THE ROLE OF THE FRONTAL EYE FIELDS
IN SELECTING MIXED-STRATEGY SACCADES

By

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“Who we are now is only a starting point in the spectrum of possibilities of who we can be”

-Aljazeera Aba
ABSTRACT

In a multi-agent environment, animals must often adopt a stochastic mixed-strategy approach to maximize reward and minimize costs; otherwise, competitive opponents can exploit predictable choice patterns. This thesis tested the hypothesis that the frontal eye field (FEF) are involved in selecting mixed-strategy saccades. To this end, I recorded preparatory activity of single FEF neurons and manipulated the preparatory activity of neuronal ensembles within the FEF while a monkey played an oculomotor version of the mixed-strategy game ‘matching-pennies’. Each trial began with fixation on a central visual stimulus which was extinguished for a predetermined warning period before two targets were presented; one in the center and the other opposite the neuron’s response field. If both the monkey and the adaptive computer opponent chose the same target, the monkey received a liquid reward; otherwise the monkey received no reward for that trial.

Like humans, monkeys chose each target in equal proportions but showed a ‘win-stay’ bias in their choice patterns. Signal detection theory was used to analyze how accurately FEF preparatory activity predicted upcoming saccade choices. My data demonstrates that the accuracy by which preparatory FEF activity predicted upcoming strategic choices gradually increased as the time of saccade execution approached. This pattern of preparatory activity is consistent with an accumulation of evidence for each potential option towards a decision threshold. Subthreshold micro-stimulation biased mixed-strategy saccadic choices, further suggesting a role
for the FEF in choosing mixed-strategy saccades, albeit unexpectedly, in favor of saccades opposite the stimulation sites.

Lastly, a particular advantage of my experiment is that the same monkey performed this task using neurophysiological experimentation in the FEF and intermediate layers of the superior colliculus (SCi). This allowed me to compare the timing and magnitude of neuronal selectivity and effects of subthreshold microstimulation across these two structures during strategic decision-making. My results indicate that the selection of mixed-strategy saccades occurred earlier and was greater in magnitude in the FEF compared to the SC, indicative of a decision process that occurs earlier in the frontal cortex before being relayed on to premotor regions in the midbrain.
CO-AUTHORSHIP

Dr. Michael C. Dorris was the principal investigator for the studies described in this thesis. Both, Dr. Dorris and I were involved in the planning and implementation of the experimental protocols. I was solely responsible for the data collection, and analysis involved in this thesis. I modified and used statistical codes that were initially written by Dr. Dorris. I produced the first draft of this thesis and subsequent drafts involved the editorial comments of Dr. Dorris.
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# Table of Content

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>CO-AUTHORSHIP</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xii</td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 DECISION THEORY</td>
<td>2</td>
</tr>
<tr>
<td>1.2 REWARD BASED LEARNING</td>
<td>3</td>
</tr>
<tr>
<td>1.3 GAME THEORY AND STRATEGIC DECISION MAKING</td>
<td>4</td>
</tr>
<tr>
<td>1.4 SACCADIC EYE MOVEMENTS AS A MODEL FOR DECISION MAKING</td>
<td>7</td>
</tr>
<tr>
<td>1.5 FRONTAL EYE FIELDS</td>
<td>8</td>
</tr>
<tr>
<td>1.5 CORTICAL REGIONS INVOLVED IN SELECTING STRATEGIC SACCADES</td>
<td>12</td>
</tr>
<tr>
<td>1.7 BIASING SACCADIC CHOICES WITH MICROSTIMULATION</td>
<td>18</td>
</tr>
<tr>
<td>1.7.1 EFFECTS OF SUPRATHRESHOLD MICROSTIMULATION</td>
<td>18</td>
</tr>
<tr>
<td>1.7.2 EFFECTS OF SUBTHRESHOLD MICROSTIMULATION</td>
<td>19</td>
</tr>
<tr>
<td>1.8 SCOPE OF THE STUDY</td>
<td>19</td>
</tr>
<tr>
<td>CHAPTER 2 METHODS</td>
<td>20</td>
</tr>
<tr>
<td>2.1 ANIMAL PREPARATION</td>
<td>20</td>
</tr>
<tr>
<td>2.2 EXPERIMENTAL PROCEDURE</td>
<td>23</td>
</tr>
<tr>
<td>2.3 BEHAVIORAL PARADIGMS</td>
<td>24</td>
</tr>
<tr>
<td>2.3.1 MIXED-STRATEGY TASK</td>
<td>24</td>
</tr>
<tr>
<td>2.3.2 UNPREDICTABLE TASK</td>
<td>25</td>
</tr>
<tr>
<td>2.3.3 PREDICTABLE TASK</td>
<td>26</td>
</tr>
<tr>
<td>2.3.4 MIXED-STRATEGY TASK: ELECTRICAL MICROSTIMULATION</td>
<td>28</td>
</tr>
<tr>
<td>2.4 NEURONAL CLASSIFICATION</td>
<td>28</td>
</tr>
</tbody>
</table>
Chapter 3 RESULTS........................................................................................................32

3.1 BEHAVIORAL RESULTS.......................................................................................32

3.2 NEURAL ACTIVITY..............................................................................................34

3.2.1 NEURONAL SELECTIVITY..................................................................................34

3.2.2 EVOLUTION OF NEURONAL PREDICTION OVER TIME.................................36

3.2.3 GRADUAL SELECTION BETWEEN FEF PREFERRED AND NON-PREFERRED
    ACTIVITIES...............................................................................................................39

3.3 BIASING OF MIXED-STRATEGY SACCADES WITH FEF STIMULATION............44

CHAPTER 4 DISCUSSION...................................................................................................47

4.1 BEHAVIORAL STRATEGIES DURING A MIXED-STRATEGY TASK.....................47

4.2 FEF ACTIVITY REPRESENTS EVOLVING SACCADIC PLANS..............................49

4.2.1 SELECTIVITY OF FEF PREPARATORY ACTIVITY...............................................49

4.2.2 STOCHASTIC MECHANISM FOR STRATEGIC SACCADIC SELECTION.........51

4.3 ARTIFICIAL MANIPULATION OF STRATEGIC SACCADES.................................54

4.4 FEF V.S. SC CIRCUIT: INFLUENCE ON MIXED-STRATEGY SACCADES............58

CONCLUSION..................................................................................................................62

REFERENCES.................................................................................................................63
List of Figures

Figure 1. Schematic of major brain areas involved in controlling saccadic eye movement

Figure 2. Schematic of the mixed-strategy “matching-pennies” task

Figure 3. Behavioral summary

Figure 4. FEF activity during the three behavioral tasks

Figure 5. Evolution of neuronal prediction over time

Figure 6. FEF activity when fixed warning period was extended

Figure 7. Two hypothetical patterns in which FEF preparatory activity increases

Figure 8. Gradual accumulation in preparatory activity for both preferred and non-preferred mixed-strategy saccades

Figure 9. Manipulating mixed-strategy saccades with microstimulation

Figure 10. Comparison of mixed-strategy saccade selection across the FEF and the SC
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Terms</th>
</tr>
</thead>
<tbody>
<tr>
<td>dACC</td>
<td>Dorsal Anterior Cingulate Cortex</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsal Lateral Prefrontal Cortex</td>
</tr>
<tr>
<td>FEF</td>
<td>Frontal Eye Fields</td>
</tr>
<tr>
<td>FP</td>
<td>Fixation Point</td>
</tr>
<tr>
<td>LIP</td>
<td>Lateral Intraparietal Cortex</td>
</tr>
<tr>
<td>RF</td>
<td>Receptive Field</td>
</tr>
<tr>
<td>RPE</td>
<td>Reward Prediction Error</td>
</tr>
<tr>
<td>SCi</td>
<td>Intermediate layer of the Superior Colliculus</td>
</tr>
<tr>
<td>SEF</td>
<td>Supplementary Eye Field</td>
</tr>
<tr>
<td>SRT</td>
<td>Saccadic Reaction Time</td>
</tr>
<tr>
<td>WARP</td>
<td>Weak Axiom of Revealed Preferences</td>
</tr>
</tbody>
</table>
Chapter 1

Introduction

Most soccer fans would agree that the penalty shoot-out between Brazil and Italy in the 1994 World Cup was one of the most decisive moments in history of the sport. The penalty shoot-out determined the winner of the World Cup, and settled a heated dispute between two long-time rivals. The face-off between the Italian striker Roberto Baggio and the Brazilian keeper Traffel highlighted the climax of the shoot-out. Baggio had to score to keep Italy's aspiration of winning alive, while Traffel could guarantee Brazil's triumph with a single save. Provided the ball is placed 12 yards from the net, and will be travelling at a high velocity, Traffel does not have time to react after the shot. Thus, Traffel must attempt to predict Baggio's intended target and proactively dive at the precise moment before Baggio's shot. Of equal importance, Traffel cannot dive before the kick, lest he give away vital information and effectively an open net. Fortunately for Brazil, Traffel predicted correctly by diving to the left, and making the save. In that moment the Brazilian team ascended to legendary status while the Italian team was left in sorrow to contemplate what could have been.

On face value this might seem like a moment good fortune mixed with great athletic ability. What is not evident is the preparatory work done by Traffel and the Brazilian coaching staff prior to the shoot-out. Professional goalkeepers meticulously study their opponents' penalty shooting habits. The history of target locations and success rate are all quantified to give the goalkeeper necessary
information to predict the likely future target location. The aim of this thesis is to study the neural mechanism associated with decision-making in such strategic competitive environments.

1.1 Decision Theory

To effectively survive in a complex environment, organisms must be capable of selecting the most appropriate course of action based on a variety of sensory and non-sensory variables. Decision-making encompasses the central step of transforming incoming information into specific motor commands.

