**1. TITLE OF PROJECT**

**Cortical and subcortical control of visual attention**

**2. RESPONSE TO SPECIFIC REQUEST FOR APPLICATIONS OR PROGRAM ANNOUNCEMENT OR SOLICITATION**

- title: [Yes or No]

**3. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR**

<table>
<thead>
<tr>
<th>Name</th>
<th>Degree(s)</th>
<th>eRA Commons User Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>McPeek, Robert M.</td>
<td>PhD</td>
<td></td>
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</tbody>
</table>

**4. HUMAN SUBJECTS RESEARCH**

- Research Exempt [Yes or No]
- Clinical Trial [Yes or No]
- NIH-defined Phase III Clinical Trial [Yes or No]
- Human Subjects Assurance No. [Yes or No]

**5. VERTEBRATE ANIMALS**

- [Yes or No]

**6. DATES OF PROPOSED PERIOD OF SUPPORT**

- From: 07/01/07
- Through: 06/30/12

**7. COSTS REQUESTED FOR INITIAL BUDGET PERIOD**

- Direct Costs: $250,000
- Total Costs: $389,026

**8. COSTS REQUESTED FOR PROPOSED PERIOD OF SUPPORT**

- Direct Costs: $1,250,000
- Total Costs: $2,067,919

**9. APPLICANT ORGANIZATION**

- Name: The Smith-Kettlewell Eye Research Institute
- Address: 2318 Fillmore Street, San Francisco, CA 94115

**10. TYPE OF ORGANIZATION**

- Public: [☐] Federal [☐] State [☐] Local
- Private: [☐] Private Nonprofit
- For-profit: [☐] General [☐] Small Business
- Woman-owned: [☐] Socially and Economically Disadvantaged

**11. ENTITY IDENTIFICATION NUMBER**

- DUNS NO. 073121105
- Cong. District CA-8

**12. ADMINISTRATIVE OFFICIAL TO BE NOTIFIED IF AWARD IS MADE**

- Name: JoAnn Yates
- Title: Sr. Research Administrator
- Address: 2318 Fillmore Street, San Francisco, CA 94115
- Tel: 415-345-2036
- Fax: 415-345-8455
- E-Mail: joann@ski.org

**13. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION**

- Name: Ruth Poole
- Title: Chief Operating Officer
- Address: 2318 Fillmore Street, San Francisco, CA 94115
- Tel: 415-345-2043
- Fax: 415-345-8455
- E-Mail: ruth@ski.org

**14. APPLICANT ORGANIZATION CERTIFICATION AND ACCEPTANCE**

- Signature of official named in 13.
- Date: 10/31/06
Attention has profound effects on visual processing, allowing us to strategically select, filter, and prioritize the vast amount of information in the visual environment. The long-term goal of this project is to understand how the brain shifts attention to visual stimuli. Attention can be shifted in two modes: it can be reflexively "captured" by a salient stimulus, or voluntarily focused by top-down mechanisms on a selected object of interest. This project investigates the roles of the superior colliculus (SC) and frontal eye field (FEF) in reflexive and top-down attention shifts. The SC and FEF are traditionally considered to be areas involved in eye movements. Recent evidence indicates that these brain areas may also be important in controlling shifts of attention in the absence of overt eye movements, but their specific contributions to attention are unclear. Based on anatomy and on their roles in eye movements, we hypothesize that the SC is involved in controlling reflexive attention shifts and the FEF is involved in top-down shifts. We will test this hypothesis, first, by determining whether neural signals recorded in the SC and FEF are appropriate for controlling reflexive or top-down attention shifts. Second, we will ascertain whether these areas play necessary functional roles in attention by examining the impact of temporarily inactivating small regions of either the SC or FEF on reflexive and top-down attention shifts. Finally, attention can produce different types of improvements in visual sensitivity, and we will investigate whether these different effects of attention on perception are separately engaged by the SC and FEF.

Relevance: Deficits in attention can seriously impair visual and cognitive functioning, interfere with activities of daily life, and have been associated with greater risk of accident and injury. We expect that the knowledge gained from this project will contribute to a better understanding of the causes of attentional manifestations of human disorders such as unilateral visual neglect, oculomotor apraxia, ADHD, and schizophrenia. Gaining an understanding of the basic neural mechanisms that control attention may provide new avenues for diagnosis, treatment, or rehabilitation of populations suffering from attentional disorders.

