, $p > 0.05$), to ensure that their isolation did not change during the recording session. Horizontal and vertical eye positions were recorded at 500 Hz using an Eyelink II system (SR Research, Kanata, Canada).

4.2.5 Spike Density Function

To evaluate the relationship between neural activity and both stimulus onset and saccade onset, continuous spike density functions were constructed. The activation waveform was obtained by convolving each spike with an asymmetric function that resembled a postsynaptic potential (Hanes and Schall, 1996; Thompson et al., 1996; Everling et al., 1999). The advantage of this function over a standard Gaussian function (Richmond and Optican, 1987) is that a spike only exerts an effect forward and not backward in time.

4.2.6 Time Course of dlPFC Deactivation

To determine the time course of the effects of dlPFC deactivation on the population of SC neurons, we performed sliding receiver operating characteristic (ROC) analyses. For the time course of dlPFC deactivation on SC activity relative to stimulus onset, the ROC value was calculated for a 10 ms epoch (centered around the time point) that started 200 ms prior to stimulus onset, using the convolved spike trains. This analysis was repeated in 1 ms increments until 300 ms after stimulus onset. For the time course of dlPFC deactivation relative to saccade onset, the analysis was conducted starting 200 ms prior

to saccade onset to 100 ms after saccade onset. An ROC time course was calculated for each neuron and then averaged separately across all SC neurons. To test whether the ROC values were significant at any time points for the population of SC neurons, we conducted bootstrap analyses. To this end, the following procedure was repeated 10,000 times: For each neuron, a random decision was made to either exchange the two activation conditions (dlPFC+ and dlPFC-) (50% probability) or leave them unchanged (50% probability). Each of the 10,000 repetitions of the analysis, performed on all SC neurons, yielded a single average time course. The distribution of the 10,000 average ROC values at each point in time was then used to calculate the 95^{th} and 5^{th} percentile values. Both were plotted together with the average ROC time course of the non-randomized data. The 95^{th} and 5^{th} percentile indicate the 5% significance criterion.

4.3 – Results

Data were obtained over a total of 52 experimental sessions. In each session, monkeys initially performed the task for 10-15 minutes. The dlPFC region was then deactivated bilaterally for 10-15 minutes by pumping chilled methanol through the implanted cryoloops (Fig. 4.1B), while the monkey continued to perform the task. In all sessions, we also recorded data for at least 10 minutes during the postcool period, and then contrasted the precool and postcool data from when the dlPFC was active (dlPFC+ period), with the cooling data during which the dlPFC was deactivated (dlPFC- period).

4.3.1 Behavioral Effects of Bilateral dlPFC Deactivation

Monkey A skipped more trials during the dlPFC- period compared with the dlPFC+ period (12.2% vs. 5.3%, $p < 0.001$; Wilcoxon signed rank test), and both monkeys broke fixation prior to peripheral stimulus presentation more often during the dlPFC- than dlPFC+ period (monkey A: 19% vs. 15.6%, $p < 0.001$, Wilcoxon signed rank test; monkey B: 29.7% vs. 20.4%, $p < 0.001$, Wilcoxon signed rank test). Although the percentage of performed trials dropped during the deactivation period, the animals continued to perform the task.

We quantified the behavioral effects of bilateral dlPFC deactivation for all 52 experimental sessions (*Appendix 6*). dlPFC deactivation increased saccadic reaction

times (SRTs), with stronger effects on anti-saccade than pro-saccade trials, and increased error rates on anti-saccade trials, i.e. an increased incidence of unwanted saccades toward the stimulus. Error rates were higher in monkey A than monkey B, and both monkeys made more errors in the 'rule memorized' than 'rule visible' condition. dlPFC deactivation also decreased the peak velocity of anti-saccades, and increased their duration. As demonstrated by their main sequence relationships, the peak velocity and duration of saccades are critically dependent on saccade amplitude. As the result of recording the activity of SC saccade neurons with different response fields, various saccade amplitudes were used across all sessions, which would consequently affect saccade peak velocity and duration as well. In this study we evaluated the effects of dlPFC deactivation within individual sessions, for which saccade amplitude was held constant, and thus was not a factor that could have affected the peak velocity or duration of saccades.

4.3.2 Effects of Bilateral dlPFC Deactivation on SC Activity

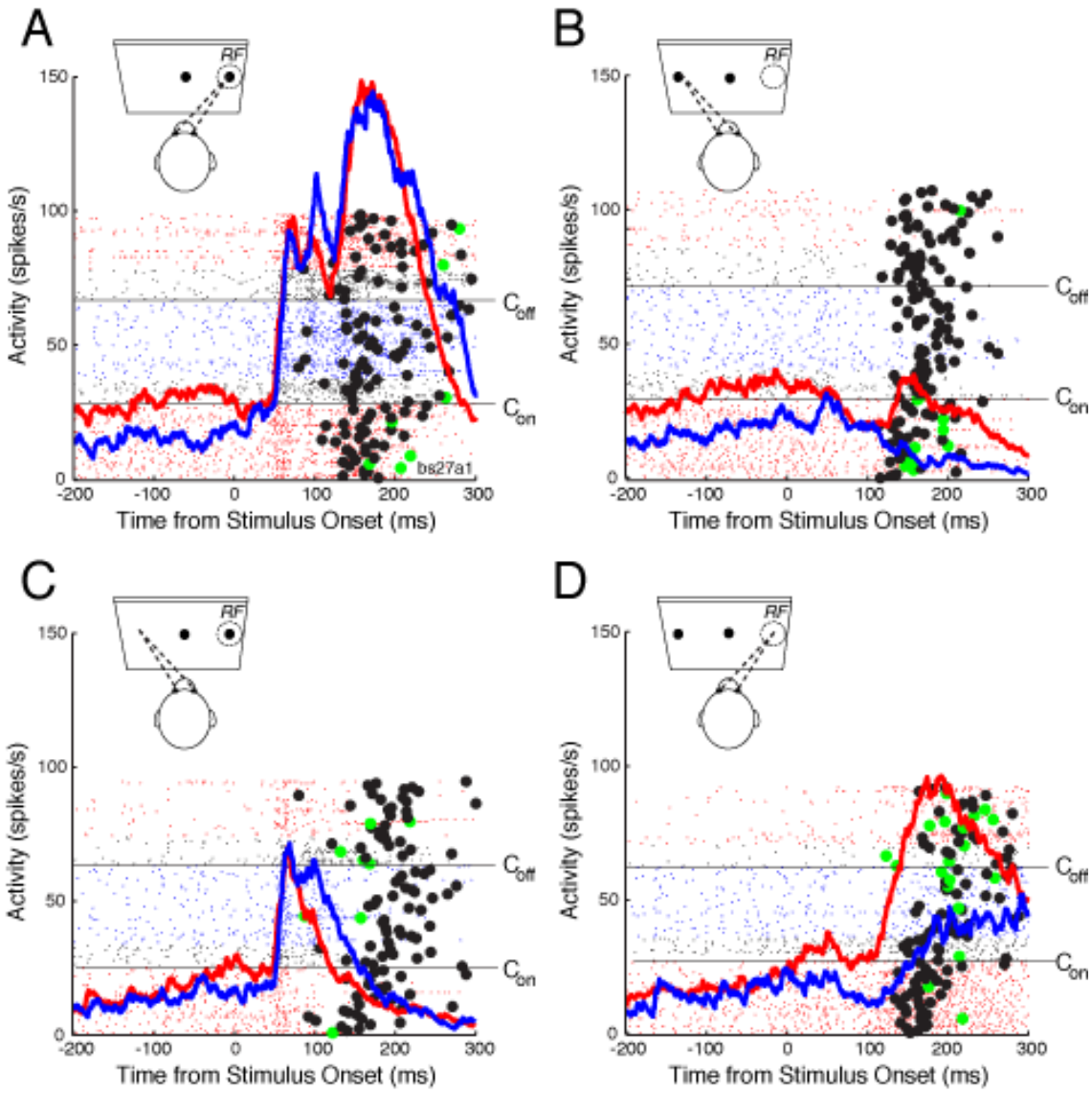
An example of the effect of bilateral dlPFC deactivation on the response of a SC saccade neuron is shown in Figure 4.3. Consistent with previous reports, the neuron had significantly higher levels of prestimulus activity on pro-saccade than anti-saccade trials (*red lines* in Fig. 4.3A,B compared with *red lines* in Fig. 4.3C,D). During the cooling period (*blue lines*), the prestimulus activity dropped, on pro-saccade trials in particular (Fig. 4.3A,B). For pro-saccade trials on which the stimulus appeared in the neuron's RF (Fig. 4.3A), the neuron had a vigorous visual response, followed by a motor burst time-locked to the saccade (*filled black circles*), while the activity decayed on pro-saccade trials when the stimulus appeared opposite to the neuron's RF (Fig. 4.3B). dlPFC deactivation had no clear effects on the initial visual response or saccade-related activity on pro-saccade trials. While this particular neuron demonstrated an increase of stimulus-related activity (i.e. a 'second-volley') following the initial visual response (Fig. 4.3A), this effect of dlPFC deactivation was not found with the population of SC saccade neurons.

Figure 4.3 – Single Neuron Example

A: Activity of a single SC neuron on pro-saccade trials when the stimulus appeared in the neuron's RF. Rasters show spikes for each trial, aligned on stimulus onset. *Black filled circles* show the onset of the saccade. *Green filled circles* show the onset of errors. C_{on} and C_{off} indicate when cooling pumps were turned on and off. *Red rasters* show activity when the dlPFC was not cooled (dlPFC+ trials), *blue rasters* show activity when the dlPFC was cooled bilaterally (dlPFC- trials), *black rasters* fall into the first 4 minutes after C_{on} and C_{off}, which were transition periods and thus excluded from all analyses. The mean spike density waveform for the dlPFC+ (*red*) and dlPFC- (*blue*) periods is overlaid.

B: Same as for *A*, but on pro-saccade trials for which the stimulus was presented opposite to the neuron's RF.

C, *D*: Same as for *A* and *B*, but for anti-saccade trials.


A
B
C
D
RF
Activity (spikes/s)
Time from Stimulus Onset (ms)
C_{off}
C_{on}
bs27a1
0
50
100
150
-200
-100
0
100
200
300

On anti-saccade trials, the neuron had a stimulus-related response on trials in which the stimulus appeared in the neuron's RF (Fig. 4.3C). The activity was then suppressed prior to saccade onset (*black filled circles*). On trials in which the monkey made an erroneous saccade towards the stimulus (green *filled circles*), the neuron displayed a burst of action potentials. On dlPFC- trials (*blue line*), the initial stimulus-related response was the same as on dlPFC+ trials (*red line*), but the neuron remained active for longer on dlPFC- trials. On anti-saccade trials for which the monkey had to generate a saccade into the neuron's RF (Fig. 4.3D), the neuron displayed a motor burst for the saccade, which on dlPFC- trials was reduced to the point of being nearly absent.

4.3.3 Prestimulus Activity

Next, we examined the effects of bilateral dlPFC deactivation on the activity immediately before the arrival of the visual signal in the SC, for our sample of 34 SC neurons that met the inclusion criteria (see *Section 4.2.4*). We measured the level of prestimulus activity in the period from 50 ms before to 50 ms after stimulus onset. SC neurons typically have visual responses greater than 50 ms (Everling et al., 1999), although this depends on the intensity of the stimulus (Bell et al., 2006), such that the activity in this analysis period reflected the activation level before stimulus onset, meaning that it was not influenced by the arrival of the visual signal. Consistent with a previous report (Everling et al., 1999), SC neurons displayed higher levels of prestimulus activity on pro-saccade trials (19.2 ± 3.2 spikes/s) than anti-saccade trials (12.1 ± 2.3 spikes/s) ($p < 0.005$, Wilcoxon signed rank test) in the 'rule visible' condition. Moreover, we found these differences were also present in the 'rule memorized' condition, in which the FP had the same colour on pro-saccade and anti-saccade trials (16.1 ± 2.8 v. 12.1 ± 2.3 spikes/s on pro-saccade and anti-saccade trials, respectively, $p < 0.05$, Wilcoxon signed rank test). The level of prestimulus activity dropped significantly ($p < 0.001$, Wilcoxon signed rank test) during the dlPFC- period on pro-saccade trials in the 'rule visible' condition (from 19.2 ± 3.2 to 11.8 ± 3.2 spikes/s; Fig. 4.4A) and 'rule memorized' condition (from 16.1 ± 2.8 to 10.3± 2.7 spikes/s; Fig. 4.4C). We also observed significant decreases of prestimulus activity on anti-saccade trials in the 'rule visible' condition (10.2 ± 2.3 vs. 12.1 ± 2.3 spikes/s;