Economic research into decision-making placed the concept of utility maximization at the heart of the field. A utility function can be experimentally obtained by varying the quantity and the types of rewards given while examining the revealed preferences. However, the benchmark of utility maximization is a bit ambiguous given ‘utility’ is not directly observable. To help put restrictions on preferences, Samuelson came up with the Weak Axiom of Revealed Preference (WARP)(Samuelson, 1938). WARP simply states that if an individual is seen selecting some object A over another object B, then that individual will not pick object B over A if put in a the same situation. If and only if the previously mentioned condition holds, then that individual is making choices to maximize some fixed utility function (Sen, 1971; Kihlstrom et al., 1976). WARP ties the idea of utility maximization closely to observable behavior, such as choices. Moreover, utility maximization represents an ordinal relationship, in that it allows for the ranking of
choices with regard to preference. This concept does not provide any cardinal information, given it does not provide the magnitude of preference between the choices (Andrew and Dean, 2009).

**1.2 Reward Based Decision-Making**

Whereas economist placed the concept of utility at the heart of decision-making, psychologist and biologist focused more on rewards. Humans and animals routinely make decision to maximize positive outcomes such as reward while minimizing punishment or losses (Lee, 2006). Thorndike’s Law Effect illustrated this adaptive strategy at the turn of the century; behaviors that lead to positive outcomes are more likely to be repeated, while the opposite is true of behaviors followed by aversive outcomes (Thorndike, 1911).

Dopamine’s role in reward-based learning has been substantiated through various experiments (Kim et al., 2009b; Shultz, 1998; Tober et al., 2005). Phasic changes in dopaminergic firing code the difference between the expected reward and the actual reward elicited from a particular outcome. The difference between the reward predicted and the reward received is referred to as the reward prediction error (RPE) (Staudinger et al., 2009; Kim et al., 2009a). RPE states that whether dopamine responds to a particular reward is dependent on whether or not the reward is expected (Shultz, 1993). Additionally, direct measurements of extracellualr dopamine in the ventral striatum (a main target for dopaminergic projections from the ventral tegmental area) have substantiated that phasic changes
of dopamine levels in target structures indeed conform quantitatively to a prediction error signal (Day et al., 2007). Furthermore, the RPE signal projecting from the ventral tegmental area to the ventral striatal and frontal regions putatively are used to accurately shape predictions and implement selections of correct reward-optimizing actions (Barto, 1994; Matsuzaki et al., 2001; Sugrue et al., 2005). The importance of this finding is highlighted in models of reinforcement learning where the RPE signal is used to update the value associated with different actions (Schultz, 2007). The dopaminergic theory of rewards provides us with a basis of examining reward related mechanisms at a neurophysiological level.

1.3 Game Theory and Strategic Decision-Making

Traditionally, decision-making experiments have examined choices with defined probabilities and outcomes, such as monetary gambles and lottery selection (for review see (Glimcher et al., 2009). However, we live in a complex social environment where optimizing decisions is complicated by the behavior of our opponents. In particular, the decisions and consequences of one's actions are not solely dependent on the individual, but in part on other agents in the environment. Likewise, the relationship between our actions and their subsequent outcomes are susceptible to changes in competitive situations with other agents (Lee et al., 2004; Seo, 2008). An example of the uncertainty that arises form social environments is illustrated in situations when we are deciding whether to ask someone out on a date. Also, another example of this uncertainty can be seen when entering a business
negotiation (Sanfey, 2007). The standard expected utility theory that underlies many earlier models of decision-making is not ideal for complex social environments. This theory is complicated by the fact that players have to infer the values and probabilities of their opponents or partners in their effort to achieve a desired outcome.

Game theory provides us with an appropriate mathematical framework to study decision-making in social situations (von Neumann and Morgenstern, 1947). In game theory, a game is defined by a definitive number of available choices and a finite number of possible choices for each player. A particular outcome is assigned to each player given the choices undertaken by all the players in the game (Lee and Wang, 2009). A strategy is defined as the complete plan of action for whatever situation might arise. A player’s strategy will determine the action the player will take at any stage of the game. Adopting an optimal strategy will result in the maximum payoff possible. Furthermore, a game is referred to as mixed-strategy when each possible action has the same probability of being selected, whereas a pure-strategy game consist of selecting a specific action consistently (Lee and Wang, 2009).

Assuming all players are rational and seek to maximize their payoffs in response to the actions selected by all players, a set of such players would adopt a strategy that is the best response given the strategies of their opponents. Game theory refers to this strategic optimization of decisions as the Nash Equilibrium. In such a situation the set of strategies by all players ensures that no individual players can
increase their respective payoffs by altering their strategy unilaterally (Lee, 2008). For example, with regards to strategy, our previous world cup soccer shoot-out is analogous to two players competing in the mixed-strategy game of “odds evens”. In this game one player is assigned the role of 'evens' and the other the role of 'odds'. Both players pump their fist, for a predetermined period of time, and simultaneously present either one or two fingers. If sum of the digits on both hands is even, then the "evens" player wins, if the sum of the digits is odd, then the "odds" player wins. The optimal strategy would involve that each player displays either one or two digits at equal proportions throughout the game and stochastically from round to round. Any other strategy would be suboptimal, in that it allows opponents to exploit the statistical biases that would exist.

Monkey experiments utilizing competitive mixed-strategy games have added further insight into the strategic decision-making process (Barraclough et al., 2004; Lee et al., 2004; Lee et al., 2005, 2008; Thevarajah et al., 2009). Monkeys present a good model for mixed-strategy behavior in humans, as they are capable of performing mixed-strategy tasks with comparable behavioral results to humans. Similar to humans, monkeys followed strategies that were relatively close to those predicted by game theory (Dorris and Glimcher 2004, Lee et al. 2004, 2005). Targets were chosen in equal probabilities, and their current trial choices were only weakly influenced by the choices in the previous trials. Also, similar to humans, monkeys’ behavior exhibited a small bias towards previously rewarded choice. The trial-to-trial bias in these results is consistent with reinforce learning models (Lee and Wang, 2009; Thevarajah et al., 2010). That is, recent actions that are previously rewarded
are more likely to be repeated relative to actions that do not yield a reward.

1.4 Saccadic Eye Movements as a Model for Decision-making

The goal of this thesis is to examine the neural mechanism underlying strategic decision-making, through saccadic eye movements. Saccades represent the behavioral manifestation of one our most common decision, choosing where and when to look. Primates perform tens of thousands of saccades per day, rapid eye movements that align targets of interest onto the fovea. The fovea is located in the eye at the center of the macula region of the retina and has the greatest visual acuity. The fovea has the highest density of retinal cone cells. Additionally, a disproportional region of the visual cortex is dedicated to processing visual information from the fovea relative to the rest of the retina (Leigh and Zee, 1999).

The saccadic system has a number of technical advantages that make it an ideal model for investigating the neural basis of decision-making. First, saccadic eye movements are ballistic and consistent in nature. Second, only three antagonistic pairs of muscles are used to rotate a spherical structure, with a consistent load and minimal inertia effects (Wurtz and Goldberg, 1989). Third, technological advancement in eye tracking technology allow for the precise and non-invasive measurement of eye position. Fourth, unlike other motor systems the neuronal circuitry for the visuosaccadic system is located entirely in the cranium, thus providing a stable platform for neural recording. Hence, the visuosaccadic system is one of the most understood sensory-to-motor transformations at a
neurophysiological level and provides an excellent framework for comprehending
the neural basis of voluntary decision-making (Wilimzig et al., 2006).

1.5 Frontal Eye Fields

The frontal eye fields (FEF) are an integral structure in the network responsible
for eliciting strategic saccades. A number of factors suggest that the FEF has a role in
the selection of strategic saccades. The unique cytoarchitecture of the neural
populations in this region appear ideal to facilitate strategic saccade selection.
Further, the FEF is robustly connected with subcortical structures that are directly
involved in generating saccades. Additionally, the FEF has bilateral projections to
frontal and parietal cortical structures that have been implicated in strategic
decision-making. These attributes may make the FEF ideally situated to be a bridge
between these higher cortical and subcortical regions.

In humans the FEF is located in the rostral bank of a portion of the precentral
sulcus at the caudal end of the middle frontal gyrus (Schall, 2002; Amiez and
Petrides, 2009). In macaque monkeys the FEF is located at the rostral bank of the
arcuate sulcus (Bruce and Goldberg, 1984; Amiez and Petrides, 2009). The FEF can
influence the generation of saccades via four main pathways. First, by projecting
directly to the intermediate layers of the ipsilateral superior colliculus (SCi). Second,
by using a pathway through the basal ganglia via the ipsilateral striatum. Third, by
projecting to the cerebellum via the pontine nuclei. Fourth, through a projection to
the mesencephalic and pontine nuclei that makes up the saccade generator circuit (Bruce et al., 1985).

The cytoarchitecture of the FEF shows a large concentration of pyramidal cells in layer 5 and a thin granular layer 4 compared to the more rostral cortex. Furthermore, neurons in the FEF that modulate movement and fixation related activity are located in layer 5 and innervate the SC and parts of the neural circuits that generate saccades (Schall, 2002). Electrophysiological recordings have indicated that these neurons, in concert with other neurons in the visuomotor circuit, influence the vector and timing of saccades (Thompson et al., 1996; Girard and Berthoz, 2005; Lee and Keller, 2008).

Fixation and movement neurons are the two primary types of neurons that control saccade generation in the FEF. Fixations neurons display an elevated discharge rate when gaze is fixed on an object. Alternatively, an increase in the discharge rate of movement neurons increases the probability a saccade will be made. Movement neurons are active before saccades of specific direction and amplitude, referred to as the receptive field (RF). The RF for neurons in the ventrolateral region of the FEF is associated with shorter saccades. This region is interconnected with the prefoveal representation in retinotopically-organized areas, with central vision in inferotemporal cortex and with other regions that are not retinotopically organized. Conversely, the mediodorsal region of the FEF produces larger amplitude saccades. This region is interconnected with the peripheral visual field representation of retinotopically organized areas, with areas highlighting
peripheral vision or have no retinotopic order and are multimodal (Bruce and Goldberg, 1984; Schall et al., 2008).