PERFORMANCE SITE(S) (organization, city, state)

The Smith-Kettlewell Eye Research Institute, San Francisco, CA
**KEY PERSONNEL.** See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator(s). List all other key personnel in alphabetical order, last name first.

<table>
<thead>
<tr>
<th>Name</th>
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<th>Organization</th>
<th>Role on Project</th>
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<tr>
<td>McPeek, Robert M.</td>
<td></td>
<td>Smith-Kettlewell</td>
<td>PI</td>
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**OTHER SIGNIFICANT CONTRIBUTORS**

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<th>Organization</th>
<th>Role on Project</th>
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<tr>
<td></td>
<td>Smith-Kettlewell</td>
<td>Associate</td>
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**Human Embryonic Stem Cells**  
☑ No  ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: [http://stemcells.nih.gov/registry/index.asp](http://stemcells.nih.gov/registry/index.asp). *Use continuation pages as needed.*

If a specific line cannot be referenced at this time, include a statement that one from the Registry will be used.
**RESEARCH GRANT**

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**Appendix** *(Five collated sets. No page numbering necessary for Appendix.)*

- Number of publications and manuscripts accepted for publication *(not to exceed 10)*
  - 5

**Checklist**

- Check if Appendix is Included
### BUDGET JUSTIFICATION PAGE

#### MODULAR RESEARCH GRANT APPLICATION

<table>
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<th>4&lt;sup&gt;th&lt;/sup&gt; Period</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; Period</th>
<th>Sum Total (For Entire Project Period)</th>
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### Personnel

**Robert M. McPeek, Ph.D., Principal Investigator**, will oversee the entire project, from the design of the experiments to the presentation of the results to the scientific community at meetings and in the form of publications. These activities will occupy all five years of the project.

**Ph.D., Research Associate**, will be involved in Aim 1 and part of Aim 2 of the project, from the design and execution of experiments to the presentation of results at meetings and in the form of publications. She is currently supported by a fellowship, and will be supported for an additional by this grant in order to complete her contributions to the first two aims of the project. Thus, she will devote of her time in year two to the project, and in year three.

**B.A., Project Assistant**, will be responsible for all the daily routine jobs in the laboratory, including: animal handling, training, and preparation; surgical assistance, ordering of supplies, and assistance with the preparation of figures for publications and slides for presentations. The project assistant will devote effort to the project for all five years.
RESOURCES

FACILITIES: Specify the facilities to be used for the conduct of the proposed research. Indicate the performance sites and describe capacities, pertinent capabilities, relative proximity, and extent of availability to the project. If research involving Select Agent(s) will occur at any performance site(s), the biocontainment resources available at each site should be described. Under "Other," identify support services such as machine shop, electronics shop, and specify the extent to which they will be available to the project. Use continuation pages if necessary.

Laboratory:
Experiments will be conducted in the P.I.'s recently-renovated recording laboratory, which includes a light-tight, electronic noise shielded experimental room and external control room. Additional rooms in the same suite include an animal prep room, electrode fabrication room, and a small mechanical/electrical shop. A shared histology laboratory with fumehoods, microscopes, and standard biochemical/histology benches is located two floors up in the building.

Clinical:
N/A

Animal:
Husbandry rooms for monkeys are located----
There is a modern, dedicated small animal surgical suite with an isoflurane gas anesthesia machine. This facility includes a separate prep/recovery room.

Computer:
Three Macintosh G4s and one G5 computer, one used for data collection in the laboratory, one in the P.I.'s office, one in the research associate's office, and one in the research assistant's office. One Pentium 4 PC is located in the laboratory to control the Plexon MAP and NAN systems, and another in the office for offline spike sorting. A networked laser printer is located in the research assistant's office.

Office:
Two adjacent offices are available to the project. One is used by the P.I. and the other by the research associate and research assistant.

Other:
A medical illustrator, a medical photographer, an electronic and computer technician, and a machinist are available to the project on a shared basis. Photocopying, word processing, and secretarial service is also available.

MAJOR EQUIPMENT: List the most important equipment items already available for this project, noting the location and pertinent capabilities of each.

The major equipment available in my laboratory includes a CNC electromagnetic field eye movement measurement system, a Plexon NAN multi-electrode microdrive, a Kopf hydraulic microdrive, a Plexon MAP multi-channel processor for recording up to 16 channels of single unit and local field potential data, FHC bipolar stimulus isolators, a 29-inch color CRT monitor, and a number of digital storage oscilloscopes.
A. Specific Aims

Visual processing resources are deployed to selected stimuli using both eye movements and shifts of attention. The primate superior colliculus (SC) and frontal eye field (FEF) play central roles in saccadic eye movements, and recent evidence suggests their involvement in visuo-spatial attention. Although their contributions to saccades have been studied extensively, we know little about their roles in attention.