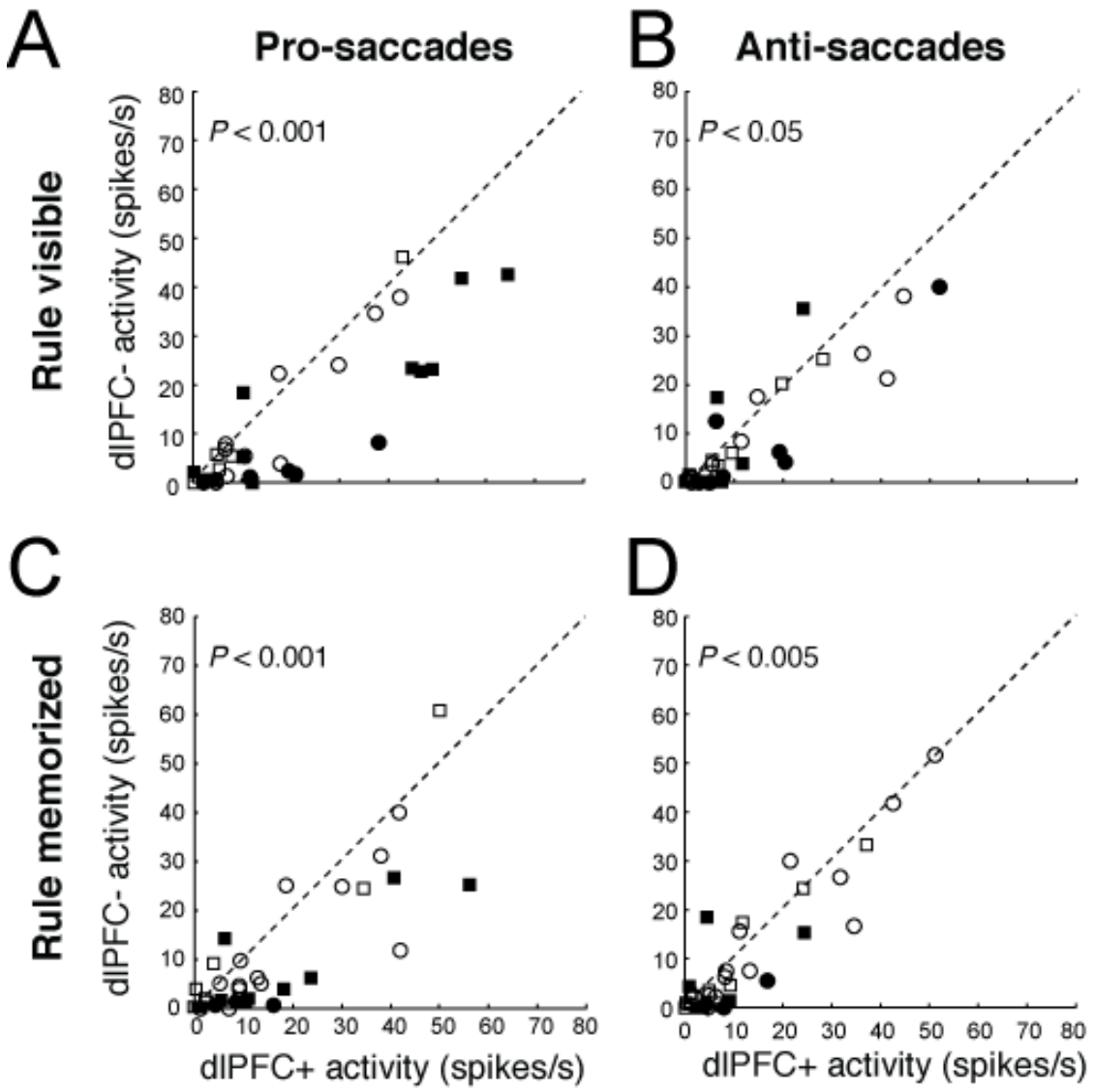


Figure 4.4 – Effects of Bilateral dlPFC Deactivation on Prestimulus Activity in the SC

A: The mean activity of individual neurons in the period 50 ms before to 50 ms after stimulus onset is plotted for the dlPFC+ period against the dlPFC- period, on pro-saccade trials in the 'rule visible' condition. *Circles* and *squares* indicate neurons recorded from monkey A and B, respectively. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $p < 0.05$). *Dashed line* is the unity line (slope, 1).

B: Same as in *A*, but on anti-saccade trials.

C, D: Same as in *A* and *B*, but in the 'rule memorized' condition.

$p < 0.05$, Wilcoxon signed rank test; Fig. 4.4B) and in the 'rule memorized' condition (8.8 ± 2.4 vs. 11.6 ± 2.4 spikes/s; $p < 0.005$, Wilcoxon signed rank test; Fig. 4.4D). As a consequence, prestimulus activity in the dlPFC- period was no longer different between pro-saccades and anti-saccades in the 'rule visible' or 'rule memorized' conditions ($p > 0.05$, Wilcoxon signed rank test), and thus was not task-selective.

4.3.4 Stimulus-related Activity

Similar to the single neuron example presented in Fig. 4.3, the population of SC neurons also responded to the presentation of the stimulus into their RFs on anti-saccade trials (Fig. 4.5B). The initial response did not vary between dlPFC+ and dlPFC- trials, however the neurons' activity remained higher on dlPFC- trials. We quantified these differences in the period 100-200 ms after stimulus onset. In the 'rule visible' condition, the mean activity was 17.7 ± 3.5 spikes/s on dlPFC+ trials, and 29.7 ± 3.5 spikes/s on dlPFC- trials ($p < 0.005$, Wilcoxon signed rank test). Significant differences ($p < 0.05$, Wilcoxon rank sum test) were obtained in 38% (13 of 34) of the neurons (Fig. 4.5D). Differences between dlPFC+ and dlPFC- trials were even stronger in the 'rule memorized' condition (Fig. 4.5E). Here, 53% (18 of 34) of neurons displayed significant differences ($p < 0.05$, Wilcoxon rank sum test), with population activity of 26.9 ± 5.2 spikes/s on dlPFC+ trials, and 42.5 ± 4.2 spikes/s on dlPFC- trials ($p < 0.001$, Wilcoxon signed rank test). These differences may have simply been the result of more erroneous saccades and therefore saccade-related bursts on dlPFC- trials. To rule out this simple explanation, we repeated the same analysis for correct trials only (Fig. 4.5C), which showed that the differences between dlPFC+ and dlPFC- trials were still present when error trials were excluded from the analysis ('rule visible' condition: 15.9 ± 3.4 vs. 22.5 ± 3.4 spikes/s, $p < 0.005$, Wilcoxon signed rank test, Fig. 4.5F; 'rule memorized' condition: 19.3 ± 3.7 vs. 29.4 ± 3.7 spikes/s, $p < 0.001$, Wilcoxon signed rank test, Fig. 4.5G).

Figure 4.5 – Effects of Bilateral dlPFC Deactivation on Stimulus-related Activity in the SC on Anti-saccade Trials

A: Cumulative distributions of correct saccadic reaction times and error saccadic reaction times on dlPFC^{+} trials (*red lines*) and dlPFC- trials (*blue lines*) in the 'rule visible' (*solid lines*) and 'rule memorized' (*dashed lines*) conditions.

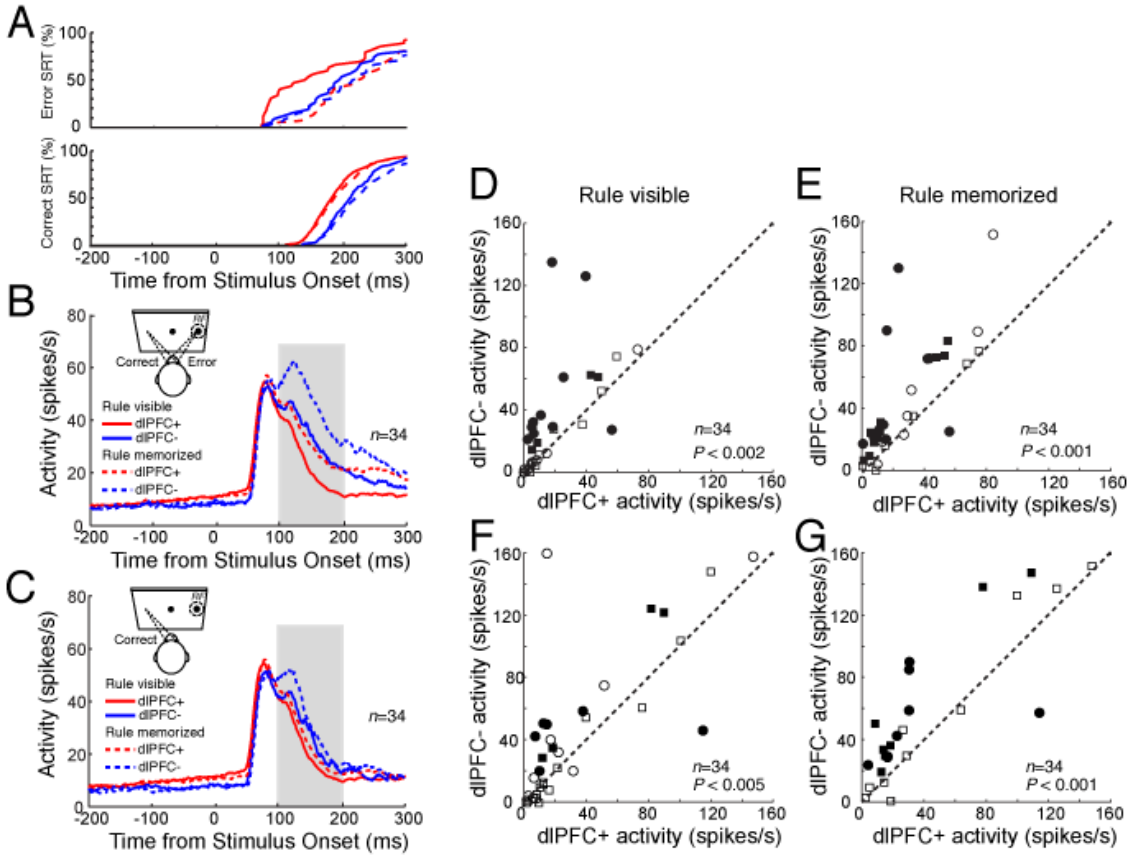
B: Mean spike density on dlPFC^{+} trials (*red lines*) and dlPFC- trials (*blue lines*) in the 'rule visible' (*solid lines*) and 'rule memorized' (*dashed lines*) conditions, for correct and error trials combined. In this and subsequent figures, the response field (RF, *dashed circle*) is displayed on the right, though the actual side varied between cells.

C: Same as in *B*, but for correct trials only.

D: The mean activity of individual neurons in the period 100-200 ms after stimulus onset (*shaded region* in *B*) is plotted for the dlPFC+ period against the dlPFC- period, in the 'rule visible' condition. *Circles* and *squares* indicate neurons recorded from monkey A and B, respectively. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $P<0.05$). *Dashed line* is the unity line (slope, 1).

E: Same as for *D*, but in the 'rule memorized condition'.

F, *G*: Same as in *D* and *E*, but for correct trials only (*shaded region* in *C*).


A
Error SRT (%)
Correct SRT (%)
Time from Stimulus Onset (ms)
B
Activity (spikes/s)
Correct
Error
Rule visible
dlPFC+
dlPFC-
Rule memorized
dlPFC+
dlPFC-
n=34
Time from Stimulus Onset (ms)
C
Activity (spikes/s)
Correct
Rule visible
dlPFC+
dlPFC-
Rule memorized
dlPFC+
dlPFC-
n=34
Time from Stimulus Onset (ms)
D
Rule visible
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
n=34
P < 0.002
E
Rule memorized
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
n=34
P < 0.001
F
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
n=34
P < 0.005
G
dlPFC- activity (spikes/s)
dlPFC+ activity (spikes/s)
n=34
P < 0.001

4.3.5 Saccade-related Activity

To test the effects of bilateral dlPFC deactivation on saccade-related activity in the SC, we examined the population activity on anti-saccade trials when the stimulus was presented opposite to the neurons' RF, i.e. saccades were directed into the RF (Fig. 4.6A). While it would be logical to think that saccade-related activity should be analysed with a neuron's activity aligned on saccade onset, we found that with this analysis of the motor burst, dlPFC deactivation had no effect. On the other hand, when we quantified neural activity in the period 100-200 ms after stimulus onset, SC neurons were more active in the dlPFC+ condition (45.3 ± 7.0 spikes/s) than in the dlPFC- condition (18.9 ± 7.0 spikes/s) for the 'rule visible' condition. These differences were significant for the population ($p < 0.001$, Wilcoxon signed rank test) and for 47% (16 of 34) of SC neurons ($p < 0.05$, Wilcoxon rank sum test) (Fig. 4.6B). Similarly, in the 'rule memorized' condition neurons were more active in the dlPFC+ condition (41.3 ± 6.5 spikes/s) than in the dlPFC- condition (19.5 ± 6.5 spikes/s) ($p < 0.001$, Wilcoxon signed rank test). Here, 53% (18 of 34) of the neurons had significant differences in their activity ($p < 0.05$, Wilcoxon rank sum test) (Fig. 4.6C). These differences were also present when only correct trials were included in the analysis ($p < 0.001$, Wilcoxon signed rank test). It should be noted, however, that the differences in this analysis are more difficult to interpret, given that dlPFC deactivation also reduced the velocity, increased the duration, and likely most relevant for this analysis, increased the gain (amplitude) for anti-saccades (see *Appendix 6*). SC saccade neurons discharge a burst of action potentials for a saccade into their broadly-tuned response field, and this discharge decreases with distance from the optimal location in their response field (Munoz and Wurtz, 1995b). Consequently the increase of saccade amplitude with dlPFC deactivation suggests that there should also be a corresponding decrease of the motor burst, however we found no such effect. This may be explained by the population coding of SC saccade neurons (Sparks et al., 1976) (see *Section 1.2.8*), whereby the variability of individual neuron discharge does not adversely affect saccade metrics, given the large population of active saccade neurons with broadly-tuned response fields.