This thesis will focus on visuomovement neurons, a subset of FEF movement neurons with visual properties. These neurons are ideal candidates for the study of preparatory process preceding overt action. Visuomovement neurons display an anticipatory discharge of activity, well in advance of future visual and memory guided saccades to their RFs. (Bruce and Goldberg, 1984; DeSouza et al., 2003; Schall et al., 2008). This preparatory activity is correlated with saccade properties such as saccade reaction time (Everling and Munoz, 2000), and strategically significant information such as expectation of reward, choice history and target presentation probability (Segraves and Goldberg, 1987; Ding and Hikosaka, 2006).

Although the FEF was initially regarded as purely a motor region, recent studies have also linked the FEF to the visual system. The FEF is interconnected to various cortical and subcortical visual areas (Barone et al., 2000; Jouve et al., 1998; Stanton et al., 1998). Additionally, most FEF neurons have a transient or sustained response to visual stimuli (Bruce and Goldberg, 1985; Schall, 1991) with relatively fast latencies up to 50-70 ms after target presentation (Liversedge, S.P., 2011; Scholesky et al., 1998).

The FEF has many important features for perceptual decision-making, decision-making based on the accumulation of sensory evidence, which can be relevant to strategic decision-making. Schall et al (1996) conducted one of the earliest neurophysiological studies linking the FEF with perceptual decision-making. Using a
perceptual discrimination task they showed that a subsequent selection process evolved as evidence accumulated, leading to the active suppression of the activity associated with the distractors and a ramping up of activity for the oddball target. The saccade towards the oddball target was executed after the level of activity reached a fixed threshold (Shcall et al., 1996). This led Schall to believe that there is a decision threshold within the FEF that allowed for only one cluster of neurons to reach the decision threshold at one time.

Additionally, experiments have linked the neuronal activity in the FEF with an internal error signal (Ferrera and Barborica, 2010). This signal may allow an organism to predict the effects of its own movements relative to the internal goals and to plan corrections in absence of sensory feedback. Evidence for internally generated error signals in the FEF implicates this region in the network that combines internal estimates of the state of the environment and of the effector systems that interact with that environment (Ferrera and Barborica, 2010; Ding and Gold, 2011).

Furthermore, FEF activity can be modulated by expectation of reward, another factor that is directly associated with strategic decision-making. FEF neuronal activity generally exhibits spatially selective reward modulation, similar to that observed in the caudate. However unlike the FEF, neurons in the caudate also display non-spatially selective reward modulation (Ding and Hikosaka, 2006).

The unique properties of the FEF make it an ideal candidate in the selection of strategic saccades. The modulation of FEF activity that reflects changes in reward,
an internal error signal and integration of evidence could stem from its bilateral projection to cortical regions such as the dorsal lateral prefrontal cortex (DLPFC), dorsal anterior cingulate cortex (dACC), supplementary eye field (SEF) and lateral intraparietal area (LIP) (Pierrot-Deseilligny, 2003; Schall et al., 2008; Amiez and Petrides, 2009; Ding and Gold, 2011). Additionally, its topographic mapping of the visual space combined with its projections to subcortical oculomotor structures also suggests that it may make it integral for the selection of strategic saccades.

1.6 Cortical Regions Involved in Selecting Strategic Saccades

Regions in the primate’s prefrontal and parietal cortex are essential for higher order cognitive function. An important characteristic of these regions is adaptive neural coding. Neurons in these cortical areas adapt their activity to carry specific information that is relevant to current concerns, producing a dense, distributed representation of related inputs, actions and rewards (for reviews see Duncan, 2001; Miller and Cohen, 2001). Imaging studies suggest that many different cognitive tasks produce the similar broad patterns of activity in these regions. Electrophysiological studies delineate this, in part, by showing extensive adaptability of function even at the level of the single neuron (Miller, 2000). This form of adaptive coding is likely to be an important component in strategic decision-making. Given its reciprocal projections to prefrontal and parietal regions, FEF activity could be modulated, in part, by the information represented in adaptive coding.
Figure 1. FEF centric schematic of brain structures and some anatomical pathways that are involved in saccade generation. Solid lines represent excitatory connections. Dashed lines represent inhibitory connections. Arrows: ACC- Anterior Cingulate Cortex, CN- Caudate Nucleus, DLPFC- Dorsal Lateral Prefrontal Cortex, FEF- Frontal Eye Field, LGN- Lateral Geniculate Nucleus, LIP- Lateral Intraparietal Area, Superior Colliculus, SEF- Supplementary Eye Field, SNr- Substantia Niagra pars Reticulata.
The primate’s DLPFC is an important source of input to the FEF. The DLPFC plays an integral role in the integration of sensory and mnemonic information and the regulation of intellectual function and actions. Of particular importance to this thesis is the modulation of DLPFC neuronal firing relative to the nature of the expected reward from an environment (Leon and Shadlen, 1999; Shultz, 1998). Further, it has been suggested that DLPFC might play an integral role in decision-making by encoding a specific state of the environment and the preference of the outcomes associated with such a state (Lee, 2009). Given its strong reciprocal connection with the FEF, such reward- and state-related information from the DLPFC could provide a source of modulation for FEF activity.

Another potentially important source of FEF input for strategic decision-making is the primate’s dACC. The dACC has been experimentally linked with the detection and prediction of error. Evidence for this stems from the observation of a RPE signal generated in the dACC upon the occurrence of errors (Gehring et al., 1993, Holroyd et al., 2004). Neurons in the dACC modulate their activity according to the contrast between the expected and actual reward (Bush et al., 2002, 2001). Varying levels of dopamine influence the optimization of this RPE system by providing expectations about the outcomes of future events (Lee and Wang, 2009; Kim et al., 2009b). Hence, dACC signals could represent a plausible source for the internal error signal observed in FEF activity (Ferrera and Barborica, 2010).

The SEF could represent another integral source of FEF input with regards to strategic decision-making (Parthasarathy et al., 1992). The SEF has afferent
projections from the amygdala and orbital frontal cortex, regions implicated in encoding the subject value associated with a reward for a particular action (Ghashghaei et al., 2007). Additionally, neural modulations in SEF activity have been linked with an oculomotor “action-value” signals, i.e., coding for the value that can be obtained by performing a particular saccade (So and Stuphorn, 2010). Given that neurons in the SEF are active much earlier than FEF neurons before value based saccades (Coe et al., 2002), this action-value signal could be an important initial source for modulation of FEF activity.

The LIP could prove to be another important source of FEF activity with regard to strategic decision-making (Stanton et al., 1995; Wurtz et al., 2001). Activity in the LIP displays a combination of sensory, motor and memory signals (Colby et al., 1996; Mazzoni et al., 1996; Li et al., 1999). Modulation of LIP activity has been linked with the probability an eye movement will yield a reward (Gold and Shadlen, 2001). Likewise, many neurons in the LIP encode the value function, an estimate of expected cumulative reward, for eye movements (Platt and Glimcher, 1999; Dorris and Glimcher, 2004); typically in conjunction with the animal’s chosen eye movement. Considering the FEF and LIP are interconnected via bilateral projections (Andersen et al., 1990; Stanton et al., 1995), the LIP could provide the FEF with an evaluation signal that aids in the selection of mixed-strategy saccades.

The basal ganglia could play an important role in the FEF’s involvement in strategic decision-making. Recent work suggests that sensorimotor and cognitive abilities of neurons in this region are strongly modulated by expected reward (Ding
and Hikosaka, 2006). Oculomotor studies show that the basal ganglia have an important role in guiding saccades to locations where reward is available. Further, neurons in the caudate nucleus and the substantia niagra reticulata are sensitive to spatial differences in expected reward (Hikosaka, 2005). This sensitivity could lead to a bias in excitability between the superior colliculi such that the saccades to rewarded position are favored. Reward related saccades could be favored via the selective disinhibition of the superior colliculi by the substantia niagra reticulata (Girard and Berthoz, 2005). It is believed that reward modulation occurs in the caudate where cortical inputs carrying spatial signals and dopaminergic inputs carrying reward signals are integrated. The spatially selective signals to saccade related neurons in the caudate have been shown to primarily originate from the FEF (Bruce et al., 1985).

To see how the dynamic nature of multi-agent decision-making is reflected in prefrontal and parietal regions, Lee and Colleagues trained monkeys on an oculomotor version of the game “matching-pennies” (Lee, 2007; Seo et al., 2009). Results indicate that neurons in DLPFC, dACC, SEF and LIP modulate their activity relative to a number of factors that are essential for optimal decision-making.

First, the firing patterns of a subset of neurons, in all four regions, were modulated by choices in the previous trial. This indicates that these neurons stored the monkey’s previous behavior, via persistent activity, resembling what’s known as an eligibility trace in reinforcement learning. Eligibility traces allow for the identification of previous actions that are relevant for a subsequent behavior after
some delay. Eligibility traces might be useful to form appropriate association between particular actions and reward (Lee and Wang, 2009; Kim et al., 2009b).

Additionally, these signals can influence the way relevant sensory stimuli is encoded and transformed to appropriate behavioral responses (Curtis and Lee, 2010).

Second, some neurons in the DLPFC, and dACC modulate their firing in accordance with the choices of the computer opponent. This pattern of firing can contribute to knowing the probability of the computer opponent’s future choices. This is relevant because in “matching-pennies” the monkey is rewarded only when they select the same target as the computer opponent. Therefore, the average reward a monkey receives is directly correlated with the target selection of the computer opponent (Lee, 2009).