Visuo-spatial attention can be driven in two different modes: reflexively, by the bottom-up salience of the stimulus, or by top-down control, based on internally-generated goals or expectations. Moreover, attention can improve visual discrimination through several mechanisms, including by filtering out irrelevant stimuli (external noise exclusion), by reducing the number of locations that must be monitored (uncertainty reduction), and by boosting responses to attended stimuli (signal enhancement).

Are these different modes and mechanisms of attention separately controlled? A starting hypothesis is that the SC, which receives short-latency visual signals, is responsible for reflexive attention, while the FEF, which is well-positioned to receive cognitive signals, is responsible for top-down attention. This project will determine whether neural signals in the SC and FEF are appropriate for controlling reflexive and top-down attention shifts. Further experiments will determine whether the SC and FEF are functionally necessary for reflexive and top-down shifts. Finally, we will investigate whether the SC and FEF engage different mechanisms for attentional improvement of visual perception. The project will distinguish the roles of the SC and FEF in directing attention and the extent to which different modes and mechanisms of attention are separately controlled by each area.

Aim 1: Do the SC and FEF contain signals appropriate for controlling reflexive and top-down attention shifts?
We will use single-unit recording to correlate SC and FEF activity with performance in reflexive and top-down attention tasks. Prior research suggests that reflexive and top-down attention have different characteristics and may be governed by distinct neural pathways. This aim compares SC and FEF activity as monkeys make reflexive and top-down attention shifts during fixation. We will measure the correlation of neural activity with the locus of attention, with the level of performance in the attention task, and with the temporal characteristics of attention shifts. Preliminary results suggest that reflexive and top-down attention shifts are processed, at least in part, by different pathways: in accordance with our hypothesis, SC activity is related only to reflexive attention. On the other hand, FEF activity is correlated with both reflexive and top-down attention.

Aim 2: Do the SC and FEF play necessary causal roles in reflexive and top-down attention shifts?
Recent microstimulation studies show that attention can be shifted by SC or FEF stimulation. However, these studies do not indicate whether these attention shifts involve reflexive or top-down attention, nor do they establish whether activity in the SC or FEF is necessary for generating an attention shift. We will use temporary chemical inactivation to test the hypothesis that the FEF plays an essential role in both reflexive and top-down attention shifts, while the SC is necessary only for reflexive shifts. Preliminary inactivation studies suggest that reflexive attention shifts are impaired following inactivation of either the SC or FEF. In contrast, the results suggest that SC inactivation does not impair top-down attention shifts. To test broadly for an influence of inactivation on attention, we use a task in which performance could be affected by several different attention mechanisms, including external noise exclusion, reduction of uncertainty, and signal enhancement.

Aim 3: Do the SC and FEF engage different mechanisms of attention? In contrast to the previous aim, this aim uses a low-noise, low-uncertainty task to specifically isolate the signal enhancement mechanism of attention. We will determine whether (and how) the signal enhancement mechanism of attention is affected by SC or FEF inactivation. If inactivation does not affect signal enhancement, it will indicate that attentional impairments in Aim 2 are likely due to deficits in external noise exclusion or uncertainty reduction mechanisms.

If signal enhancement is impaired following inactivation, our analysis will distinguish between two different components of the enhancement mechanism: contrast gain and response gain. Contrast gain refers to an increase in the effective contrast of attended signals, while response gain describes a multiplicative increase in response to attended signals across all contrasts. Preliminary results suggest that when the SC and FEF are intact, reflexive attention engages both contrast gain and response gain, while top-down attention engages only contrast gain. We will determine whether inactivation of either structure preferentially affects contrast gain or response gain. Given the preliminary evidence for selective involvement of the SC in reflexive attention, we will test the hypothesis that the SC is necessary for engaging the response gain component of signal enhancement, which is present for reflexive attention shifts, but absent from top-down shifts, while the FEF is necessary for engaging the contrast gain component.
B. Background and Significance

Spatially-selective attention allows us to strategically select, filter, and prioritize the vast amount of information in the visual environment. Attention’s critical role in mediating perception has been appreciated for more than a century, as William James (1890) observed that: “millions of items... are present to my senses which never properly enter into my experience... My experience is what I agree to attend to... without selective interest, experience is an utter chaos.” Disorders of visual attention, such as spatial neglect and extinction, can cause serious impairments in activities of daily life. Attentional deficits are seen in individuals who have suffered stroke or other damage to frontal, parietal, or subcortical regions (e.g., Mesulam 1981; Posner et al. 1982; Sapir et al. 1999; Husain et al. 2000; Weddell 2004; Sereno et al. 2006). Gaining an understanding of the neural mechanisms involved in controlling shifts of attention is essential if we are to make progress in treating disorders of attention.