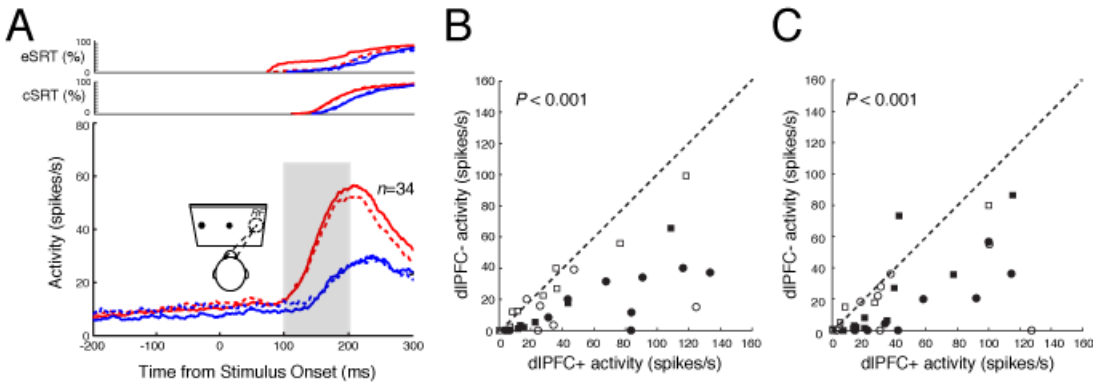


Figure 4.6 – Effects of Bilateral dlPFC Deactivation on Saccade-related Activity in the SC on Anti-saccade Trials

A: Mean spike density for anti-saccades directed into the SC neuron's response field (RF), in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods of the 'rule visible' (*solid lines*) and 'rule memorized' (*dashed lines*) conditions. Cumulative distributions of saccadic reaction times for correct (cSRT) and error (eSRT) trials are shown in the *top panels*.

B: The mean activity of individual neurons in the period 100-200 ms after stimulus onset (*shaded region* in *A*) is plotted for the dlPFC+ period against the dlPFC- period in the 'rule visible' condition. *Circles* and *squares* indicate neurons recorded from monkey A and B, respectively. *Filled symbols* indicate neurons with significant differences (Wilcoxon rank sum test, $p < 0.05$). *Dashed line* is the unity line (slope, 1).

C: Same as in *B*, but for the 'rule memorized' condition.

4.3.6 Time Course of Bilateral dlPFC Deactivation on Population Activity in the SC

To perform a more principled analysis of the time course of dlPFC deactivation effects on SC activity, we performed an ROC analysis on the convolved activity in 10 ms time bins, shifted by 1 ms (Fig. 4.7). To test whether these ROC values were significantly different from chance, we also conducted a bootstrap analysis (see *Section 4.2.6*). These analyses confirmed significant differences in neural activity starting 100 ms after stimulus onset between the dlPFC- and dlPFC+ periods, on anti-saccade trials in which the stimulus was presented either in (Figs. 4.7 A,B) or opposite to (Figs. 4.7 C,D) the neuron's RF.

4.3.7 Error Trials

To directly test whether errors on anti-saccade trials are the result of an increased motor preparation during the prestimulus period, or a failure to suppress the stimulus-related response, we compared correct and error trials in the dlPFC- period (Fig. 4.8). This analysis was performed on the 16 SC neurons for which we had obtained at least 4 errors on anti-saccade trials during the dlPFC- period. Data from the 'rule visible' and "rule memorized" conditions were combined for this analysis. In contrast to our previous study that compared the prestimulus activity of SC neurons for correct anti-saccades and anti-saccade errors on a gap saccade task (Everling et al., 1998), in the present study we did not find any differences of prestimulus activity between correct and error trials in the dlPFC- period (Fig. 4.8A, *left panel*). It should be noted, however, that the errors in the gap saccade task were mainly short-latency express saccades (Everling et al., 1998), whereas the errors during dlPFC deactivation had longer reaction times (Fig. 4.5A). Differences between correct and error trials emerged following the initial stimulus-related response, when the activity was suppressed on correct trials but continued to increase on error trials to culminate in a motor burst (Fig. 4.8A, *right panel*). Note that although the activity was suppressed on correct dlPFC- trials (*solid blue line*), this suppression was faster on correct dlPFC+ trials (*red line*). When tested by an ROC analysis with a 10 ms sliding window (Fig. 4.8B), differences between correct anti-saccades (*solid blue line*) and anti-saccade errors (*dashed blue line*) in Fig. 4.8A became statistically significant 103 ms following stimulus onset, and 90 ms before

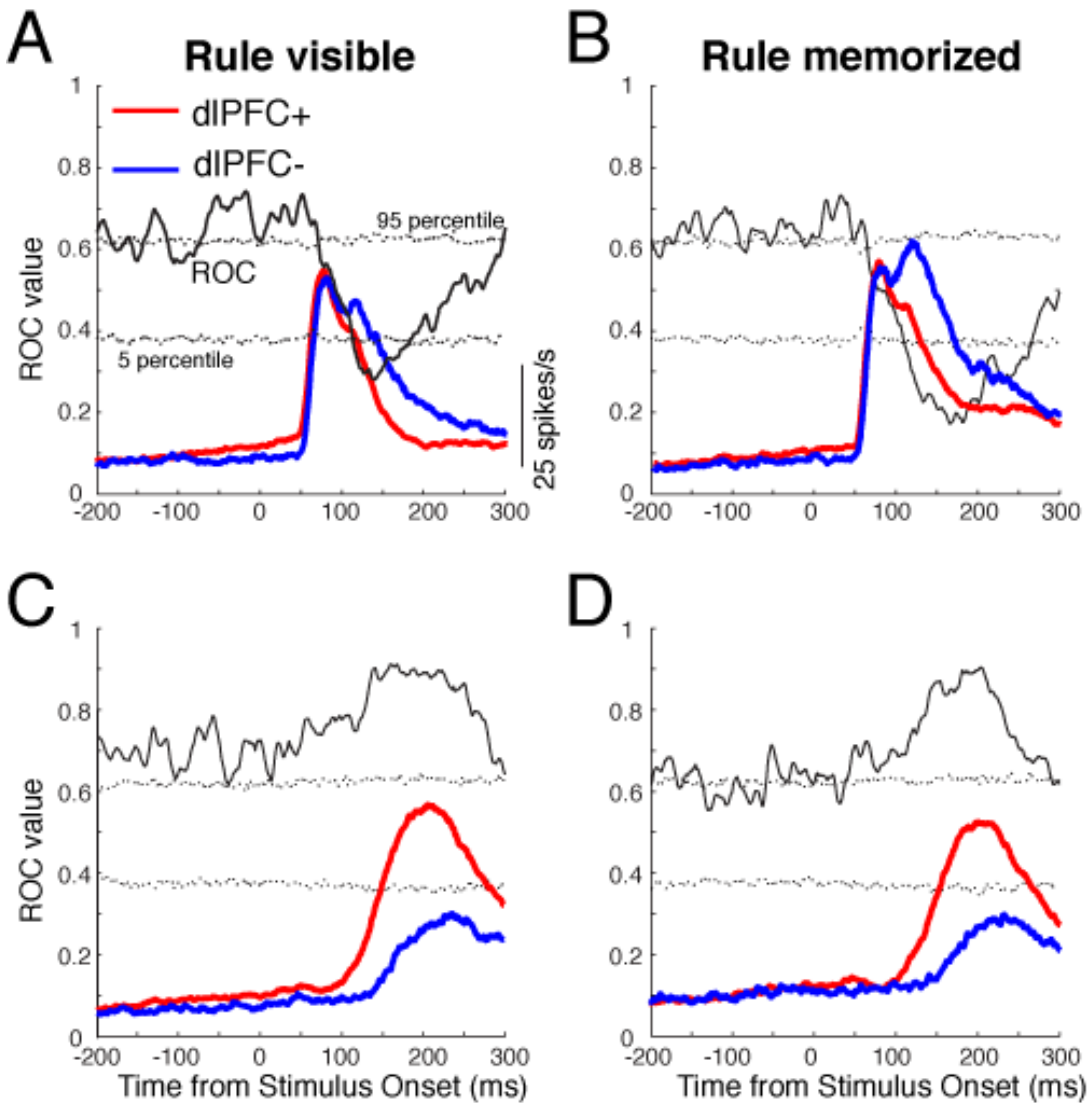


Figure 4.7 – Time Course of Bilateral dlPFC Deactivation Effects

A: Mean spike density on anti-saccade trials when the stimulus appeared in the neurons' RF, in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods for the 'rule visible' condition. Also plotted is the time course of the average population ROC values for comparison of the dlPFC+ and dlPFC- periods (*solid line*). *Dotted lines* represent percentile values obtained from a bootstrap analysis, and thus time points with significant differences ($p < 0.05$) are found where the *solid line* is either above or below the *dotted lines*.

B: Same as in *A*, but for the 'rule memorized' condition.

C, *D*: Same as in *A* and *B*, but for anti-saccades directed into the neurons' RF.

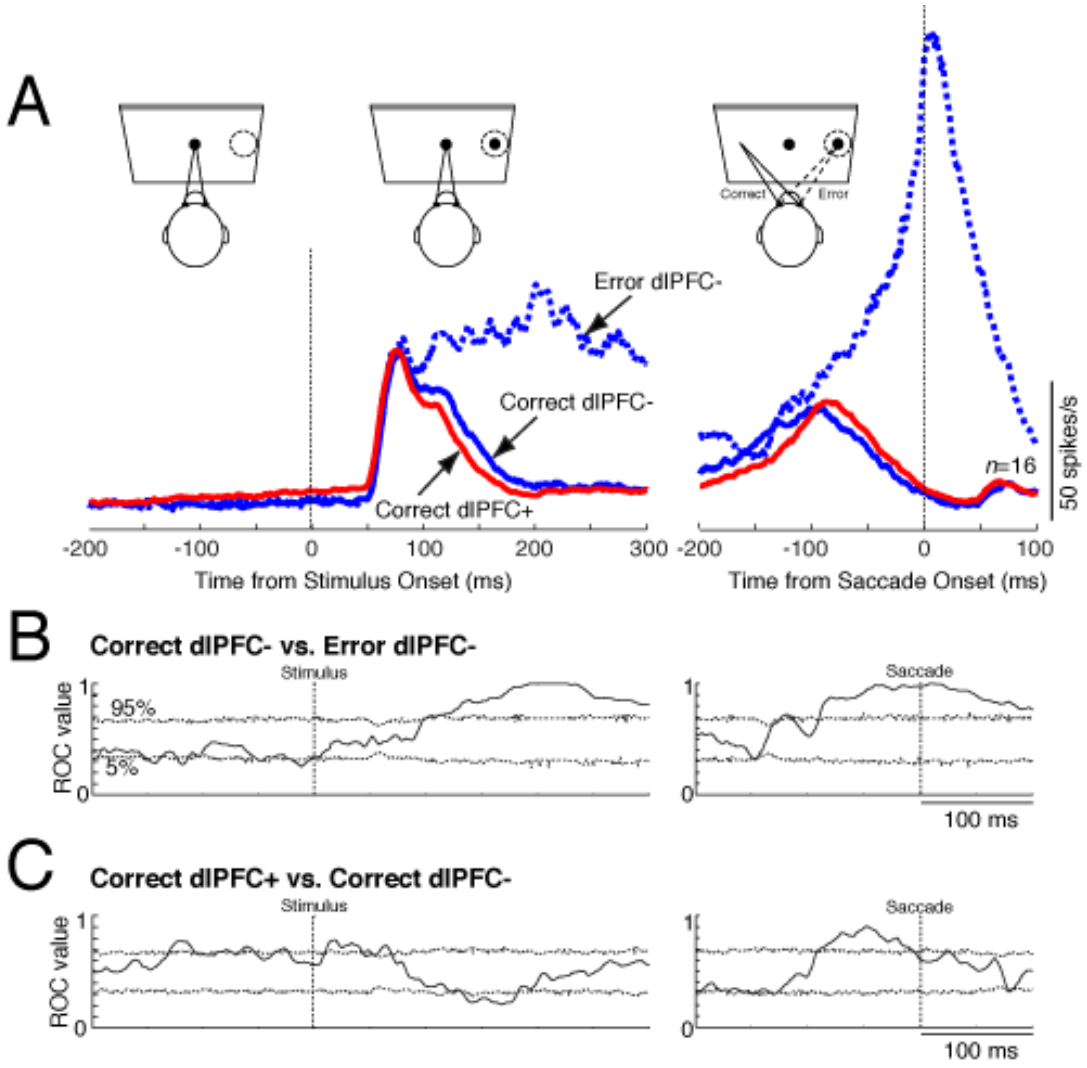


Figure 4.8 – SC Activity on Correct and Error Trials

A: Mean spike density for correct trials in the dlPFC+ period (*red lines*), and both correct (*solid blue lines*) and error (*dashed blue lines*) trials in the dlPFC- period, aligned on stimulus onset (*left panel*) and saccade onset (*right panel*).

B: Time course of the average population ROC values for comparison of correct and error trials in the dlPFC- period (*solid line*), aligned on stimulus onset (*left panel*) and saccade onset (*right panel*). *Dotted lines* represent percentile values obtained from a bootstrap analysis, and thus time points with significant differences ($p < 0.05$) are found where the *solid line* is either above or below the *dotted lines*.

C: Same as in *B*, but for the comparison of correct trials in the dlPFC+ and dlPFC- periods.

saccade onset. The differences between correct dlPFC+ and correct dlPFC- trials (Fig. 4.8A, *right panel*) were significant in the period 136-182 ms after stimulus onset, and in the 100 ms preceding saccade onset (Fig. 4.8C). These findings demonstrate that with bilateral dlPFC deactivation, anti-saccade errors occurred when the activity of SC saccade neurons was not suppressed at about 100 ms after stimulus onset. The data also demonstrate there is a difference of neural activity in the SC between correct anti-saccades in the dlPFC+ and dlPFC- periods.