Lastly, in all four regions, neural activity during the feedback period was regularly modulated by whether the monkey was rewarded on the previous trial or not. The activity of some neurons reflected whether the monkey was rewarded on the last two to three previous trials, indicating that reward information can be temporally integrated in the prefrontal and lateral parietal cortex. This reward related modulation of activity could serve as a monitoring system for the rate of reward received by the animal, and can trigger adaptive responses necessary for optimizing the animal’s decision-making strategy (Lee, 2008). For example, dACC neurons that fluctuate their activity accordingly with the reward associated with the current and previous trials could be encoding the average reward rate. In some instances neurons in the dACC respond antagonistically, i.e. decrease in discharge
rate, to the reward in the current. The antagonistic activation of these neurons could represent coding the degree to which the reward in the current trial deviates from the reward rate established in the previous trials (Matsumoto et al., 2007; Seo and Lee, 2008)

In conclusion, during the ‘matching-pennies’ task activity in the lateral (DLPFC), medial (dACC) and dorsal-medial (SEF) frontal combined with the lateral parietal (LIP) cortex is regulated by three different signals that are associated with the animal’s decisions, their outcomes and the computers choice. Due to robust projections with the FEF, this evidence suggest that FEF activity could reflect higher order statistics of strategic saccade selection via afferent projections from the DLPFC, dACC, SEF and LIP.

1.7 Biasing Saccadic Choices with Microstimulation

1.7.1 Effects of Suprathreshold Microstimulation

The possible involvement of the FEF in saccade selection was first brought to light in the late 1800’s by David Ferrier who noticed that microstimulation of the frontal cortex of anesthetized monkeys elicited movements of the eyes to the visual field contralateral to the stimulated hemisphere (Segraves and Goldberg, 1987; Stanton et al., 1995). Later, suprathreshold, microstimulation that automatically elicits saccades, stimulation experiments helped characterized the neuronal population within this region (Bruce and Goldberg, 1984; Bruce et al., 1985; Schall
et al., 1995). Additionally, suprathreshold experiment added more insight about the
saccade selection mechanism within the FEF (Sparks, 1983; Gold and Shadlen, 2000).

1.7.2 Effects of Subthreshold Microstimulation

Recent work has examined the effects of subthreshold, microstimulation that
does not automatically elicits saccades, stimulation of the FEF in perceptual
decision-making. Subthreshold stimulation refers to microstimulation that increases
the firing rate of neurons at the stimulated loci without reaching levels sufficient to
trigger a saccade. Subthreshold stimulation of the FEF biases attentional
mechanisms in perceptual tasks (Moore and Fallah, 2001; 2004) (Schafer and Moore,
2007). Likewise, subthreshold stimulation of the FEF results in modulations of V4
neuronal activity similar to known effects of voluntary attention to the RF that
corresponds with the stimulated FEF loci (Moore et al., 2003; Armstrong and
Fitzgerald, 2006). Given the effects of subthreshold microstimulation in the
previous experiments, we expect subthreshold stimulation to bias strategic saccadic
choices, in our oculomotor mixed-strategy task.

1.8 Scope of the Study

My study will examine the activity of FEF neurons that precedes the selection of
strategic saccades. I will utilize a set of electrophysiological experiments on the FEF
while a monkey competes against an adaptive computer opponent in the oculomotor mixed-strategy game of ‘matching-pennies’.

The first hypothesis is that the premotor activity in the FEF is predictive of upcoming mixed-strategy saccades. This will be substantiated by a gradual increase in preparatory activity during the warning period that will be significantly greater if the monkey selects the target in the response field.

The second hypothesis is the FEF is causally involved in the selection of mixed strategy saccades. This will be affirmed by microstimulation biasing the proportion of mixed-strategy saccadic choices in the ‘matching-pennies’ task.

Lastly, I will compare previous experiments from our laboratory, conducted on the superior colliculus (SCi), with my current results. Throughout the two sets of experiments we have utilized the same tasks and same monkey, which resulted in similar behavioral strategies across the two sets of experiments. The continuity between the two sets of experiments allows for the delineation of the processing differences in these two areas that cannot be explained by discrepancies in strategies between monkeys.
Chapter 2

Materials and Methods

General Methodology

In this study, I detected extracellular activity of single cells in the FEF and microstimulated populations of FEF neurons from a male rhesus monkey (*maccaca mulatta*). The monkey weighed an average of 10 kg throughout the study. All procedures were approved by the Queen's University Animal Care Committee and complied with the guidelines of the Canadian Council on Animal Care. Animals were under close supervision of the university veterinarian.

2.1 Animal Preparation

The monkey underwent two surgeries in order to record single neurons from the FEF. The first surgery attached a head fixation post, and the second the placed a microelectrode chamber. The monkey was anaesthetized by intramuscular injections of Ketamine HCl (10mg/kg), followed by an I.V. administration of a bolus of Propofol (10mg/mL). Subsequently, the trachea was intubated with an endotracheal tube to allow for air and 1-2% isofluorane gas, an anesthetic, to the lungs during surgery. Moreover, the hair around the scalp was shaved and the vitals (heart rate, respiratory rate and body temperature) were closely monitored.
The two surgeries performed on the monkey utilized similar procedures. To place the head fixation post, the monkey was secured in a stereotaxic frame and a longitudinal incision was made along the midline of the scalp to expose the cranium. The cranium was cleaned and titanium screws were inserted into the bone to act as anchors for the dental acrylic. Thereafter, the head fixation post was placed atop the skull and implanted in the dental acrylic.

For the second surgery, microelectrode chamber placement, the monkey was secured in a stereotaxic frame and the area atop the coordinates of the FEF was cleared of dental cement using an electrical drill. A trephine was used to make a circular opening in the skull that encompassed the stereotaxic FEF coordinates (A23 and L20) (Mayo & Sommer, 2008). Following, a plastic chamber was placed in the circular cavity and anchored down to the skull by dental acrylic. The microelectrode chamber, which covered the exposed dura, was cleaned every day with sterile saline, peroxide and betadine to prevent infection.

After each surgery, injections of the anti-inflammatory drug Anafen (100 mg/ml), the antibiotic Baytril (50 mg/mL), and the analgesic Bupenorphine (0.3mg/mL) were administered. The monkey was closely monitored until he was fully conscious and regained mobility. Experiments commenced two weeks post microelectrode chamber placement surgery.

The monkey was placed on water control by the removal of water from his cage throughout the time he participated in an experiment. The monkey acquired water during the experimental sessions as a reward. During the experiments, the monkey
was allowed to work to satiation. The weight and health of the monkey was monitored carefully to ensure he was adequately hydrated, and supplementary water and fruits were given as required.

### 2.2 Experimental Procedure

The monkey subject was seated in a primate chair with his head firmly attached to the chair by the implanted head-post, while participating in the experimental session. The monkey faced a tangent screen 57 cm away, which spanned ± 42° of the central visual field. Throughout the experiment the right eye position was recorded at 250 Hz, with a resolution of 0.1° RMS, using an infrared eye tracker system (EYELINK II. Research). A sipper tube was placed in the monkey's mouth to administer the liquid reward. Stimuli presentation, data collection and delivery of reward were managed by real-time data acquisition software (Gramalkn Ryklin Software).

The activity of single neurons was recorded by tungsten microelectrodes (Frederick Haer, 1-2 MΩ at 1kHz) that were lowered through 23 gauge stainless steel guide tubes by a hydraulic microdrive (Narishige) attached to the recording chamber. Guide tubes with an average length of 28 mm were placed just within or through the dura. The guide tubes were positioned inside the recording chamber using a 2 mm grid spacer (Rist et al., 1988). Single-cell discharges were sampled at 4kHz and passed through 1kHz digital window discriminator that excludes trials that did not meet amplitude and time constraints (Plexon, Inc.).
2.3 Behavioral Paradigms

Each experimental session began with target directed saccades as I searched for neurons in the FEF. Monkeys were required to fixate at a central fixation point (FP) then make a saccade towards a target. The target was presented at various locations in the visual field for 50-100 trials. The center of the neurons response field (RF) was defined by the target location that evoked the highest activity associated with target directed saccades, relative to the central FP. In the three behavioral tasks that followed, one target was placed in center of the neurons RF while another target was presented at a mirror-image location in the opposite hemifield.

2.3.1 Mixed-Strategy Task

The monkey competed in the oculomotor version of the ‘matching-pennies’ game against an adaptive computer opponent (Fig.2). Each trial began with the presentation of a single FP. The monkey was required to foveate to this point and maintain central gaze fixation throughout the 800ms presentation of FP. Next, the fixation point was extinguished leaving a blank screen, signaling the beginning of a 600ms warning period that preceded target presentation. The monkey was required to maintain central gaze fixation throughout the warning period. Afterwards, two targets were simultaneously presented with the monkey free to saccade to either. One target was presented in the preferred direction of the neuron, while the other was presented in the non-preferred direction of the neuron. The fixed warning
period and the certainty of the target location facilitated advanced selection and preparation for saccades (Dorris and Munoz, 1998). After the monkey fixated on the target, a red box appeared around one of the targets indicating the computer’s choice. The computer’s choice influenced whether the monkey received a reward on the current trial (see below). If the saccade was initiated too early (i.e. less than 70 ms after target presentation), or too late (i.e. more than 350 ms after target presentation), the trial was aborted and the reward was withheld on that trial. The inter-trial interval was fixed at 1000 ms.

The monkey received ~ 0.3 mL liquid reward on trials in which both players chose the same target and nothing otherwise. An algorithm designed to exploit statistical patterns in the monkey’s choices dictated the computer’s choices throughout the task (Barraclough et al., 2004). The algorithm takes into account the monkey’s history of choices and rewards to compute conditional probabilities of the monkey’s response and, consequently, identify the option that would be advantageous for the computer opponent (Lee et al., 2004). Thus, the monkey would maximize its reward within a block by choosing both targets in equal proportions and stochastically from trial to trial.