Spatial attention modulates processing of stimuli based on their locations in the visual field, while feature-based attention modulates processing of particular visual features, regardless of their location. This project examines the neural mechanisms controlling shifts of spatial attention without eye movements. To understand attention, we must consider both the effects of attention on visual processing and the source of the signals that control attention. Recent years have witnessed exciting progress on both fronts. Psychophysics, single-unit, and fMRI studies have led to the development of quantitative accounts of the effects of attention on responses in visual areas of the brain (e.g., Treue and Maunsell 1996; Treue and Martinez-Trujillo 1999; McAdams and Manusse 1999; Reynolds et al. 2000; Somers et al. 1999; Ress et al. 2000; Saenz et al. 2002), and on behavioral performance (e.g., Lu and Dosher 1998; Palmer et al. 2000; Huang and Dobkins 2005). At the same time, fMRI studies of the signals controlling attention have lent support, at least in general terms, to the long-debated pre-motor theory of spatial attention (Corbetta et al. 1998; Kastner and Ungerleider 2000; Giteiman et al. 2002). This theory holds that attention shifts are controlled by the same brain regions that mediate saccadic eye movements (e.g., Posner 1988; Rizzolletti et al. 1987).

The role of parietal cortex in attention is well established (e.g., Bushnell et al. 1981; Constantinidis and Steinmetz 2005; Bisley and Goldberg 2006; Balan and Gottlieb 2006), and recent evidence suggests that the primate superior colliculus (SC) and frontal eye field (FEF) are also be involved in controlling shifts of covert attention. The contributions of the SC and FEF to saccades have been studied extensively over the last 35 years, but we still know very little about their roles in attention. This project will illuminate the different contributions of the SC and FEF to attention, and reveal whether they play essential functional roles.

**Reflexive and top-down attention shifts**

Spatial attention can be shifted either reflexively, by the bottom-up salience of the immediate stimulus, or top-down, by internally-generated goals, expectations, and memories (Posner 1980). Reflexive attention has also been called exogenous, involuntary, or transient attention, while top-down attention is also known as endogenous, voluntary, or sustained attention. Psychophysics and fMRI data suggest that reflexive and top-down attention shifts have different characteristics and may be governed, to some extent, by different neural pathways (Nakayama and Mackeben 1989; Kastner and Underleider 2000; Lu and Dosher 2000; Corbetta and Shulman 2002; Ling and Carrasco 2006). Whereas most prior animal studies of attention have only considered reflexive or top-down attention shifts alone, this project will directly compare the neural mechanisms underlying both modes of attention.

Following the onset of a salient cue, such as a bright figure or a color oddball, an improvement in perception due to reflexive attention can be measured by presenting a discrimination target at the cued location. The time between cue onset and target onset is known as the “cue lead time.” When improvement at the cued location is measured for different cue lead times, a characteristic time-course is seen (Nakayama and Mackeben 1989; Müller and Rabbitt 1989; Posner et al. 1982): visual performance begins to improve ~75 ms after cue onset, is best at cue lead times of 150-250 ms, and gradually becomes worse at longer cue lead times (Fig. 1). This transient time-course has been seen in studies of reflexive attention in monkeys as well as humans (Lee and McPeek 2006; Bisley and Goldberg 2006; Golla et al. 2004; Balan and Gottlieb 2006; Fecteau et al. 2004), and appears to be an intrinsic property that is not under voluntary control (Jonides 2006).
Salience maps and the hypothesized role of oculomotor areas in controlling attention

Research on the control of spatial attention has been strongly influenced by the pre-motor theory, which posits that shifts of attention are triggered by sub-threshold saccadic commands in oculomotor areas (e.g., Rizzolatti et al. 1987). Support for this idea has come from observations that attention and eye movements are strongly linked behaviorally (e.g., Kowler et al. 1995; McPeek et al. 1999; Sheligia et al. 1995), from fMRI recording studies of visual attention showing activation in eye-movement areas for attention tasks (e.g., Corbetta et al. 1998; Beauchamp et al. 2001; Nobre et al. 2000), from stimulation studies showing that activation of neurons in the SC, FEF, and area LIP can change the focus of attention (Moore and Fallah 2001; Müller et al. 2005; Cavanaugh and Wurtz 2004; Cutrell and Marrocco 2002), and from neurological studies of patients with attentional disorders following damage to frontal cortex, parietal cortex, or the midbrain (e.g., Posner et al. 1982, 1984; Housain and Kennard 1996; Sapir et al. 1999; Sereno et al. 2006).