4.3.8 Unilateral dlPFC Deactivation

Though we have focused on the effects of bilateral dlPFC deactivation, we also performed unilateral dlPFC deactivations in the 'rule visible' condition (Fig. 4.9). Like with bilateral deactivation, unilateral deactivations were associated with prolonged stimulus-related activity on anti-saccade trials in the SC ipsilateral ($p < 0.05$, Wilcoxon Signed Rank Test) (Fig. 4.9D) and contralateral ($p < 0.05$, Wilcoxon Signed Rank Test) (Fig. 4.9C) to the deactivated hemisphere. Unilateral deactivation also had strong lateralized effects, with higher levels of prestimulus activity in the contralateral SC ($p < 0.05$, Wilcoxon Signed Rank Test) (Figs. 4.9A, C), and lower saccade-related activity in the ipsilateral SC for anti-saccades directed contralateral to the deactivated hemisphere ($p < 0.05$, Wilcoxon Signed Rank Test) (Fig. 4.9D). These findings demonstrate that unilateral dlPFC deactivation caused an imbalance of activity at the level of the SC: saccade neuron activity decreased in the ipsilateral SC, and increased in the contralateral SC. This is consistent with the increased incidence of ipsilateral errors on anti-saccade trials that was found with unilateral injections of muscimol into the ventral bank of the principal sulcus (Condy et al., 2007). Not surprisingly, such an imbalance was not observed with bilateral dlPFC deactivation.

4.4 – Discussion

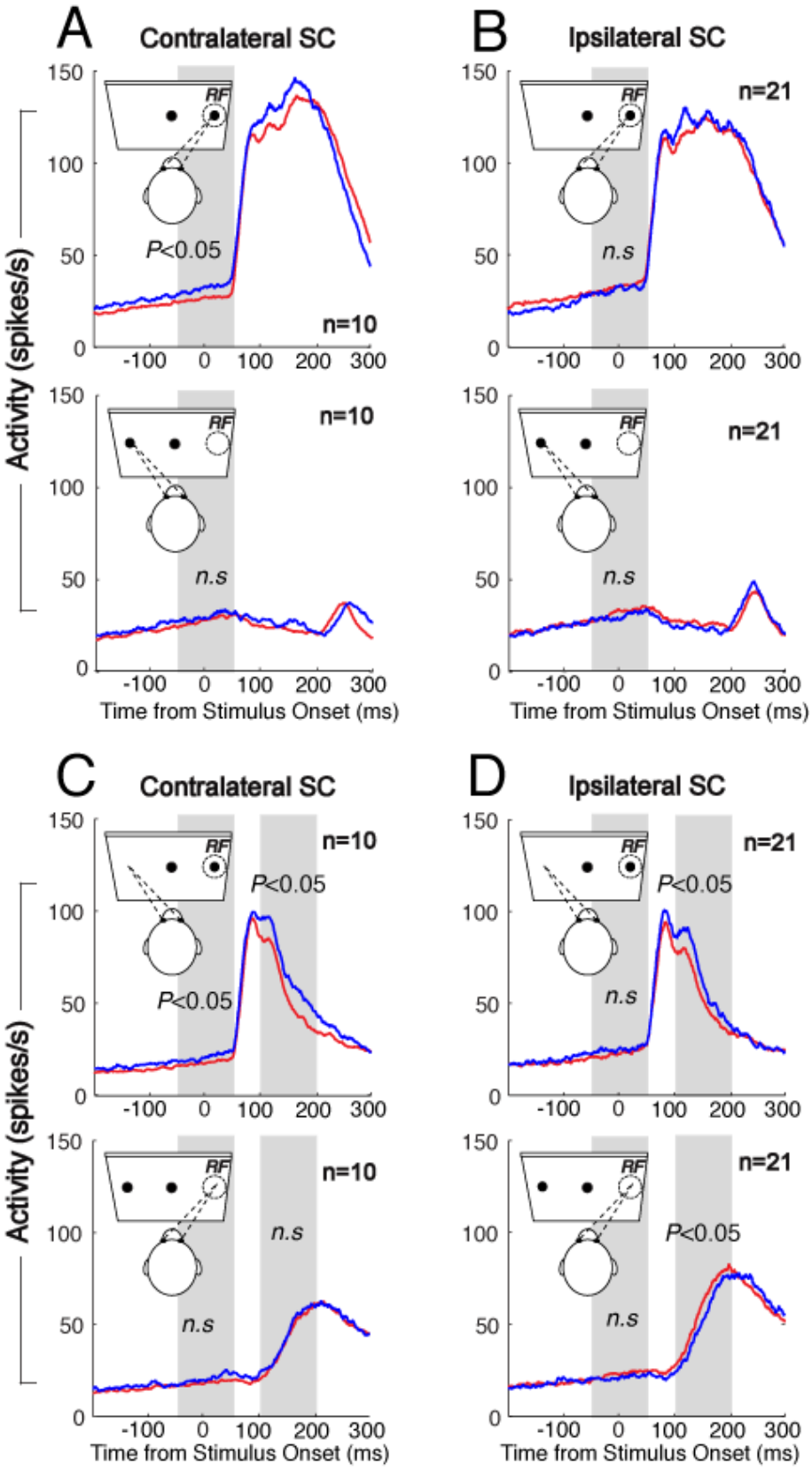
An influential hypothesis of dorsolateral prefrontal cortex (dlPFC) function has emphasized its role in biasing the activity of sensory and motor areas, which depends on behavioral rules and goals (Miller and Cohen, 2001). While previous studies have used delayed response and delayed-match-to-sample tasks to describe the effects of dlPFC

Figure 4.9 – Effects of Unilateral dlPFC Deactivation on SC Activity

A: Mean spike density of saccade neurons in the SC contralateral to dlPFC deactivation, aligned on stimulus onset, in the dlPFC+ (*red lines*) and dlPFC- (*blue lines*) periods, on pro-saccade trials when the stimulus appeared in the neurons' response field (RF) (*top panel*), and opposite to the RF (*bottom panel*), in the 'rule visible' condition.

B: Same as in *A*, but for saccade neurons in the SC ipsilateral to dlPFC deactivation.

C, *D*: Same as in *A* and *B*, but on anti-saccade trials.


A
Contralateral SC
B
Ipsilateral SC
C
Contralateral SC
D
Ipsilateral SC
RF
P<0.05
n.s
n=10
n=21
Activity (spikes/s)
Time from Stimulus Onset (ms)
150
100
50
0
-100
0
100
200
300

deactivation on neural activity in the thalamus (Fuster and Alexander, 1973), parietal cortex (Chafee and Goldman-Rakic, 2000), and inferotemporal cortex (Fuster et al., 1985), it was not known how the dlPFC modulates neural activity to establish rule-dependent mappings between inputs and outputs. Here we investigated the effects of dlPFC deactivation on neural activity in the superior colliculus (SC), using two simple oculomotor tasks with different stimulus-response (SR) mapping rules. On pro-saccade trials, monkeys had to follow a congruent SR mapping rule by looking towards a flashed stimulus, while anti-saccade trials used an incompatible SR mapping rule that required looking away from the stimulus and in the opposite direction (Hallett, 1978; Munoz and Everling, 2004). We found that bilateral dlPFC deactivation a) eliminated the differences in neural activity between the two SR mapping rules during the prestimulus period, b) impaired suppression of the stimulus-driven response, and c) delayed generation of the motor response on anti-saccade trials. These findings support the hypothesis that the dlPFC plays an important role in arbitrary SR mappings (Miller and Cohen, 2001; Sakai, 2008), and reveal a neural mechanism by which the dlPFC exerts task-dependent control on neural activity in the SC.

Neural correlates of behavioral rules have been found in many studies of PFC function (Hoshi et al., 1998; White and Wise, 1999; Asaad et al., 2000; Wallis et al., 2001; Everling and DeSouza, 2005). Recently, Buckley and colleagues (2009) tested the effects of circumscribed PFC lesions on separable task components in a monkey analogue of the Wisconsin Card Sorting Test. Animals with lesions restricted to the principal sulcus made more errors after a brief interruption of the task. This is consistent with our finding that principal sulcus deactivation was associated with more errors in the ‘rule memorized’ as compared with the ‘rule visible’ condition. Both findings support a role of the dlPFC in working memory for rules.

Indeed, task-related differences between pro-saccade and anti-saccade trials during the prestimulus period have been described in dlPFC neurons (Everling and DeSouza, 2005; Johnston and Everling, 2006; Johnston et al., 2007; Johnston et al., 2009). Moreover, a subset of dlPFC neurons sends these task-selective signals directly to the SC (Johnston and Everling, 2006). Low-frequency activity in the SC that occurs well in advance of the saccade has been termed “prelude” (Glimcher and Sparks, 1992)

or "buildup" (Munoz and Wurtz, 1995a), and associated with motor preparation (Dorris et al., 1997; Everling et al., 1999), target probability (Basso and Wurtz, 1998; Dorris and Munoz, 1998), covert shifts of attention (Kustov and Robinson, 1996; Ignashchenkova et al., 2004), and target selection (Horwitz and Newsome, 2001; McPeek and Keller, 2002).

Consistent with a previous study (Everling et al., 1999), we found that SC saccade neurons display higher levels of prestimulus activity on pro-saccade trials than anti-saccade trials in the precool and postcool periods. Moreover, we have shown here that SC neurons display these differences even when the monkeys had to briefly memorize the instruction during a delay period. These task-selective differences in prestimulus activity have been interpreted to reflect reduced motor preparation on anti-saccade trials. Munoz and Everling hypothesized that anti-saccade task performance requires the suppression of neural activity in the SC prior to stimulus appearance, to prevent the stimulus-driven burst of visual activity from reaching saccade threshold and triggering a short-latency express saccade (Munoz and Everling, 2004). The authors hypothesized that prefrontal lesions would lead to more response errors in the anti-saccade task by allowing prestimulus activity in the SC to increase (Munoz and Everling, 2004). Here we demonstrate this is not the case: bilateral deactivation of dlPFC area 46 reduced prestimulus activity in the SC, and eliminated the difference of prestimulus activity between pro-saccade and anti-saccade trials. Considering that the level of prestimulus activity of SC neurons is negatively correlated with saccadic reaction times (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1999), this finding can explain the increased reaction times of pro-saccades and anti-saccades during dlPFC deactivation in monkeys, and in patients with prefrontal disorders. In a more general framework, reduced motor preparation may underlie hypokinesia and thus delayed response initiation in these patients. While the reduction in prestimulus activity can explain the longer reaction times, it cannot account for the increased error rates during dlPFC deactivation. In fact, a direct comparison of correct and error trials during dlPFC deactivation did not show any differences in prestimulus activity.

Our data show that dlPFC deactivation impaired suppression of the stimulus-related response in SC neurons. While the amplitude of the initial visual response did

not differ between the cooling and noncool periods, with dlPFC deactivation there was a larger 'second volley' following the initial visual response, such that SC neurons remained active longer on cooling than noncool trials. The comparison of correct trials and error trials demonstrated that on correct trials, the activity was suppressed starting about 100 ms after stimulus onset, whereas the activity continued to increase on error trials. An impaired ability to efficiently suppress the stimulus-driven signal during dlPFC deactivation may be a neural correlate for the known role of the lateral PFC in the inhibition of "prepotent" response tendencies (Diamond and Goldman-Rakic, 1989). This finding is also reminiscent of the increased amplitude of auditory evoked potentials in patients with prefrontal lesions (Knight et al., 1989), and may underlie the inability of patients with prefrontal damage to filter irrelevant stimuli (Fuster, 1997).

Antidromically-identified corticotectal neurons have demonstrated that the dlPFC sends a mixture of prestimulus, stimulus-related, and saccade-related signals directly to the superior colliculus (Johnston and Everling, 2006, 2009). The most prevalent task-selective signals of corticotectal dlPFC neurons were higher levels of prestimulus activity, and an enhanced visual response to stimuli presented in the contralateral hemifield, for anti-saccades compared with pro-saccades (Johnston and Everling, 2006). This activity pattern, which may be shaped by the microcircuitry of the dlPFC (Johnston et al., 2009), has been interpreted as a signal that suppresses SC activity on anti-saccade trials. Our finding of reduced prestimulus activity and prolonged stimulus-related activity during dlPFC deactivation suggests that the influence of the dlPFC on the SC may be excitatory prior to stimulus onset, and inhibitory after stimulus onset. Although the direct projections from layer V of the dlPFC to the intermediate layers of the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981) are excitatory (Jones, 2004), it is unknown whether these axons synapse directly on saccade neurons, or inhibitory interneurons that mediate local and long-range inhibition in the SC (Sooksawate et al., 2011). Corticocortical neurons in primates are also excitatory, and mainly form synapses with other excitatory neurons (Somogyi et al., 1998), although there are projections to inhibitory neurons which have been proposed to improve response selectivity in behavioral tasks (Medalla and Barbas, 2009). Moreover, although it is tempting to speculate that the effects of dlPFC

deactivation on SC activity result from elimination of direct prefrontotectal projections, prefrontal cooling has also been shown to alter thalamic activity (Alexander and Fuster, 1973). The dlPFC could also influence SC activity indirectly by way of the frontal eye field, supplementary eye field, and basal ganglia, which also carry task-related signals for pro-saccades and anti-saccades (Schlag-Rey et al., 1997; Everling and Munoz, 2000; Ford and Everling, 2009; Watanabe and Munoz, 2009; Yoshida and Tanaka, 2009). It is therefore conceivable that the dlPFC has both inhibitory and excitatory influences on the SC, depending on the neural circuits that are recruited for particular task requirements.