### 2.3.2 Unpredictable Task

The unpredictable task was identical to the mixed-strategy task, with two key differences. First, only a single saccade target was presented on each trial. The target was equally likely to be presented in the preferred or non-preferred direction on each trial. Second, reward was equally likely to be given or withheld for a successful
completion of each trial. Thus, the overall pattern of choices and reward schedule was comparable for both the unpredictable and the mixed-strategy task but saccadic choice was under sensory instructions in the former and voluntary control in the latter.

### 2.3.3 Predictable Task

The predictable task was also identical to the mixed-strategy task except that a single target was presented on each trial, always at the same location. During one block, the target would consistently be presented in the neuron’s preferred direction. In the next block the target would consistently be presented in the neuron’s non-preferred direction. To produce a reward rate that was comparable to the other two tasks, reward was given on each trial, but the magnitude of the reward was halved. Thus, both saccade direction and reward were maximally certain on each trial. An alternative way of providing a comparable reward schedule relative to the other tasks would have consisted in giving the same magnitude of reward and halving the number of trials to be rewarded. Thus, the reader should keep in mind that the behaviour and FEF neuronal activity could have responded differently to the two reward schedules.
Figure 2. Mixed Strategy Task. Each panel represents a successive screen presented to the subject. The two red arrows represent the possible choices the monkey subject can choose from. One of the targets was always placed in the center of the neuron’s response field (preferred direction) as indicated by the dashed circle, and the other in the mirror location (non-preferred direction). The red box indicates the choice of the computer opponent.
2.3.4 Mixed-Strategy Task: Electrical Micro-stimulation

I applied sub-threshold stimulation to the FEF in order to determine whether pre-saccadic activity in this region was casually involved in the selection of upcoming mixed-strategy saccades. The FEF movement vectors were found using 0.3 ms biphasic pulses that were applied at a fixed frequency of 300Hz and current levels <50 μA (Bruce and Goldberg 1985; Bruce et al. 1985). Once the suprathreshold saccade vector was determined for a given stimulation site, stimulation threshold was defined as the minimum current required to consistently produce the characteristic saccadic vector for a given FEF loci. In all cases, the stimulation vectors were in close agreement with the preferred vector of neurons recorded just before stimulation.

Subthreshold stimulation was applied for the first 500 ms of the 600 ms warning period, in the mixed-strategy task. The stimulation was applied to a FEF site that coincided with one of the mixed-strategy saccade targets. Each block consisted of approximately 200 trials, with stimulation applied randomly to 50% of trials. Current intensity and frequency were altered so that the threshold required for eliciting saccades was approached but not crossed. A standard frequency of 100 Hz and an average current of 28 ± 4.3 μA were used during the mixed-strategy stimulation task blocks.
2.4 Neuronal Classification

I recorded from saccade related FEF neurons located between 5 to 11 mm below the surface in which the first neural activity was detected below the dura. All neurons used in this thesis displayed saccade related activity (10-20ms before saccade onset) above 25 spikes/s, (paired t test, p<0.01), for saccades in the center of the neuron’s RF. Moreover, all neurons had early pre-target activity, which I referred to as preparatory activity, prior to target onset at the end of the gap period. This preparatory activity was significantly greater at the end of the warning period (50ms after target presentation) than during the initial visual fixation (100 ms preceding FP disappearance; paired t-test, p<0.01) and above 15 spikes/sec (paired t-test, p<0.01).

2.5 Data Analysis

Trials were aborted online if the monkey’s eye position was not maintained within ± 3° of the appropriate spatial location, or if saccades were not initiated in a 70 – 300 ms temporal window after target presentation. Computer software determined the beginning and end of each saccade using velocity, acceleration, threshold and template matching criteria (Waitzman et al., 1991). The experimenter examined trials offline to verify accuracy.

Statistical analyses were performed offline on a personal computer with an intel Core 2GHz processor running Matlab version 7.11 (Mathworks Inc). The first 20 trials of each block were discarded from analysis off-line to allow the monkey time to adjust to the new task conditions. Majority of the analysis consisted of paired t-
test of the null hypothesis that two matched samples, in the vectors X and Y, come from distributions with equal means. The distributions of neural activity and saccade choices are normally distributed unless otherwise stated.

Signal Detection Theory (Green and Swets, 1966) was used to quantify the degree to which an ideal observer of the FEF preparatory activity could predict which choice the monkey would make. The separation between the two distribution of activity between the preferred and non-preferred was estimated from the area under the receiver operating characteristic (ROC) curve. Any differences in neuronal selectivity across the three behavioural tasks were tested using a one-way Anova analysis. In all cases the null hypothesis was rejected for all p values less than 0.05.

A Hartigan Dip Test of Unimodality was used for my inter-spike interval analysis (Fig. 8). The dip test looks for multimodality in a sample by examining the maximum difference, over all sample points, between the empirical distribution function, and the unimodal distribution function that minimizes that maximum difference. Additionally I tested the asymmetry of the ISI distribution around the sample mean via a skewness test. The skewness of the distribution was defined as

$$s = \frac{E(x - \mu)^3}{\sigma^3}$$

where $\mu$ is the mean of $x$, $\sigma$ is the standard deviation of $x$, and $E(t)$ represents the expected value of the quantity $t$.

Analysis of the neuronal data was achieved via convolving each spike train by using a post-synaptic function with a rise of 1 ms and a decay of 20 ms (Hanes and Schall, 1996):
where $t$ is the time the spike occurred. The mean discharge rate from FP offset to 50 ms after target presentation was defined as the preparatory activity. None of the neurons recorded during the warning period had target aligned visual responses that occurred before 65 ms when tested with a Poison spike train analysis technique (Hanes et al.1995). Thus, sampling within this epoch yielded the activity of these neurons immediately before any change that can be induced by the appearance of the target.
Chapter 3

Results

3.1 Behavioral Results

First I examined how closely the monkey’s behavior approached the predicted
game theoretic equilibrium of choosing both the preferred and non-preferred target
in equal proportions and stochastically from trial to trial. Previous work illustrates
that animal subjects often display significant deviations from this equilibrium
strategy (Barraclough et al., 2004; Lee et al., 2004). Our data shows that the
proportions of choices did not differ from the preferred and non-preferred direction
in both the mixed-strategy ($P_{\text{preferred}} = 49.5 \pm 0.7\%$) and unpredictable task ($P_{\text{preferred}}
= 50.6 \pm 0.6\%$) (Fig. 3A) (paired t test, $p=0.44$). However, the monkey did not
perfectly choose each target stochastically from trial to trial evidenced by a win-stay
bias ($P_{\text{Win-stay}} = 55 \pm 0.7\%$) (binomial test, $p < 10^{-3}$) during the mixed-strategy task.
The computer opponent exploited predictability in monkey’s choice patterns as
displayed by the lower percentage of rewarded choices in the mixed-strategy task
($P_{\text{reward}} = 40.8 \pm 0.7\%$) relative to the unpredictable task ($P_{\text{reward}} = 50.6 \pm 0.01\%$)
(binomial test, $p< 10^{-7}$ and $p=0.4$, respectively) (Fig. 3C). The monkey had a faster
saccadic reaction time in the predictable ($256.11 \pm 10.36$ ms) compared with the
unpredictable task ($281.01 \pm 6.76$ ms) (Fig. 3D) (paired t test, $p<10^{-9}$) indicating the
monkey used task predictability to bias motor preparation. In particular, the
Figure 3. Behavioral summary. **A-C**, The three panels compare the percentage of preferred direction of the neuron (A), win-stay/lose-shift choices (B), and rewarded choices (C) during the experimental session where the mixed-strategy and unpredictable tasks were performed. The dashed lines represents expected results attributable to randomization in the unpredictable task and the game theoretical solution in the mixed-strategy task. **D**, A comparison of saccadic reaction time for the unpredictable and the predictable task.
monkey exploited target probability in preparing saccades (Dorris & Munoz 1998). There was no significant difference between SRTs for the preferred and non-preferred in the mixed-strategy task (paired t test, p=0.3).

3.2 Neural Activity

3.2.1 Neuronal Selectivity

Preparatory activity was recorded from 35 FEF neurons from one monkey, of which 27 met our criteria and were included in the further analysis. Of the neuronal population all 27 were recorded in the mixed-strategy task, 22 in the unpredictable and a further 12 in the predictable task. I compared the FEF activity for the preferred and non-preferred directions across the three tasks. Preparatory activity was calculated as the discharge rate from fixation point offset to 50 ms after target onset. Preparatory activity increased gradually during the warning period for all tasks as the time of target presentation approached (Fig. 4A-C). To delineate the effects of target direction, neural activity was separated on whether a saccade was made for the preferred (Fig. 4A-C; top panels, dark blue) or the non-preferred (Fig. 4A-C; top panels, light blue) direction. Preparatory activity in the 50 ms after target presentation (gray bar) was predictive of upcoming saccades in the mixed-strategy task. By the end of the warning period, the mean preparatory activity was significantly greater if preferred rather than non-preferred saccades were chosen for the mixed-strategy ($P_{\text{preferred}} = 66.05 \pm 5.2$, $P_{\text{non-preferred}} = 28.27 \pm 2.5$; spikes/s) (paired t test, $p < 10^{-9}$) and predictable ($P_{\text{preferred}} = 52.88 \pm 5.2$, $P_{\text{non-preferred}} = 23.6 \pm$
Figure 4. FEF activity during the three behavioral tasks. A-C, Activity of a single representative FEF neuron during the mixed-strategy (A), predictable (B) and unpredictable (C) task. Rasters (top panels) and postsynaptic activation functions (bottom panel) are sorted based on preferred (dark blue) and non-preferred (light blue) saccades. D-F, Preparatory activity from neuronal sample during the mixed-strategy (D) (N=27, 267 ± 7 trials per neuron), predictable (E) (N= 12, 60 ± 5 trials per neuron), unpredictable task (F) (N=22, 206 ± 15 trials per neuron). The diagonal line represents the line of unity. The black squares represents the mean + SEM for all neurons. The filled data points represent statistically significant differences (paired t test, p<0.01). Grey bars represent preparatory activity 50 ms after target presentation.
3.1; spikes/s) (paired t test, p < 10^-5) tasks (Fig. 4 D, E). In contrast, there was no significant difference for the mean preparatory activity for the preferred and non-preferred direction in the unpredictable (P_{preferred} = 19.3 ± 2.4, P_{non-preferred} = 18.7 ± 2.1; spikes/s) (paired t test, p = 0.17) task (Fig. 4F). Moreover, the mean preparatory activity was greater in the mixed-strategy relative to the unpredictable task for saccades made to the preferred directed of the neuron (paired t test, p<0.01). These findings indicate that advance selection and preparation for saccades occur within the FEF preceding mixed-strategy saccades.

Receiver Operating Characteristics (ROC) analysis, a technique from signal detection theory, was used to quantify the degree of selectivity that FEF preparatory activity predicted upcoming saccadic choices (Fig. 5A). Sampling successive 50 ms epoch throughout the warning periods illustrates that the neuronal prediction gradually increased throughout the warning period for the mixed-strategy (green circles; maximum, 71%, prediction; paired t test, p<10^-3) and predictable (blue circles; maximum, 67%, prediction; p<0.01) task, but remained near to below chance levels for the unpredictable task (red circles; maximum, 49%, prediction; p=0.2).

**Evolution of Neuronal Prediction over Time**

The increase in neural selectivity observed during the mixed-strategy task could have occurred at a relatively fixed rate, from the beginning of the warning period, or
Figure 5. Evolution of neuronal prediction over time. **A.** Aggregated ROC analysis of mixed-strategy (N=27; green), predictable (N=12; blue) and unpredictable (N=22, red) task across all neurons. The circles represent neuronal predictions based on successive 50 ms epochs throughout the warning period. **B.** Comparison of neuronal prediction when the warning period was extended from 600ms (green) to 1200 ms (light blue) during two blocks of mixed-strategy task. Only neurons that were recorded from in both conditions are shown (N=6). The asterisk represents time points where neuronal prediction significantly differed from each other across the timing conditions (test p<0.01). The filled data points represent predictions that are significantly greater than chance prediction denoted by the dashed line (paired t test, p<0.01).
Figure 6. A representative FEF cell when the fixed warning period was extended from 600 ms (black) to 1200 ms (blue) across two blocks of the mixed strategy task.
it could have been tailored to the expected time of the response. To differentiate between these two possibilities, I added an additional mixed-strategy block; where the warning period was extended from 600 ms to 1200 ms. My data suggest that the time at which the preparatory activity becomes selective is delayed when the expected time of the response is prolonged (Fig. 6). ROC analysis was done to quantify the delay of neuronal selectivity across our small sample of cells (N=6) (Fig. 5B).

**Gradual Selection between the FEF Preferred and Non-Preferred Activities**

The gradual selectivity of FEF preparatory activity observed in the previous ROC does not fully address how neuronal selection occurred on each trial. This selection process could have occurred via an abrupt transition in FEF firing rate that is averaged across trials. If abrupt selection took place in the FEF, I would expect to see an abrupt transition in the firing rate, with an abrupt increase if the preferred saccade was selected and an abrupt decrease if the non-preferred saccade is selected. Selection would appear gradual on aggregated, given that the transition occurred later in the warning period (Fig. 7A, Abrupt selection). Alternatively, the selection would also appear gradual if, on each trial, there was indeed a gradual selection of FEF activity associated with the two potential saccadic targets (Fig. 7B, Gradual selection).
Figure 7. Two hypothetical patterns in which FEF preparatory activity could increase. (A,C) Abrupt Selection. (B,D) Gradual Selection. Each tick on the top panels represents an action potential. The thin gray lines represent the average of those across all trials. Note that average accumulation of activity is the same in both conditions. Each vertical dashed line in (A) highlights the time at which the abrupt transition in activity occurred on each trial. Bottom Panels- Theoretical results from the inter spike interval (analysis) that would support each of the two hypotheses. ISI\textsubscript{N} denotes the time between the current pair of spikes and ISI\textsubscript{N+1} denotes the time between subsequent pair of spikes. (C) For abrupt selection, ISI\textsubscript{N} and ISI\textsubscript{N+1} would be of the same duration and thus be clustered around the diagonal line of unity. Furthermore, the data would be bimodally distributed with modes associated with low and high frequency of activities, respectively. (D) For gradual selection, ISI\textsubscript{N+1} data would generally be shorter than ISI\textsubscript{N} data and therefore concentrated below the line of unity. These data would unimodally distributed and skewed rightward reflecting the gradual transition from low to high.
To distinguish between these two alternatives an analysis of inter-spike interval (ISI) was performed on the preparatory activity during the warning period.

An ISI represents the time between two successive action potentials (ie. spikes). The ISI corresponding with to current pair of spikes (ISI\(_N\)) was plotted against the ISI corresponding to the subsequent pair of spikes (ISI\(_{N+1}\)). Abrupt selection would be characterized by bimodal ISI distributions and ISI\(_N\) values that were equal to ISI\(_{N+1}\) values (Fig. 7C,). A gradual selection process would be characterized by a decrease, on average, of successive ISIs in the warning period. This would result in a positively skewed unimodal ISI distribution, with shorter ISI\(_{N+1}\) values relative to ISI\(_N\) values.

Analysis for ISI distributions of a representative cell illustrates the great deal of variability in the ISI distribution (Fig. 8 A, B, grey dots). However, on average, successive ISI decreased by 1.6 ms when a saccade was made towards the preferred direction of the neuron (Fig 8, A, red dot) (paired t test, p<0.05). Likewise, successive ISIs for saccades made to the non-preferred direction of the neuron decreased by 1.54 ms (Fig. 8, B, red dot) (paired t test, p<0.05). Mean ISIs for the population of 27 neurons decreased by 4.37 ms for the preferred direction (Fig. 8C,) and 2.52 ms for the non-preferred direction (Fig. 8D,) (p >0.05). Additionally, 48 % of our neurons had significant decreases in successive ISI pairs (paired t test, p<0.05) and none had a significant increase. Mean ISIs were longer for the non-preferred relative to preferred saccades (55.10 ms vs. 46.24 ms; paired t test, p<10\(^{-6}\)), which aligns well with the greater preparatory activity preceding preferred
Figure 8. Gradual accumulation in preparatory activity for both preferred and non-preferred mixed-strategy saccades. (A,B) Inter-spike interval (ISI) analysis conducted on preparatory activity for representative neuron. (A) Neuron’s preferred direction. (B) Neuron’s non-preferred direction. Each grey data point represents an ISI from a pair of spikes (ISI\textsubscript{N}) plotted against the ISI from the successive pair of spikes (ISI\textsubscript{N+1}). Red dots represent the mean data for this neuron. Diagonal lines represent lines of unity. Histogram scale bars in top right corners equals 100 observations. (C,D) ISI analysis for the sample of 27 neurons with
preparatory activity. (C) Neuron's preferred direction. (D) Neuron's non-preferred direction. Each data point represents mean data from a single neuron. Filled circles represent significant differences between ISI_N and ISI_N+1 for individual neurons (paired t-test, p<0.05). The red squares represent the mean of the entire neuronal sample.
saccades. The ISI distributions were generally unimodally shaped and positively skewed, suggesting gradual transition from a low to high firing rate. The mean skewness was significantly greater than zero (skewness of 2.42 ± 0.09; p<10\(^{-8}\)).

### 3.3 Biasing of Mixed-Strategy Saccades with FEF Stimulation

The previous selectivity observed in the preparatory activity suggests that the FEF is involved in the selection of mixed-strategy saccades. However, the results obtained to this point can also be explained via the FEF passively reflecting the sum of inputs received in real time. I applied subthreshold stimulation in order to probe the role of FEF preparatory activity in biasing mixed-strategy saccadic choices. If subthreshold microstimulation biased saccadic choices, then it would support our hypothesis that the FEF is located in the circuit that is involved in selecting mixed-strategy saccades.

Stimulation was used to artificially increase activity for the preferred direction of the population of neurons at the stimulated FEF site. Crucially, the stimulation was applied at levels below threshold and in advance of the saccadic eye movement. The fact that the stimulation only biased a proportion of saccades (Fig 9, C, D) suggests that it was sub-threshold. Whereas suprathreshold stimulation would automatically elicit saccades towards the movement vector corresponding with the stimulated FEF loci (Bruce and Goldberg, 1984). Furthermore, the stimulation was terminated 100ms before target onset.
Unexpectedly, subthreshold stimulation biased the selection of mixed-strategy saccades away from the stimulation vector (57%), relative to interleaved no stimulation trials (43%), (Fig. 9,B; paired t test, p<0.007). Additionally, SRTs for saccades made towards the stimulation vector were significantly greater on stimulation than non-stimulation trials (277.09 ± 6.0 ms vs. 191.86 ± 5.0 ms, respectively)(paired t test, p<10^{-4}).
Figure 9. Manipulating mixed-strategy selections with subthreshold stimulation. (A) Behavioral paradigm. Sub threshold stimulation was applied for the first 500 ms of the warning period in the mixed-strategy task (N=9 stimulation sites). (B) Percentage of choices allocated towards the stimulation vector during stimulation and non-stimulation trials. The diagonal line represents the line of unity. N= 9 stimulation sites. (C,D) Endpoints of saccades during stimulated (C) and non-stimulated (D) trials for a representative FEF stimulation site.
Chapter 4
Discussion

My results demonstrate that the FEF is involved in the selection of strategic saccades. This is substantiated, first, by the evolution of a selection process through the gradual accumulation of neural activity for one option over another, in the time preceding the saccade. Overall, preparatory activity of single neurons, at the end of the warning period, predicted mixed-strategy choices with seventy-one percent accuracy. Second, subthreshold microstimulation biased saccadic choices away from vector coded by the stimulated FEF loci.