Alternatively, impaired inhibition of the stimulus-driven response may be directly related to an impairment of generating the motor command for the anti-saccade. According to Desimone and Duncan's biased competition model (1995), inhibition occurs as the result of local competition between conflicting representations. In the anti-saccade task, this could be viewed as a competition between the representations of the motor programs for the stimulus-driven pro-saccade and the goal-driven anti-saccade. The neural mechanism by which the dlPFC exerts task-dependent control, and thus facilitates anti-saccade task performance, could be an influential biasing of the motor command for anti-saccades, such that removal of the bias signal would delay motor responses, prolong stimulus-driven responses, and allow an increase of response errors. Therefore deficits in generating a motor command for the anti-saccade could be directly responsible for the prolonged stimulus-related response that we observed, and may explain the robust behavioral deficits of patients with neurological or psychiatric disorders that affect the prefrontal cortex (Everling and Fischer, 1998; Broerse et al., 2001; Hutton and Ettinger, 2006). This hypothesis is supported by the evidence that we found in *Chapter 3* which suggests that the dlPFC has an excitatory rather than inhibitory influence on SC saccade neurons. Whether this excitatory influence is exerted by a direct prefrontotectal pathway, or indirectly by way of other saccade-related areas that also send direct projections to the SC, remains to be determined.

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Chapter 5

General Discussion

The prefrontal cortex plays an important role in the cognitive control of saccadic eye movements (Johnston and Everling, 2008; McDowell et al., 2008; Muri and Nyffeler, 2008), a well-established test of which is the anti-saccade task that instructs subjects to look away from a suddenly-appearing stimulus (Hallett, 1978). It has been proposed that anti-saccade task performance requires the inhibition of a prepotent pro-saccade toward the stimulus, inversion of the saccade vector from toward the stimulus to away from the stimulus, and generation of a voluntary anti-saccade away from the stimulus (Munoz and Everling, 2004). The dorsolateral prefrontal cortex (dlPFC) and anterior cingulate cortex (ACC) are prefrontal saccade-related areas that have been implicated in the inhibitory component of anti-saccade task performance (see *Sections 1.3.13 and 3.1*). By either direct or indirect pathways, these prefrontal areas are connected with the superior colliculus (SC), which is a midbrain oculomotor structure that sends saccade commands to the brainstem saccade generator (Munoz et al., 2000; Scudder et al., 2002; Sparks, 2002; Gandhi and Katnani, 2011). Human neuroimaging studies have found a higher prestimulus BOLD signal at the dlPFC and ACC for anti-saccades than pro-saccades (Ford et al., 2005; Brown et al., 2007), while a monkey electrophysiology study found that SC saccade neurons have lower prestimulus activity for anti-saccades than pro-saccades (Everling et al., 1999). These correlational studies suggest that the dlPFC and ACC are engaged, and SC saccade neurons suppressed, on anti-saccade trials. Therefore an inhibitory model of prefrontal function was proposed by which the prefrontal cortex inhibits an unwanted saccade toward the stimulus, by suppressing the activity of SC saccade neurons on anti-saccade trials (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009). To establish a causal relationship between the prefrontal cortex, SC saccade neurons, and anti-saccade task performance, we deactivated either of these prefrontal areas with the cryoloop method of reversible cryogenic deactivation (Lomber et al., 1999). This enabled us to identify the neural

mechanism by which the prefrontal cortex facilitates anti-saccade task performance, which was the **Main Objective** of this dissertation.

The **first Specific Aim** was to assess the roles of the dlPFC and ACC in saccade control, by directly comparing the behavioural effects of unilateral dlPFC and ACC deactivation on pro-saccades and anti-saccades. Both dlPFC and ACC deactivation increased the incidence of ipsilateral saccades, but only dlPFC deactivation impaired contralateral saccades. The **second Specific Aim** was to perform a direct test of the inhibitory model by deactivating the dlPFC unilaterally, and recording the activity of SC saccade neurons, while the monkey performed the same anti/pro-saccade task. Unilateral dlPFC deactivation caused a neural imbalance at the SC, which enabled us to identify an excitatory influence of the dlPFC on saccade neurons in the SC ipsilateral to deactivation. Bilateral dlPFC deactivation, on the other hand, was designed to not cause a neural imbalance, and thus was used for the **third Specific Aim**, which was to identify the effects of dlPFC deactivation that were related to cognitive control impairments. Bilateral dlPFC deactivation increased the stimulus-related activity, and decreased the saccade-related activity, of SC saccade neurons. Furthermore, an increase of anti-saccade errors was more substantial in a "rule memorized" condition, which suggests that the dlPFC plays an important role in rule maintenance. Given an excitatory influence of the dlPFC on SC saccade neurons, these findings suggest that the dlPFC facilitates anti-saccade task performance by first maintaining the relevant rule in working memory, then implementing the rule by enhancing the saccade-generating signal at the SC.

5.1 – Integration of the Results

The dlPFC and ACC are prefrontal components of a cortical saccade control network, including the posterior parietal cortex (PPC), frontal eye field (FEF), and supplementary eye field (SEF), that facilitates contralateral saccades (see *Section 2.1*). Here we found that dlPFC deactivation both increased the incidence of ipsilateral saccades and impaired contralateral saccades, whereas ACC deactivation only increased the incidence of ipsilateral saccades. The lack of contralateral saccade impairments with ACC deactivation may be explained by either the behavioral task that we used, or the

particular area of the ACC that we deactivated (see *Section 5.3*). The contralateral saccade impairments that we observed with dlPFC deactivation were an increase of contralateral saccade latency, a decrease of contralateral saccade velocity, and an increase of contralateral anti-saccade duration. All contralateral saccade impairments were greater for anti-saccades than pro-saccades, which supports a role of the dlPFC in the performance of more cognitively-demanding tasks. We found no effect of unilateral dlPFC deactivation on saccade gain (i.e. the amplitude of a saccade), whereas bilateral dlPFC deactivation increased the gain and thus decreased the accuracy of anti-saccades. This may be explained by the response fields of dlPFC neurons which are predominantly contralateral, while some are ipsilateral (Boch and Goldberg, 1989; Funahashi et al., 1989, 1990, 1991). Therefore with unilateral dlPFC deactivation there remains one side of the dlPFC that encodes one hemifield (contralateral to the active dlPFC), and to a lesser extent the opposite hemifield (ipsilateral to the active dlPFC), whereas with bilateral dlPFC deactivation, neither dlPFC is active, and thus neither hemifield is encoded.

The contralateral saccade impairments that we found were behavioral effects which suggested that dlPFC deactivation decreased the activity of saccade neurons in the SC ipsilateral to deactivation. This implies that the dlPFC has an excitatory influence on the oculomotor system, and thus supports an excitatory model of prefrontal function (Fig. 3.1). An inhibitory model of prefrontal function, on the other hand, proposed that the dlPFC has an inhibitory influence on the oculomotor system by suppressing the activity of SC saccade neurons (Fig. 3.1) (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009). We performed a direct test of these models by recording the activity of SC saccade neurons, and found that unilateral dlPFC deactivation delayed the onset of saccade-related activity at the SC ipsilateral to deactivation, which corresponds with an increase of contralateral saccade latency.

Unilateral dlPFC deactivation also increased the prestimulus and stimulus-related activity of saccade neurons in the SC contralateral to deactivation. This was likely the result of interhemispheric inhibition at either the cortical or collicular level (Munoz and Istvan, 1998; Schlag et al., 1998; Takahashi et al., 2005; Palmer et al.,

2012), such that a decrease of activity on the ipsilateral side would allow an increase of activity on the contralateral side. Now while we did not find a decrease of activity at the SC ipsilateral to deactivation per se, the delayed onset of saccade-related activity implies that SC saccade neurons were impaired in their ability to send a saccade command to the brainstem saccade generator. This we interpret as a reduction in the activity related to saccade generation at the SC. We also found a decrease of ipsilateral pro-saccade latency that corresponded with both an increase of prestimulus activity and an earlier onset of saccade-related activity at the contralateral SC. However, there was also a later onset of saccade-related activity at the ipsilateral SC, which suggests these effects of unilateral dlPFC deactivation on saccade-related activity may have been caused by a neural imbalance at the SC. To test this idea we used bilateral dlPFC deactivation, which was designed to not cause a neural imbalance, and found a decrease of both the prestimulus and saccade-related activity in SC saccade neurons. This corresponded with an increase of saccade latency, which suggests that a neural imbalance at the SC did not cause the delayed onset of saccade-related activity that we observed with unilateral dlPFC deactivation. Unilateral dlPFC deactivation also increased prestimulus activity in the contralateral SC, which persisted beyond stimulus onset (see *Section 3.3.3*), and thus implies that the effects we found on saccade-related activity were directly related to the effects on prestimulus activity. Together these effects of dlPFC deactivation have demonstrated that saccade latency is inversely related to the prestimulus activity of SC saccade neurons, which agrees with previous findings (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1999).

With unilateral dlPFC deactivation, we also found an increase of stimulus-related activity at the contralateral SC, which corresponded with an increased incidence of ipsilateral errors on anti-saccade trials. Similarly we found that bilateral dlPFC deactivation increased both stimulus-related activity and errors on anti-saccade trials. We then directly compared the activity of SC saccade neurons on correct and error trials, and found that with both unilateral and bilateral dlPFC deactivation, there was a higher level of stimulus-related activity for anti-saccade errors than correct anti-saccades. This demonstrates that anti-saccade errors are correlated with the stimulus-related activity of SC saccade neurons. With unilateral dlPFC deactivation there was

also a higher level of prestimulus activity, and a greater visual response, for anti-saccade errors, however these effects were not found with bilateral dlPFC deactivation, which suggests they may have been caused by a neural imbalance instead.

In summary, unilateral dlPFC deactivation created a neural imbalance at the SC which suggests that the dlPFC enhances the activity of SC saccade neurons, and thus has an excitatory influence on the oculomotor system. We believe that bilateral dlPFC deactivation, on the other hand, did not create a neural imbalance, but rather reduced the prestimulus activity of SC saccade neurons which consequently reduced the saccade-generating signal and thus increased anti-saccade latency. The reduced saccade-generating signal also allowed more time for the competing stimulus-driven signal (i.e. the 'second volley') to reach saccade threshold and trigger an erroneous saccade toward the stimulus. Therefore we propose that the dlPFC facilitates anti-saccade task performance by enhancing the saccade-generating signal in the SC. Furthermore, an increase of anti-saccade errors was more substantial in a "rule memorized" condition, in which the task instruction was not available at the time of the response, than in a 'rule visible' condition, in which the task instruction was visible for the entire duration of the trial. This suggests that the dlPFC plays an important role in rule maintenance. Given an excitatory influence of the dlPFC on SC saccade neurons, these findings suggest that the dlPFC facilitates anti-saccade task performance by first maintaining the relevant rule in working memory, then implementing the rule by enhancing the saccade-generating signal at the SC.

5.2 – Implications

The anti-saccade task requires both the inhibition of a prepotent saccade toward the stimulus, and generation of a voluntary saccade away from the stimulus. While patients with prefrontal disorders have been found with both longer anti-saccade reaction times and more anti-saccade errors than healthy subjects (Everling and Fischer, 1998; Broerse et al., 2001; Hutton and Ettinger, 2006), the impairment most discussed has been that of inhibition rather than initiation, and this seems to have been perpetuated in the anti-saccade literature. For example, a greater prestimulus BOLD signal at the dlPFC for anti-saccades than pro-saccades (Ford et al., 2005; Brown et al., 2007), and dlPFC

neurons with greater prestimulus activity for anti-saccades than pro-saccades (Everling and DeSouza, 2005; Johnston and Everling, 2006), have been interpreted as support for the inhibition of a saccade toward the stimulus, rather than the initiation of a saccade away from the stimulus. In light of our findings, however, it appears that the prefrontal cortex facilitates anti-saccade task performance by first enhancing the saccade-generating signal at the SC, which by interhemispheric inhibition then suppresses the stimulus-driven signal at the opposite SC, to inhibit an unwanted saccade toward the stimulus. This suggests that the role of the prefrontal cortex in the cognitive control of saccades is primarily implemented by an excitatory rather than inhibitory influence on the oculomotor system.

5.2.1 Prefrontal Bias Signals

Cognitive control enables us to guide our behaviour in an appropriate manner. A proposed mechanism for cognitive control is top-down bias signals from the prefrontal cortex that influence processing at other brain areas which are more directly involved in the generation of a response (Miller and Cohen, 2001). These prefrontal bias signals are proposed to facilitate both selective attention, which enhances the processing of task-relevant information, and behavioral inhibition, which suppresses the processing of information that is not relevant. This biased competition is necessary due to the brain's inherently limited capacity to process information, and furthermore the winner-take-all mechanism which determines the information that is selected. Prefrontal bias signals could influence response selection in either of three ways: a) directly suppressing the unwanted response, which allows the desired response to occur; b) directly enhancing the desired response, which does not allow the unwanted response to occur; c) both directly suppressing the unwanted response, and directly enhancing the desired response.