4.1 Behavioral Strategies During a Mixed-Strategy Task

The monkey was found to approach but deviate significantly from the predicted mixed-strategy Nash Equilibrium. The Nash Equilibrium strategy, for the mixed-strategy task, is for each subject to choose both targets in equal proportion and stochastically from trial to trial. In our experiment the monkey did choose each target in equal proportions, but however he exhibited a win-stay bias that resulted in non-stochastic choices on a trial-by-trial basis. This win-stay bias has been reported in similar monkey (Barraclough et al., 2004; Lee et al., 2004; Lee et al., 2005; Thevarajah et al., 2009) and human (Camerer, 2011) behavioral studies.

Importantly, there is difference between asking a subject to choose randomly from alternatives and having a subject play a game against an adaptive computer opponent, as
in the ‘matching-pennies’ task. In the latter, the aim of randomizing choices from trial to trial is aided by an online dynamic interaction (Rapoport and Budescu, 1992). The subject can utilize the online feedback from the computer opponents choices (Armantier, 2004; Barraclough et al., 2004; Mookherjee and Sopher, 1991). Likewise, monkeys’ choices become more stochastic as the computer opponent becomes more ‘intelligent’, specifically, when the computer utilizes a better algorithm for exploiting patterns in the monkeys’ choices (Barraclough et al., 2004).

Alternatively, the difficulty in producing a stochastic set of choices could stem from processing limitations. In the ‘matching-pennies’ task, the computer retains a complete record of the history of choices and rewards, while the monkey’s brain is constrained with a limited memory capacity (Courtney, 2010; Ramsey, 2003; Marois and Ivanoff, 2005). The ability to recall and perform statistical analysis on the complete history of choices and rewards is critical to the computer's advantage in the task. The effects of reward and choice history on behavior are temporally discounted (Seo, 2008; Thevarajah et al., 2010). In particular, the more recent an event is the greater influence it will have on present behavior, with events further back having a reduced effect on current behavior (Kim et al., 2009b; Rushworth et al., 2011)

Humans playing ‘matching-pennies’ against an adaptive computer opponent generally displayed both a win-stay and a loose-shift bias (Cohen and Ranganath, 2007). The lack of a lose-shift bias in my version of ‘matching-pennies’ maybe due to it not being a pure zero-sum game. A loss resulted in the neutral outcome of
withholding a reward rather than punishing the subject, via the withdrawal of a reward. Further, this is supported by research on humans (Kahneman et al., 1991; Genesove et al., 2001; Weller et al., 2007) and monkeys (Chen et al., 2006; Brosnan et al., 2007; Seo and Lee, 2009) illustrating that both are generally sensitive to losses.

4.2 FEF Activity Represents Evolving Saccadic Plans

4.2.1 Selectivity of FEF Preparatory Activity

There was a significant ramping up of preparatory activity in the warning period, when the monkey made a saccade towards the preferred direction. This activity reflected the temporal and spatial development of upcoming strategic saccades. The accumulation of activity towards the preferred direction increased at a gradual rate. This was consistent with a previous study conducted by Mikulic and Dorris (2007), their results indicate that saccade preparation, in the SCi, during a competitive game was differentially allocated towards one target over another in advance of target onset. Albeit in the SCi there was also a ramping up of activity for non-preferred saccades. Moreover, FEF activity corresponding with the preferred direction of the neuron predicted the spatial direction of the upcoming saccade at a rate that is tailored to expected saccade timing. The above results suggest that the FEF reflects both the direction and timing of strategic saccades.

The selectivity of the preparatory activity for the preferred direction could stem from a competitive selection process in the FEF (Schall and Hanes, 1993; Schall,
1995). FEF neurons interact, in both a competitive and cooperative manner, when selecting saccades (Cohen et al., 2010). Pairs of neurons with overlapping RF cooperate through spike timing synchrony around the time of target selection. Conversely, neurons compete through spike timing asynchrony when neurons have no overlapping RF. The pattern of interaction between FEF neurons is consistent with network models in which neurons with spatially overlapping RF excite each other as well as a population of interneurons that inhibit neurons with non-overlapping RF (Cohen et al., 2010). This indicates that FEF neurons cooperate and compete with each other to select targets in visual space, further suggesting that timing and spike rate influence the process that selects strategic saccades.

FEF preparatory activity, during a free viewing task, indicates that the neural activity preceding a saccade is modulated by the target location of the upcoming saccade (Burman and Segraves 1994a, 1994b). Additionally, more recent work, utilizing a sequential saccade task, indicates that the preparatory activity during the initial fixation period could predict the vector of the second saccade of a pair of successive saccades (Phillips and Segraves, 2010). Therefore, my data combined with the aforementioned studies indicate that FEF preparatory activity is predictive of upcoming voluntary saccades.

Two control tasks allowed me to contrast the neural process preceding strategic saccades with those preceding saccades whose target location was predictable and unpredictable. If the neural selectivity preceding the strategic task resembles that in the predictable task, then saccades are selected and prepared in advance in both
cases. If the neural selectivity in the strategic task resembles that in the unpredictable task, then strategic saccades are not selected in advance of target presentation and preparation was not initially biased. The results indicate that the pattern of neuronal selectivity in the mixed-strategy task is similar to the predictable task. This suggests that preparation and target selection of strategic saccades are initially biased becoming increasingly selective as the time of target presentation occurs.

Perhaps surprisingly, the level of selectivity was greater for the mixed-strategy than the predictable task. I speculate that this could be due to the cognitive demands of the tasks, which may recruit more FEF resources, with regards to updating reward and choice history in order to optimize future choices. The properties of the FEF neuronal population in conjunction with its afferent cortical projections could suggest that the activity of this region is biased, preferentially responding to mixed-strategy than predictable saccades.

4.2.2 Stochastic Mechanism for Strategic Saccade Selection

Analysis of ISI indicates that the selection process occurred gradually, via the accumulation of activity at an FEF loci corresponding with one of the targets. This gradual transition from a low to high firing rate is also consistent with a process where the decision is made in other cortical structures and transmitted into the FEF in a continuous manner. However, the fact that FEF stimulation biased the selection
of strategic saccades suggests that FEF is directly involved rather than passively reflecting a completed selection process.

Sensory percepts could not have caused the accumulation in the mixed-strategy task, due to the accumulation of activity occurring in the warning period, where the monkey faced blank screen. Therefore, upon target presentation, both targets were equally salient and the subject was free to saccade to either. However, the neural mechanisms underlying these strategic decisions may share some properties with perceptual decision-making. Common models of perceptual decision-making assume a gradual differential accumulation of sensory evidence across the sensory maps. The appropriate action is elicited once the stochastic build up reaches a particular threshold (Gold and Shadlen, 2002; Hanes and Schall, 1996).

Computational models posit that the relative rate of accumulation for neural activity in favor of potential percepts hinges on the quality and duration of the sensory evidence corresponding to the choices (Gold and Shadlen, 2001; Huk, 2005; Palmer et al., 2005).

The FEF selection process could be delineated via a modified version of a network model proposed by Wang (2002). In brief, two neural pools are selective for either the preferred or the non-preferred direction, each pool is comprised of a number of spiking neurons. The circuit contains strong direct excitatory connections between pyramidal neurons with similar receptive fields (within each pool) and weak connections for neurons with non-overlapping receptive fields (between pools). Further, there is a balance between excitatory and inhibitory connection,
with inhibitory neurons inducing competition between distant neural pools (Wang, 2002). Recent work suggest that there is a similar recurrent circuit within the FEF, where pyramidal cells connect mainly with other pyramidal cells (87%), the remaining synapses being with GABAergic neurons (13%) (Anderson et al., 2011). The inhibition produces the winner-take all property, so that an increase in activity for one pool of neurons leads to a suppression of the other competing pool of neurons.

The results are consistent with the aforementioned model, given that the threshold of FEF activity required for pushing the downstream SC activity past threshold is adjustable. Tuning the synaptic strength of the cortical-striatal pathway can modify the threshold. This is possible if the synapses are plastic, binary and gated by a dopaminergic reward signal (Soltani et al., 2006). Cortical-striatal synapses represent a major target of innervation by dopamine neurons, which play a critical role in the reinforcement learning signal (Reynolds and Wickens, 2002).

For example, in our “matching-pennies” task, if the monkey selects the right target and he is rewarded, then the cortical-striatal synapses in the left hemisphere would be potentiated via the associated release of dopamine. This would lead to a small bias on the subsequent trial for the right target. Conversely, if selection of the right target does not yield a reward, then there would be a depression of the cortical-striatal synapses in the left hemisphere that are associated with the right target. This would make the right target less likely to be selected on the subsequent trial (Hikosaka et al., 2006). Given there is no sensory input, firing rates of input
neurons to both the selective pools of excitatory neurons are similar, so the difference in the overall inputs to the two neural pools is depends only on the state of their plastic synapses (Soltani et al., 2006). In such a scenario the noise and the competitive nature of the FEF map would make it more likely that the skewed option would be chosen, but not necessarily guarantee that the selection will favor the initial biased option. This model is consistent with the behavior seen here and related experiments (Lee et al., 2004; Seo et al., 2009; Thevarajah et al., 2009).

4.3 Manipulation of Strategic Saccades with Subthreshold Microstimulation

The administration of subthreshold stimulation to bias saccade selection supports that the FEF is within the neural circuit responsible for the selection of strategic saccades. Surprisingly, subthreshold stimulation biased choice probability away from the stimulated movement vector. Previous work suggests that suprathreshold stimulation of the FEF triggers saccades towards the movement vector (Barborica, 2004) (Bruce et al., 1985), and sometimes results in averaging effect when administered concomitantly with a voluntary saccade (Gold and Shadlen, 2000). More pertinent to this study, subthreshold stimulation of the FEF is capable of augmenting attentional mechanisms of V4 neurons that are associated with the stimulated movement vector (Armstrong and Fitzgerald, 2006; Clark et al., 2011; Elkstorm et al., 2009). The current runs in contrast to previous studies that probed
FEF attentional mechanism via subthreshold stimulation, in that; stimulation did not bias movement towards the vector coded for by the stimulated FEF loci or movement field.

A number of possible explanations for these stimulation results are proposed here. The different effects of FEF subthreshold stimulation on the SC and V4 can be delineated, in part by, the heterogeneity of the intrinsic and extrinsic circuitry of the FEF in macaque monkey. The nature of the cytoarchitecture of the FEF provides further insight to the contrast between my results and other subthreshold perceptual decision-making tasks. Efferent cortical (e.g., V4) and subcortical (e.g., SC) projections of the FEF are distinct and are distributed within different layers of the FEF (Barone et al., 2000; Builler et al., 1984). Specifically, only layer 5 neurons are the source of projections to the SC, whereas the neurons projecting to V4 are mainly in the supragranual layers of the FEF, with few in layer 5. Additionally, the supragranular pyramidal neurons project to the extrastriate cortex and the infragranual pyramidal neurons project to the SC are in embedded in quite different local inhibitory circuits (Pouget et al., 2009).

My data is more in line with those of Opris and colleagues (2005). They examined the effect of subthreshold stimulation on delay/preparatory activity, during a memory guided saccade task. For remembered targets near the preferred location, microstimulation biased saccadic choices by deflecting them away from the preferred location. Additionally, saccade latency increased as the target of the memory guided saccades overlapped with the stimulated FEF loci. Furthermore,
they found an inverse relationship between preferred direction of the delay/preparatory activity and the direction shift caused by subthreshold microstimulation on memory guided saccades. The inverse relationship was not observed in other high threshold regions in the peri-arcuate cortex.

Similar results in a memory guided saccade task suggest that subthreshold stimulation of the FEF could mimic a signal indicating that a small saccade has occurred, through a stimulation induced corollary discharge signal (White and Snyder, 2007). That is, the target location in spatial memory could have been updated due to the fictive eye movement. In order to compensate for the supposed shift in gaze, the resultant saccade to the target would be in opposite direction of the initial stimulated movement field. The corollary discharge signal is believed to originate from the superior colliculus via the medial dorsal thalamus. This pathway is known to contribute to spatial updating (Girard and Berthoz, 2005; Berman et al., 2009). Evidence for spatial updating can be seen in studies where pharmacological inactivation of the medial dorsal thalamus leads to subjects being unable to fully compensate for the intervening eye movements during a sequential saccade task (Sommer, 2004; Crapse and Sommer, 2008).

Additionally, an experiment utilizing a fixation task suggests that subthreshold microstimulation of the FEF can possibly shift saccades away from the stimulated movement field (Seidemann, 2002). Transient microstimulation in FEF can be followed by a prolonged hyperpolarization of the cortex. Saccade peak velocity decreased during the drop in depolarization and then reversed to the ipsilateral
direction during hyperpolarization. Further, saccades that were initiated during the strong hyperpolarization were almost always large return saccades in the ipsilateral direction. Further, many FEF neurons with presaccadic activity are actively suppressed following a saccade into their RF (Bruce et al., 1985; Goldberg and Bruce, 1990). It’s probable that the time of saccade selection in my task fell within the time frame of hyperpolarization, resulting in a stimulation-induced bias for ipsilateral saccades.

Lastly, subthreshold microstimulation has been shown to have suppressive effects of on saccade related FEF activity (Burman, 1997; Izawa et al., 2004; 2011). Microstimulation is capable of suppressing ipsilateral, contralateral and bilateral saccade generation. My results substantiated these findings by having longer SRTs for stimulation than non-stimulation trials. The increase in saccade latency may happen because the stimulating current disrupts circuitry involved in the planning and control of saccades (Opris et al., 2005). Likewise, the suppression could result from the activation of the GABAergic inhibitory circuit within the FEF (Pouget et al., 2009). The preferential activation of the axons and axon terminals of GABAergic afferents could be a result of the lower excitability and/or increased number of inhibitory interneurons relative to excitatory pyramidal neurons in the stimulated FEF loci.
4.4 FEF v.s SC Circuit Influence on Mixed-Strategy Saccades

Neuronal signals in the cortex are generally thought to regulate signals in the subcortical regions. The FEF and SCi are pivotal nodes in the visuosaccadic systems, and provide ideal regions for the examination of strategic saccades. The SCi, a sensorimotor structure in the midbrain, has afferent projections from the FEF (Berman et al., 2009; Ding and Hikosaka, 2006; Schall et al., 2008). Moreover, the SC projects signals to the FEF via the mediodorsal nucleus of the thalamus (Munoz et al., 2000; Sommer, 2004; Sommer and Wurtz 1998; Sommer, 2004).

A major strength in the comparison of our FEF and SCi results is the continuity between both sets of experiments. We utilized the exact same ‘matching-pennies’ task on the same monkey (Thevarajah et al., 2009). Further, our behavioral analysis confirms that the monkey utilized the same behavioral strategies. The monkey allocated choices, in both the FEF and SCi experiments, in equal proportions to the preferred and non-preferred directions. Likewise, in both cases the reward rate was <50% because the monkey displayed a statistical bias that the computer was able to exploit. Therefore, it’s likely that the contrast in neural activity across the FEF and SCi is due to the difference in cortical processing across the two regions, not due to any difference in the monkeys or strategy.

The baseline firing rate of the SCi is higher than that of the FEF (Fig 10, A-B). Preparatory activity during the warning period became gradually predictive in both structures, with substantial ramping up of activity if the monkey chose a target in the neuron’s RF. However, the ramping up of activity in the SC was observed for
both preferred and non-preferred saccades, while this accumulation of activity was restricted to preferred saccades in the FEF. The magnitude and time course of selectivity differed across the two structures. The selection of mixed-strategy saccades occurred earlier and was in greater magnitude in the FEF (71%) compared to the SCi (62%) (Fig 10, C). The time course of the selectivity in the SCi was affected by the degree and duration with which the non-selected saccade was represented in advanced SCi activity. Substantial neural activity was present just before the selection of non-preferred saccades.

The greater magnitude of selectivity within the FEF might appear counterintuitive, given that the SCi is located close to the motor output of the visuosaccadic circuit (Wurtz et al., 2001). I expected the signal to be more accurate as it propagates down to the level of the SCi. However, I think the discrepancy exists because neuronal selectivity was tailored in a manner that reflected the time of the expected saccade. The neural representation of saccades well advance of the target presentation in the SCi is a risky venture. Given the proximity of the SCi to the brainstem saccade generator, fluctuations due to inherent noise could surpass the threshold required and trigger premature saccades. Hence, the relative decrease of SCi selectivity could be a result of tonic inhibition from the basal ganglia (Purcell et al., 2010).

Alternatively, the FEF could inherently be more selective than the SCi due to its unique robust afferent frontal and parietal projections from DLPFC, dACC, SEF and LIP; structures that code for higher level statistics of ‘matching-pennies’. The
difference in selectivity across the FEF and the SCi could stem from the unique afferent inputs to each structure. This view is consistent perhaps with FEF activity more closely representing the decision and the SCi enacting that decision with respect to the timing and location of saccades.

Stimulation experiments were utilized in an attempt to bias the selection of strategic saccade in both the FEF and SCi. In the SCi, subthreshold stimulation biased saccadic choices towards the stimulated movement vector on majority of trials, with 60% of stimulated trials towards and 40% away from the stimulated movement vector. Conversely, I observed the opposite effect within the FEF. Stimulation biased majority of saccadic choices away from the movement vector, with 43% of trials towards and 57% away from the stimulated movement vector (Fig 10, D). The difference between the results may be attributed to subthreshold stimulation exerting its effects locally within the SCi. This can result in artificially biased activity being maintained, and possibly augmented through recurrent network of local excitation and distal inhibition (Lee and Wang, 2009). The stimulation can circumvent the tonic basal ganglia inhibition on the stimulated SCi loci. Thus, causing a significant difference of activity across the stimulated and non-stimulated SCi loci. After which the random fluctuations in noise are more likely to push the activity in the stimulated loci past the required threshold to elicit a saccade.
Figure 10. Comparison of mixed-strategy saccade selection across the FEF and the SC. (A), Mean preparatory activity of FEF neurons for the preferred (dark blue) and non-preferred (light blue) directions across the warning period. (B), Mean preparatory activity of SC neurons for the preferred (red) and non-preferred (pink) directions across the warning period. (C), Evolution of neuronal prediction over time, ROC analysis for the FEF (blue) and SC (red) during the mixed-strategy task. (D), Manipulating mixed-strategy selection with subthreshold stimulation. Percentage of FEF (blue) and SC (red) choices allocated towards the stimulation vector during stimulation and non-stimulation trials.
Conclusion

This study indicates that the FEF is involved in the circuit that is responsible for the selection of strategic saccades. My results suggest that FEF preparatory activity is predictive of upcoming mixed-strategy saccadic choices. I speculate that the accumulation of activity, for one target over another, is governed by the competitive nature of the FEF map and the stochastic nature of cortical firing. Further, the data indicates that FEF subthreshold stimulation biases saccadic choices away from the preferred direction of the stimulated FEF loci. I speculate that subthreshold stimulation perturbs the circuitry associated with saccade planning at the stimulated loci, resulting in a bias favoring saccades towards the non-stimulated FEF loci. Additionally, the selection of mixed-strategy saccades occurred earlier and was in greater magnitude in the FEF than the SC, indicative of a decision process that occurs earlier in the frontal cortex before being relayed on to premotor regions in the midbrain.
References