In support of the latter, a match/non-match task identified 'look' and 'don't look' neurons at the very caudal extent of the dlPFC, bordering on the FEF, which encoded stimuli that either should or should not be selected (Hasegawa et al., 2004). This suggests that both selective attention and behavioral inhibition are directly facilitated by the dlPFC. On the other hand, Fuster (1997) has proposed that the dlPFC

plays a role in selective attention, and thus directly enhances the desired response, whereas the orbitomedial prefrontal cortex is responsible for inhibitory control. This is not to say that the dlPFC has no effect on the unwanted response, given as how selective attention and behavioral inhibition can be considered as "two sides of the same coin", such that enhancement of task-relevant representations further biases the competition by also inhibiting conflicting representations (Desimone and Duncan, 1995). In agreement with this, we found that dlPFC deactivation reduced the saccade-generating signal, which allowed an increase of the stimulus-driven signal that would otherwise have been suppressed. Therefore it appears that the influence of prefrontal bias signals on response selection is mediated by directly enhancing the task-relevant and thus desired response, which indirectly inhibits unwanted responses (Munakata et al., 2011).

5.2.2 Rule-guided Behaviour

How then does a particular response come to be identified as task-relevant? The prefrontal cortex plays an important role in the cross-temporal association of a stimulus with a response (Fuster, 1997). These associations are guided by rules, and strengthened by the reinforcement of a successful and thus task-relevant response. The context in which this occurs becomes associated with the rule, and therefore determines the task-relevant response. For example, when driving on to the University of Western Ontario campus, most times I will turn right to go to my office, but in the context of a Monday night, the rule is that I am going to play hockey, and thus the relevant response is to turn left and drive to the hockey arena instead. Prefrontal support is particularly important when a weak but task-relevant behaviour, such as getting out of bed to go to school in the morning, is in competition with a stronger, more habitual response, such as staying in bed and going back to sleep. In the absence of prefrontal support to implement the appropriate rule, we are unlikely to perform the appropriate response, and thus much more likely to perform the prepotent but inappropriate response instead.

In addition to implementing the rules that guide our behaviour, the dlPFC also encodes these rules (Hoshi et al., 1998; White and Wise, 1999; Asaad et al., 2000; Wallis et al., 2001; Everling and DeSouza, 2005; Mansouri et al., 2006), and when

relevant maintains them in working memory (Mansouri et al., 2006; Buckley et al., 2009). Here we found further support for a role of the dlPFC in the encoding and maintenance of rules, as demonstrated by the effects of bilateral dlPFC deactivation on pro-saccades and anti-saccades in both a 'rule visible' and 'rule memorized' condition. The 'rule memorized' condition required that a task instruction be held in working memory for a brief period of time, and thus had greater cognitive demands than the 'rule visible' condition for which the task instruction was available at the time of the response. We found only a small increase of anti-saccade errors with bilateral dlPFC deactivation in the "rule visible" condition, which suggests that the dlPFC does not play a critical role in saccade inhibition, and thus does not have an inhibitory influence on the oculomotor system. Alternatively, this weak effect of dlPFC deactivation on pro-saccades and anti-saccades may be explained by a role of the dlPFC in learning new rules as opposed to performing well-learned rules. This is supported by the finding that a rCBF signal at the dlPFC was greater at the time of initial learning, as compared to when the task had been practiced and thus well-learned (Raichle et al., 1994; Shadmehr and Holcomb, 1997). Furthermore dlPFC neurons were found to have a greater response for novel as compared to familiar stimuli (Asaad et al., 1998), whereas patients with prefrontal damage that included the dlPFC had a lower ERP response to novel stimuli as compared to healthy control subjects (Knight, 1984). An additional possibility is that this weak effect of dlPFC deactivation in the "rule visible" condition could have been the result of impaired task encoding, the impact of which may be lessened by rule-encoding neurons in the orbitofrontal cortex, ventrolateral prefrontal cortex, premotor cortex, posterior parietal cortex, and to a lesser extent, the dorsal striatum (Wallis et al., 2001; Wallis and Miller, 2003; Stoet and Snyder, 2004; Muhammad et al., 2006).

5.2.3 Rule Maintenance

The increase of anti-saccade errors with dlPFC deactivation was greater in the 'rule memorized' condition than the 'rule visible' condition, which suggests that the dlPFC plays a greater role when the task instruction is not available at the time of the response. This is supported by previous findings of dlPFC neurons which maintained rule-selective activity across an entire block of trials, and dlPFC lesions that impaired rule

maintenance, when performing an analogue of the Wisconsin Card Sorting Test (Mansouri et al., 2006; Buckley et al., 2009). Together these findings indicate that the dlPFC plays an important role in executive control, a cardinal function of which is the maintenance of rules that guide our behaviour in the appropriate manner (Miller and Cohen, 2001; Munakata et al., 2011).

We also found that one of our monkeys demonstrated both an increase of errors toward the stimulus on anti-saccade trials, and a decrease of errors away from the stimulus on pro-saccade trials. While the increased anti-saccade errors may suggest that inhibition was impaired, the decreased pro-saccade errors suggest that it was not. Instead, this increased incidence of prepotent saccades toward the stimulus demonstrates that regardless of the task instruction, the animal was simply more likely to perform the prepotent response, rather than having an impaired ability to suppress a saccade toward the stimulus. Therefore rule maintenance, rather than response inhibition, was impaired by dlPFC deactivation in the 'rule memorized' condition.

5.2.4 Anti-saccade Errors

With regards to the cause of anti-saccade errors, an accumulator model was proposed by which an increased level of prestimulus activity in SC saccade neurons enables a stimulus-driven burst of visual activity to reach saccade threshold and trigger an erroneous saccade toward the stimulus (Munoz and Everling, 2004). An inhibitory model then proposed that the dlPFC suppresses the prestimulus activity of SC saccade neurons to inhibit an unwanted saccade toward the stimulus (see *Section 3.1*). Here we found with bilateral dlPFC deactivation that there was an increase of anti-saccade errors, a decrease of prestimulus activity, and no difference of prestimulus activity between correct anti-saccades and anti-saccade errors. Furthermore, anti-saccade error reaction times were not in the range of express saccades, and thus we found neither an increase of prestimulus activity nor express-latency anti-saccade errors with dlPFC deactivation. This suggests that anti-saccade errors were not automatically driven by the appearance of a stimulus. Instead we found that anti-saccade errors corresponded with a larger 'second volley' and increase of stimulus-related activity which started at around 100 ms after stimulus appearance. To explain this, we propose that with dlPFC

deactivation there was a reduction of the saccade-generating signal which allowed more time for the stimulus-driven signal to increase toward saccade threshold and trigger a saccade toward the stimulus, which is an error on the anti-saccade task. In support of this, we performed a direct comparison of SC saccade neuron activity between anti-saccade errors and correct anti-saccades, and found there was a higher level of stimulus-related activity for anti-saccade errors than correct anti-saccades.

5.2.5 Models of Prefrontal Function

As discussed in *Chapter 4* (see *Section 4.4*), the increase of stimulus-related activity that we observed with bilateral dlPFC deactivation could be the result of either an impaired suppression of stimulus-related activity, or an impaired saccade-generating signal that allows more time for the stimulus-driven signal to increase. The first interpretation is supported by an inhibitory model of prefrontal function in which the dlPFC suppresses the activity of SC saccade neurons (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009), whereas the latter interpretation suggests that the dlPFC enhances the activity of SC saccade neurons instead. With regards to the inhibitory model, the dlPFC sends direct projections to the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981) and thus could have an inhibitory influence on SC saccade neurons by synapsing directly with either fixation neurons in the rostral SC, or inhibitory interneurons in the caudal SC, both of which suppress the activity of SC saccade neurons (Munoz and Istvan, 1998). The dlPFC also sends indirect projections to the SC by way of other cortical saccade-related areas such as the frontal eye field and supplementary eye field (Selemon and Goldman-Rakic, 1988), which themselves have direct projections to the SC (Stanton et al., 1988; Shook et al., 1990). The dlPFC also sends projections to the basal ganglia (Selemon and Goldman-Rakic, 1985), which play a modulatory role in saccade control by the disinhibition of SC saccade neurons via a direct pathway, and the inhibition of SC saccade neurons via indirect and hyperdirect pathways (Hikosaka et al., 2000; Watanabe and Munoz, 2011). The dlPFC also influences thalamic activity (Alexander and Fuster, 1973), and thus could modulate subcortical input to cortical saccade-related areas (Tanaka and Kunimatsu, 2011).

Therefore both direct and indirect pathways could mediate an inhibitory influence of the dlPFC on SC saccade neurons.

In *Chapter 3*, however, we found evidence to suggest that the dlPFC has an excitatory rather than inhibitory influence on SC saccade neurons. This implies that rather than suppressing the stimulus-driven signal directly, the dlPFC enhances the saccade-generating signal at the SC, which by reciprocal inhibitory mechanisms at the level of either cortical areas (Schlag et al., 1998; Palmer et al., 2012) or collicular structures (Munoz and Istvan, 1998; Takahashi et al., 2005), indirectly suppresses the competing stimulus-driven signal. Consequently dlPFC deactivation would reduce support for the saccade-generating signal, which would allow more time for the stimulus-driven signal to reach saccade threshold and trigger an unwanted saccade toward the stimulus. While this interpretation does not agree with the aforementioned inhibitory model of prefrontal function, on the other hand it does agree with an indirect competitive inhibition model, according to which the dlPFC directly enhances task-relevant representations (Munakata et al., 2011). The indirect collateral effect of this targeted enhancement is the inhibition of competing representations that are not task-relevant. In support of this, excitatory pyramidal neurons send long-range cortical efferent projections that show no evidence of preferential connectivity with inhibitory interneurons at the target region (Jones, 2004; Tamamaki and Tomioka, 2010). Furthermore, inhibitory interneurons appear to play a role in diffuse lateral inhibition, rather than targeting specific representations within an area (Markram et al., 2004). Finally, computational models have demonstrated that impaired goal maintenance produces deficits similar to those demonstrated by patients with prefrontal disorders which have previously been attributed to impairments of inhibitory control (Cohen and Servan-Schreiber, 1992; Morton and Munakata, 2002). The indirect competitive inhibition model suggests that these deficits occur not as the result of impaired inhibition, but rather as the result of reduced support for the task-relevant behaviour when in competition with a stronger, more habitual response (Miller and Cohen, 2001; Braver et al., 2007; Munakata et al., 2011).

In *Chapter 4* we also found that with bilateral dlPFC deactivation there were greater anti-saccade task impairments in a ‘rule memorized’ condition when the task

instruction was not available at the time of the response, than in a 'rule visible' condition when the task instruction was visible throughout the trial, which supports a role of the dlPFC in rule maintenance (Fuster, 1997; Miller and Cohen, 2001; Mansouri et al., 2006; Braver et al., 2007; Sakai, 2008; Buckley et al., 2009; Munakata et al., 2011). This follows from earlier hypotheses of prefrontal contributions to working memory, in which the dlPFC a) plays a role in the maintenance of spatial representations in working memory (Goldman-Rakic, 1995), b) monitors and manipulates representations held in working memory (Petrides, 1995), and c) mediates the cross-temporal integration of task-relevant information by maintaining the appropriate stimulus-response mapping rules in working memory (Fuster, 1997). Miller and Cohen (2001) then proposed that the dlPFC both maintains behaviour-guiding rules in working memory, and implements them by influencing the activity at other brain areas that are more directly involved in the processing of sensory information and generation of a response. More recently, Braver and colleagues (2007) posited a "dual mechanisms of control" theory in which the dlPFC actively maintains rules in working memory as a form of proactive control that mediates the anticipation and prevention of interference, while transient activation of the dlPFC is a form of reactive control that mediates the resolution of interference. Of additional importance is that this transient dlPFC activation follows a transient activation of the ACC which mediates the detection of interference, which has been proposed by various theories of conflict monitoring (Botvinick et al., 2001), performance monitoring (Holroyd and Coles, 2002), and error likelihood (Brown and Braver, 2005). Therefore many theories of prefrontal function agree that the dlPFC actively maintains abstract information such as context, goals, and rules that determine relevance for the cognitive control of behaviour (Fuster, 1997; Miller and Cohen, 2001; Braver et al., 2007), which includes but is not limited to inhibitory control (Munakata et al., 2011).

5.3 – Future Research

5.3.1 Anterior Cingulate Cortex

Here we found that ACC deactivation increased the incidence of ipsilateral saccades, whereas dlPFC deactivation both increased the incidence of ipsilateral saccades and impaired contralateral saccades. While this may suggest that the ACC has a relatively weak effect on the oculomotor system, there are some alternative explanations as well. First, we may have used a behavioral task that did not sufficiently probe ACC function. In a previous study we found rule-selective prestimulus activity in the ACC with an uncued blocked task (Johnston et al., 2007), whereas when deactivating this same area of the ACC in the present study, we found only weak effects on an interleaved cued task. The ACC has been implicated in reinforcement-guided behavior (Kennerley et al., 2006; Buckley et al., 2009), which is probed by the uncued blocked task, and thus deactivation of this same ACC area would likely demonstrate more effects on the uncued blocked task than those we observed with the interleaved cued task.

On the other hand, rather than by using a different task, we may find more effects by deactivating a different area of the ACC instead. The ACC is a heterogenous area that consists of rostral and dorsal subregions (Paus, 2001). Two distinct cingulate eye fields (CEF) have been identified in the dorsal ACC (Wang et al., 2004), although given the ambiguity of their locations, the area that we deactivated could have corresponded with either the rostral CEF, or an area between the rostral and caudal CEFs that is not involved in saccade control (see *Section 2.4.4*). Alternatively, it is possible that we may have deactivated slightly different areas of the ACC in our monkeys, given that our landmark for cryoloop implantation in the anterior cingulate sulcus was the posterior end of the principal sulcus, whereas in the two monkeys studied by Wang and colleagues (2004), the locations of the CEFs relative to the posterior principal sulcus were different. Therefore determining the precise location of the CEFs would help to both interpret our results, and guide future studies of dorsal ACC contributions to saccade control. Furthermore, patients with lesions of either the dorsal or rostral ACC had impaired suppression of prepotent saccades (Paus et al., 1991; Gaymard et al., 1998; Milea et al., 2003), and human neuroimaging studies have

shown that healthy subjects have an increased rCBF at both the dorsal and rostral ACC when performing oculomotor tasks, including anti-saccades (Paus et al., 1993). Therefore the rostral ACC should also be examined for a role in anti-saccade task performance.

5.3.2 Corticotectal Pathways: Direct vs. Indirect

Even though the dlPFC sends direct projections to the SC (Goldman and Nauta, 1976; Leichnetz et al., 1981), and we found there were effects of dlPFC deactivation on the activity of SC saccade neurons, it cannot necessarily be assumed that these effects were mediated by dlPFC corticotectal projections. This is because the dlPFC also sends projections to the FEF, SEF, PPC, and basal ganglia (Selemon and Goldman-Rakic, 1985, 1988; Cavada and Goldman-Rakic, 1989), which themselves send direct projections to the SC (Jayaraman et al., 1977; Lynch et al., 1985; Stanton et al., 1988; Shook et al., 1990). Therefore the effects of dlPFC deactivation on SC saccade neurons could have been mediated by corticotectal, corticostriatal, and intracortical projections, or some combination thereof. Intracortical output neurons are found in the supragranular layers of cortical tissue, while corticotectal and corticostriatal output neurons are found in the infragranular layers, and thus the contributions of intracortical and corticofugal projections could be differentiated by comparing the effects of deactivating either the supragranular or infragranular layers, with the effects of deactivating all cortical layers.

Deactivation of only the supragranular layers, however, would further require that deactivation be restricted to only intracortical output neurons, given that input from cortical afferent projections is still required to generate corticofugal signals. The selectivity of a) cryogenic deactivation, which disrupts synaptic transmission, b) muscimol, which binds GABA receptors, and c) lidocaine, which blocks sodium channels, would therefore be insufficient for these purposes. Instead, optogenetics would be required to selectively deactivate intracortical output neurons in the supergranular layers. This could be done in a manner similar to Cavanaugh and colleagues (2012), who targeted saccade neurons in the intermediate layers of the SC by injecting an adeno associated virus incorporating the light-driven outward proton pump

ArchT, fused to GFP, and expressed under a pan-cellular promoter. If the effect of deactivating the supragranular layers in such a manner was the same as the effects of deactivating all cortical layers, this would suggest that the role of the dlPFC in saccade control is mediated by intracortical projections. On the other hand, if there were no effects of deactivating only the supragranular layers, then it would appear that corticofugal projections mediate the role of the dlPFC in saccade control instead.

Deactivation of the infragranular layers, on the other hand, need not be restricted to corticofugal neurons. Instead, muscimol or lidocaine injections could be used to deactivate infragranular layer 5, which contains subcortical output neurons inclusive of corticotectal and corticostriatal neurons. While preventing spread to the adjacent granular layer 4 and infragranular layer 6 is not imperative for the purposes of this comparison, what is most important is that the supragranular layers remain unaffected. If the effect of deactivating the infragranular layers is the same as the effects of deactivating all cortical layers, this would suggest that the role of the dlPFC in saccade control is mediated by corticofugal projections. On the other hand, if there were no effects of deactivating only the infragranular layers, then it would appear that intracortical projections mediate the role of the dlPFC in saccade control instead.

Therefore, if the role of the dlPFC in saccade control is mediated by intracortical projections, then deactivation of only the supragranular layers would be expected to have the same effects as deactivating all cortical layers, while deactivation of only the infragranular layers would be expected to have no effects. On the other hand, if the role of the dlPFC in saccade control is mediated by corticofugal projections, then deactivation of only the infragranular layers would be expected to have the same effects as deactivating all cortical layers, while deactivation of only the supragranular layers would be expected to have no effects. Finally, if the role of the dlPFC in saccade control is mediated by both intracortical and corticofugal projections, then deactivation of only either the supragranular or infragranular layers would be expected to have a lesser effect than deactivation of all cortical layers, but an effect nonetheless.

5.3.3 dlPFC vs. FEF

In the dlPFC we deactivated the cortex of the posterior sulcus principalis, which is immediately adjacent to the anterior bank of the arcuate sulcus that contains the saccade-related area of the FEF (Bruce and Goldberg, 1985; Bruce et al., 1985). In humans, the analogous areas are located in the middle frontal gyrus (Petrides and Pandya, 1999), and at the junction of the superior frontal sulcus and precentral sulcus (Paus, 1996). Given the close proximity of these two areas, and their extensive reciprocal connections, it is important to distinguish their functional roles in saccade control. While both have been implicated in anti-saccade task performance, patients with FEF lesions have increased anti-saccade reaction times but not errors (Pierrot-Deseilligny et al., 1991; Rivaud et al., 1994; Gaymard et al., 1999), whereas patients with dlPFC lesions have increased anti-saccade errors but not reaction times (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003), with a possible exception (Ploner et al., 2005; Ettinger et al., 2008, p.1156). Furthermore, human neuroimaging studies have shown a greater response-related BOLD signal for correct anti-saccades than anti-saccade errors at the FEF but not the dlPFC (Curtis and D'Esposito, 2003; Brown et al., 2007), and a greater prestimulus BOLD signal for correct anti-saccades than anti-saccade errors at the dlPFC but not the FEF (Ford et al., 2005). Another prominent distinction is that saccades are evoked by electrical microstimulation of the FEF (Bruce et al., 1985), but not the dlPFC (Boch and Goldberg, 1989), even though both have direct corticotectal projections (Goldman and Nauta, 1976; Leichnetz et al., 1981; Stanton et al., 1988).

Therefore it seemed that these areas could easily be differentiated from each other based on an excitatory role of the FEF, and an inhibitory role of the dlPFC, however here we have found evidence to suggest that the dlPFC, like the FEF, has an excitatory influence on the oculomotor system. While this may suggest that the effects of cooling could have spread from the banks of the posterior principal sulcus to the adjacent anterior bank of the arcuate sulcus, we are confident that they did not because the spread of deactivation with cryoloops is limited to approximately the thickness of the cortical gray layer (Lomber and Payne, 1996; Payne et al., 1996; Lomber et al., 1999). It would be nonetheless informative to deactivate the anterior bank of the arcuate

sulcus and thus compare the effects of dlPFC and FEF deactivation on anti-saccade task performance and the activity of SC saccade neurons.

5.3.4 SC Fixation Neurons

dlPFC corticotectal projections have been proposed to synapse with fixation neurons in the rostral SC, which by reciprocal inhibition suppress the activity of saccade neurons in the caudal SC (Munoz and Everling, 2004; Johnston and Everling, 2006; Johnston et al., 2009). This inhibition model of prefrontal function would predict a decrease of fixation neuron activity, and an increase of saccade neuron activity, with dlPFC deactivation (Fig. 3.1). Conversely here we found that dlPFC deactivation reduced the activity of saccade neurons, which suggests that dlPFC corticotectal projections synapse directly with saccade neurons, and thus supports an excitation model of prefrontal function instead (Fig. 3.1). Given the reciprocal inhibition between them, this decrease of saccade neuron activity would be expected to allow the activity of fixation neurons to increase (Munoz and Istvan, 1998). Fixation neurons may also be inhibited by the dlPFC, given that dlPFC corticotectal neurons have been identified by antidromic stimulation of the rostral SC (Johnston and Everling, 2006, 2009). Consequently dlPFC projections to the rostral SC could synapse with inhibitory interneurons that suppress fixation neurons (Takahashi et al., 2005), which would reduce their inhibition of saccade neurons (Munoz and Istvan, 1998), coincident with dlPFC signals to the caudal SC that enhance saccade neurons directly. However, because all axon fibres enter the SC at the rostral pole (Stanton et al., 1988), antidromic stimulation of the rostral SC could activate axons that terminate in either the rostral SC or caudal SC, and thus the actual rostrocaudal extent of corticotectal axon terminals in the SC is unknown. Assuming that the dlPFC sends projections to both the rostral SC and caudal SC, dlPFC deactivation would be expected to reduce prefrontal input to both interneurons in the rostral SC, and saccade neurons in the caudal SC, which would delay both the suppression of fixation neurons, and discharge of saccade neurons. In support of this, here we found that dlPFC deactivation delayed onset of saccade-related activity in the caudal SC, and furthermore would be expected to delay the pause of fixation-related activity in the rostral SC. This could easily be determined by combining dlPFC

deactivation with the recording of SC fixation neurons, and would be a worthwhile contribution to our understanding of the neural mechanism by which the prefrontal cortex facilitates anti-saccade task performance.

5.4 – Summary and Conclusion

The prefrontal cortex has for over 20 years been implicated in the inhibitory control of saccades by anti-saccade task performance. Studies of patients, neuroimaging, and transcranial magnetic stimulation with humans, in addition to neuroimaging, electrophysiology, electrical microstimulation, and reversible deactivation studies with monkeys, have supported an inhibitory model of prefrontal function (see *Sections 1.3.13 and 3.1*). This inhibitory model proposed that on anti-saccade trials, the prefrontal cortex suppresses the activity of SC saccade neurons, to inhibit an unwanted saccade toward the stimulus (Pierrot-Deseilligny et al., 1991; Pierrot-Deseilligny et al., 2003; Munoz and Everling, 2004; Ploner et al., 2005; Johnston and Everling, 2006; Johnston et al., 2009). Here we performed a direct test of this inhibitory model by deactivating the dlPFC and recording the activity of SC saccade neurons, and found evidence to suggest that the dlPFC has an excitatory rather than inhibitory influence on the oculomotor system. This prompted us to propose an excitatory model by which the dlPFC enhances rather than suppresses the activity of SC saccade neurons (Fig. 3.1). These prefrontal signals are proposed to implement the behaviour-guiding rules that are encoded and maintained by the dlPFC (Miller and Cohen, 2001; Mansouri et al., 2006; Buckley et al., 2009). We found more anti-saccade errors when the task instruction was not available at the time of the response, and thus proposed that the dlPFC facilitates anti-saccade task performance by first encoding and maintaining the behavior-guiding rule, then implementing the rule by enhancing the saccade-generating signal at the SC. This may explain the increased anti-saccade reaction times and errors of patients with prefrontal disorders, and more generally suggests that the inappropriate behavioral responses of patients with prefrontal disorders such as schizophrenia and Tourette's syndrome may be caused by an impaired ability to maintain and implement the appropriate rule.

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Appendix 1

Ethics Approval Form

Western

Dec. 30, 2008

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2012

Dear Dr. Everling:

Your Animal Use Protocol form entitled:
Role of Frontal Cortex in cognitive control
Funding Agency CIHR - Grant #R3104A12; NSERC DISCOVERY - Grant - PYFRR

has been approved by the University Council on Animal Care. This approval is valid from **Dec. 30, 2008 to Dec. 31, 2009**. The protocol number for this project is **#2008-125 and replaces #2004-099-12.**.

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 4 Years

Species	Strain	Other Detail	Pain Level	Animal # Total for 4 Years
Other, add to detail	NHP - Macaca mullata or Macaca fascicularis	3-16 kg	D	20

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.

c.c. Approved Protocol - S. Everling, T. Admans
Approval Letter - S. Everling, T. Admans

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, • London, Ontario • CANADA – N6A 5C1
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Appendix 2

Behavioral Effects of Unilateral dlPFC Deactivation in the Overlap Condition

	Pro-saccades							Anti-saccades						
	Contralateral				Ipsilateral			Contralateral				Ipsilateral		
	Pre	Cool	Post		Pre	Cool	Post	Pre	Cool	Post		Pre	Cool	Post
Monkey A (n=23)														
Correct (%)	93.6	93.8	95.2		93.6	97.0	94.8	93.9	80.9	89.2	**	92.6	94.1	87.0
SRT (ms)	172.9	207.2	179.0	***	173.8	163.0	179.5	***	175.5	202.6	188.1	*	180.7	189.9	195.2
Velocity (◦/s)	308.2	294.0	298.6	*	305.6	299.8	297.4	269.2	223.6	272.8	***	261.8	271.2	268.9
Duration (ms)	35.5	36.2	36.1		35.5	35.9	36.1	48.0	58.4	48.6	***	48.8	48.7	49.5
Accuracy (gain)	1.00	0.98	1.00		1.00	1.00	1.00	1.40	1.51	1.50		1.42	1.48	1.51
Monkey C (n=35)														
Correct (%)	98.0	95.2	95.6		97.2	98.7	98.2	93.7	85.0	86.9		96.1	98.1	93.4
SRT (ms)	285.3	334.6	291.0	***	267.4	257.2	268.1	*	285.7	308.3	306.7		292.2	311.2	305.3
Velocity (◦/s)	293.8	282.5	294.2	**	319.9	320.1	316.7	243.8	212.5	244.3	***	269.1	281.9	271.2	*
Duration (ms)	44.0	46.4	45.0	**	44.8	44.6	45.3	50.2	57.9	52.9	***	50.1	51.6	53.4	
Accuracy (gain)	1.04	1.04	1.05		1.04	1.04	1.04	1.04	1.02	1.06		1.08	1.15	1.14	
Monkey D (n=1)														
Correct (%)	65.8	82.4	82.8		94.3	91.7	90.6	85.2	70.0	64.2		84.8	92.3	81.4
SRT (ms)	218.1	202.6	219.0		181.9	180.5	185.5	198.4	259.3	207.4		174.9	199.1	191.2
Velocity (◦/s)	274.7	273.9	280.9		239.9	230.9	232.2	273.3	346.1	298.8		260.6	226.6	229.3
Duration (ms)	36.9	36.7	36.6		34.5	34.2	34.7	41.1	41.0	41.0		39.4	38.8	39.2
Accuracy (gain)	1.16	1.17	1.15		1.00	0.97	0.98	1.28	1.59	1.42		1.24	1.12	1.10

*p<0.05, **p<0.01, ***p<0.001, paired t- test.

Appendix 3

Behavioral Effects of Unilateral dlPFC Deactivation in the Gap Condition

	Pro-saccades								Anti-saccades							
	Contralateral				Ipsilateral				Contralateral				Ipsilateral			
	Pre	Cool	Post		Pre	Cool	Post		Pre	Cool	Post		Pre	Cool	Post	
Monkey A (n=23)																
Correct (%)	94.2	94.4	95.4		94.4	98.3	95.9	**	85.1	77.2	78.7		87.2	90.9	79.7	
SRT (ms)	139.1	164.8	144.2	***	136.0	128.4	144.2	***	144.8	165.0	155.3	***	147.2	152.3	155.6	
Velocity (◦/s)	309.0	295.0	298.4		305.8	299.8	296.5		268.1	228.2	271.8	***	264.2	269.8	271.0	
Duration (ms)	35.5	36.1	36.2		35.4	35.9	36.0		46.7	56.5	47.5	***	46.5	48.1	47.9	
Accuracy (gain)	1.01	0.98	1.00		0.99	1.00	0.99		1.13	1.20	1.18		1.35	1.47	1.45	
Monkey C (n=35)																
Correct (%)	92.8	79.8	89.9	**	93.8	98.5	94.4	***	69.4	52.4	62.3	***	75.9	79.4	70.3	
SRT (ms)	229.2	286.5	244.7	***	209.2	194.8	221.4	**	255.4	292.0	270.8	**	260.2	256.1	268.0	
Velocity (◦/s)	279.7	264.2	280.7	**	301.6	305.7	300.3		244.9	218.4	245.8	***	277.6	288.5	278.1	*
Duration (ms)	45.4	48.1	46.9		45.7	45.8	46.4		48.7	56.1	51.2	***	50.5	51.6	51.7	
Accuracy (gain)	1.02	1.02	1.04		1.02	1.03	1.02		1.01	1.02	1.05		1.12	1.17	1.14	*
Monkey D (n=1)																
Correct (%)	82.0	66.7	86.2		96.0	100	97.8		63.1	0	37.7		56.1	50.0	62.7	
SRT (ms)	151.9	129.8	138.4		133.1	125.9	135.5		n/a	n/a	n/a		176.3	155.0	178.9	
Velocity (◦/s)	276.6	280.2	283.2		242.5	239.7	239.6		n/a	n/a	n/a		236.0	243.7	225.6	
Duration (ms)	36.5	39.2	36.0		34.4	34.6	34.6		n/a	n/a	n/a		37.8	41.7	37.7	
Accuracy (gain)	1.15	1.13	1.15		1.00	1.00	1.00		n/a	n/a	n/a		1.08	1.21	1.04	

*p<0.05, **p<0.01, ***p<0.001, paired t- test.

Appendix 4

Behavioral Effects of Unilateral ACC Deactivation in the Overlap Condition

	Pro-saccades Contralateral			Ipsilateral				Anti-saccades Contralateral			Ipsilateral			
	Pre	Cool	Post	Pre	Cool	Post		Pre	Cool	Post	Pre	Cool	Post	
Monkey A (n=21)														
Correct (%)	95.4	97.5	96.0	96.0	98.7	95.7	*	95.1	94.3	86.4	94.8	96.2	81.5	
SRT (ms)	170.0	176.2	176.7	165.5	178.1	178.5		174.5	194.8	198.8	169.6	193.5	194.2	
Velocity (◦/s)	311.8	306.3	301.9	294.1	286.9	283.9		273.8	280.7	293.1	255.1	257.4	252.7	
Duration (ms)	35.2	35.1	35.7	35.3	35.6	36.1		46.8	47.9	49.7	47.6	48.2	51.0	
Accuracy (gain)	1.01	1.00	1.00	0.98	0.97	0.98		1.32	1.48	1.58	1.44	1.47	1.52	
Monkey C (n=12)														
Correct (%)	96.5	96.5	95.5	97.0	96.8	96.7		91.2	85.9	79.3	93.9	94.0	89.1	
SRT (ms)	301.7	303.3	308.8	258.9	258.8	270.2		289.9	294.7	323.2	310.2	299.7	316.0	
Velocity (◦/s)	279.8	290.4	283.7	318.9	323.0	317.9		231.4	232.4	230.4	266.0	279.8	277.5	
Duration (ms)	46.9	46.3	47.5	46.7	46.1	46.1		51.4	51.1	54.8	51.3	51.6	54.0	
Accuracy (gain)	1.03	1.04	1.04	1.03	1.03	1.05		1.00	1.01	1.05	1.08	1.14	1.16	
Monkey D (n=10)														
Correct (%)	95.9	89.9	88.6	79.0	96.0	75.4	***	95.0	87.3	86.2	88.5	89.8	88.4	
SRT (ms)	181.7	184.5	196.7	243.8	222.3	251.6	*	173.5	206.2	194.3	*	196.8	201.8	201.8
Velocity (◦/s)	247.1	246.3	239.3	292.4	279.9	287.8	*	246.5	260.9	245.1	***	283.5	275.9	297.2
Duration (ms)	36.1	35.8	35.8	38.5	38.9	38.7		40.2	40.7	41.2	42.6	43.6	43.0	
Accuracy (gain)	0.99	0.99	0.97	1.15	1.13	1.14		1.13	1.23	1.16	1.27	1.28	1.33	

*p<0.05, **p<0.01, ***p<0.001, paired t- test.

Appendix 5

Behavioral Effects of Unilateral ACC Deactivation in the Gap Condition

	Pro-saccades Contralateral				Ipsilateral				Anti-saccades Contralateral				Ipsilateral			
	Pre	Cool	Post		Pre	Cool	Post		Pre	Cool	Post		Pre	Cool	Post	
Monkey A (n=21)																
Correct (%)	92.4	96.0	95.6		96.9	97.3	96.7		87.3	81.1	70.6		91.2	89.7	77.0	
SRT (ms)	141.9	147.3	147.1		127.5	133.5	137.7		150.8	172.5	162.8		138.9	149.7	151.6	
Velocity (◦/s)	313.1	305.5	303.4		292.0	286.1	282.6		270.1	263.0	281.1	*	260.7	262.9	262.7	
Duration (ms)	35.4	35.5	35.8		35.3	35.5	35.9		46.1	47.8	48.6		45.9	46.8	49.2	
Accuracy (gain)	1.02	1.01	1.02		0.97	0.97	0.97		1.36	1.36	1.47		1.35	1.40	1.52	
Monkey C (n=12)																
Correct (%)	88.5	88.4	84.0		91.6	93.5	92.7		68.9	59.1	59.0		76.5	70.5	66.1	
SRT (ms)	254.1	261.4	269.5		209.2	214.3	227.3		268.2	271.5	296.7		284.0	279.7	290.5	
Velocity (◦/s)	269.8	274.6	275.0		302.7	301.9	303.3		225.8	240.0	237.8		265.1	281.2	272.2	
Duration (ms)	47.6	48.6	48.5		46.8	47.0	47.9		50.7	48.0	53.4	**	51.9	52.0	54.6	
Accuracy (gain)	1.02	1.03	1.01		1.02	1.02	1.03		0.98	0.97	1.04		1.11	1.10	1.16	
Monkey D (n=10)																
Correct (%)	96.1	94.1	89.9		80.9	99.2	81.9	***	82.7	43.1	71.3	***	64.5	59.5	56.7	
SRT (ms)	130.3	137.6	141.9		168.0	128.0	156.9	***	164.3	175.9	186.1		178.9	176.6	191.6	
Velocity (◦/s)	250.2	258.2	245.8	**	293.1	279.4	291.2	**	240.4	252.9	235.9		287.3	274.9	300.4	
Duration (ms)	36.0	36.7	35.8		38.4	38.6	38.4		38.8	41.5	39.8		42.0	41.5	42.7	
Accuracy (gain)	0.99	1.02	0.98	**	1.14	1.10	1.13	*	1.10	1.18	1.09	*	1.24	1.24	1.34	

*p<0.05, **p<0.01, ***p<0.001, paired t- test.

Appendix 6

Behavioral Effects of Bilateral dlPFC Deactivation

	Pro-saccades						Anti-saccades					
	Rule visible			Rule memorized			Rule visible			Rule memorized		
	dlPFC+	dlPFC-		dlPFC+	dlPFC-		dlPFC+	dlPFC-		dlPFC+	dlPFC-	
Monkey A												
SRT (ms)	187.8	199.4	***	206.9	205.5		209.7	254.3	***	200.5	241.2	***
Error (%)	3.4	0.5	***	18.6	7.7	***	6.9	22.2	***	20.7	42.5	***
Velocity (◦/s)	252.0	244.5	***	251.4	243.8	***	235.9	205.7	***	241.7	222.8	***
Duration (ms)	35.9	36.4	***	35.6	36.1	***	51.1	65.2	***	50.2	63.2	***
Amplitude (gain)	1.00	0.98	**	0.99	0.99		1.40	1.64	**	1.40	1.59	***
Skipped trials (%)	4.4	8.8	***	4.5	9.2	**	6.4	14.6	***	6.1	16.3	***
Broken fixation (%)	12.7	19.6	***	15.6	16.7		15.4	22.0	**	19.1	17.7	
No response (%)	0.5	1.3		0.6	0.5		1.2	2.3		1.0	0.8	
Monkey B												
SRT (ms)	154.6	174.7	***	184.4	236.5	***	188.9	215.7	***	194.1	232.7	***
Error (%)	0.1	0.1		14.0	15.8		1.0	2.0		8.6	13.1	*
Velocity (◦/s)	325.4	298.9	***	329.7	311.7	***	339.9	303.4	***	338.3	323.5	***
Duration (ms)	35.9	39.5	***	35.2	38.2	***	44.5	59.5	***	42.9	56.2	***
Amplitude (gain)	0.97	0.95	**	0.94	0.94		1.24	1.43	***	1.18	1.42	***
Skipped trials (%)	4.3	2.9	*	3.5	3.4		3.0	3.0		3.0	3.1	
Broken fixation (%)	17.2	23.4	***	22.3	29.0	***	19.8	31.3	***	22.3	35.1	***
No response (%)	0.0	0.1		0.0	0.1		0.2	0.5		0.1	0.4	

*p<0.05, **p<0.01, ***p<0.001, Wilcoxon signed rank test.

Curriculum Vitae

Dr. Michael J. Koval

Postdoctoral Researcher at Duke University

EDUCATION

Doctor of Philosophy: Neuroscience **2012**
University of Western Ontario, London, ON, Canada
Advisor: Dr. Stefan Everling
Dissertation: *An investigation of the neural mechanism by which the prefrontal cortex facilitates anti-saccade task performance.*

Master of Science: Neuroscience **2006**
University of Western Ontario, London, ON, Canada
Advisor: Dr. Stefan Everling
Thesis: *Functional classification of rule-selective neurons in the lateral prefrontal cortex.*

Bachelor of Science: Honors Physiology and Psychology **2004**
University of Western Ontario, London, ON, Canada
Advisor: Dr. Stefan Everling
Thesis: *Effect of saccade direction probability on performance of the anti-saccade task.*
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ACADEMIC AWARDS

- NSERC Canada Graduate Scholarship 2009 – 2012
- Ontario Graduate Scholarship (OGS) (declined) 2009 – 2010
- University of Western Ontario Graduate Research Scholarship 2008 – 2012
- Ontario Graduate Scholarship (OGSST) (declined) 2006 – 2007
- University of Western Ontario Graduate Research Scholarship 2004 – 2006
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RESEARCH EXPERIENCE

Research Assistant for Dr. Stefan Everling, University of Western Ontario, 2006–2008.

PUBLICATIONS

Johnston K, **Koval MJ**, Lomber SG, and Everling S (2013) Macaque dorsolateral prefrontal cortex does not suppress saccade-related activity in the superior colliculus. *Cerebral Cortex* (in press).

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PRESENTATIONS

Koval MJ, Lomber SG, and Everling S (2010). Modulation of neuronal activity in the primate superior colliculus during pro- and anti-saccades by reversible bilateral principal sulcus inactivation. *Society for Neuroscience*, San Diego, USA.

Koval MJ, Lomber SG, and Everling S (2010). Pro-saccades, anti-saccades, and maintenance of task set are affected by principal sulcus cooling. *Primate Neurobiology*, Tübingen, Germany.

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Koval MJ, Lomber SG, and Everling S (2009). Anti-saccade task performance deficits during reversible deactivation of anterior cingulate cortex or lateral prefrontal cortex. *Neural Control of Movement*, Hawaii, USA.

Johnston KD, Levin HE, **Koval MJ**, and Everling S (2006). Neural activity in primate prefrontal cortex and anterior cingulate associated with the performance of pro- and antisaccades. *Society for Neuroscience*, Atlanta, USA.

Koval MJ, Thomas BS, and Everling S (2005). The effects of social attention on saccadic reaction times are task-dependent. *Society for Neuroscience*, Washington, USA.