However, more fine-grained analysis at the single-neuron level suggests that the subset of SC and FEF neurons showing attention-related activity is not the same as the subset of neurons thought to carry the saccadic command signal (Murthy et al. 2001; Sato and Schall 2003; Ignashchenkova et al. 2004; Juan et al. 2004; Thompson et al. 2005). Thus, the emerging hypothesis is that sub-threshold movement commands are not required to shift attention, and instead, that visual activity in the SC, FEF, and area LIP represents the salience of stimuli in the visual field (Thompson and Bichot 2005; Fecteau and Munoz 2006; Goldberg et al. 2006). Many neural models posit that spatial attention and saccades are guided by such a salience map (e.g., Koch and Ullman 1985; Itti and Koch 2000; Desimone and Duncan 1995; Wolfe 1994). Salience maps do not encode specific visual attributes, but rather, encode the behavioral significance of stimuli, mediated through reflexive or top-down mechanisms (e.g.: reflexive: Yantis and Jonides 1984; Sagi and Julesz 1987; Nothdurft 2000; top-down: Tsotsos 1995; Rao et al. 2002). Thus, under this view, bottom-up salience signals (from cortical visual areas) and top-down signals related to behavioral relevance (presumably from pre-frontal or other cortical areas) feed into salience maps, and the allocation of attention is determined by the activity in these maps. Signals from the salience maps then modulate processing at corresponding locations in visual areas, presumably via feedback pathways.

An attractive starting hypothesis is that the SC is involved in controlling reflexive attention and the FEF is involved in top-down attention (e.g., Sereno 1992). The SC is well-positioned to react to reflexive cues, because it receives short-latency visual input directly from the retina (e.g., Schiller and Malpeli 1977). Furthermore, lesions of the SC permanently abolish short-latency express saccades, while FEF lesions have a less pronounced effect on saccade latency (Schiller et al. 1987), but produce major deficits in more complex situations, including memory-guided saccades, double-step saccades, and anti-saccades (Braun et al. 1992; Dias et al. 1995; Sommer and Tehovnik 1997; Deng et al. 1986; Guitton et al. 1985). Indeed, in the anti-saccade paradigm, where reflexive responses must be inhibited, SC cells show reduced activity (Everling et al. 1999).

Background to Aim 1: Recording studies of attention in SC and FEF

Importance of behavioral measures of attention

One stumbling block in identifying the sources of attentional control signals is that single-unit studies have not always made behavioral measurements to establish that attention is being allocated as expected. Indeed, early studies in the SC and FEF concluded that neither area is involved in controlling covert attention (e.g., Wurtz and Mohler 1976; Goldberg and Bushnell 1981), perhaps because the tasks used did not sufficiently engage attention, but more recent studies have supported the idea of SC and FEF involvement in attention (e.g., Kustov and Robinson 1996; Kodaka et al. 1997; Thompson et al. 1997). In this project, we will record behavioral measures of attention in parallel with neural activity, and will correlate the neural signal with performance.

Neural correlates of attention during visual search

Attention-related activity in the FEF during visual search was shown in an elegant study by Thompson et al. (2005), who trained monkeys to maintain fixation and discriminate a pop-out stimulus presented with distractors in a visual search array. They found that the later visual responses of FEF neurons were greater for the target than for distractors, consistent with a role of the FEF in visual attention. Our experiments will build on this finding by using reflexive and top-down cues to manipulate attention in advance of the onset of the discrimination target. This scheme has several advantages: (1) it will reveal how activity differs for reflexive and
top-down attention; (2) it separates the attentional cue and the discrimination target in time, allowing us to better distinguish attention-related activity from visual responses related to target onset; (3) it allows us to vary the effectiveness of the attention cues without varying the properties of the target. This permits us to parametrically manipulate the level of attention allocated to the cell’s RF to examine the correlation between activity and attention. At the extreme, we can cue attention away from the location of the target using invalid cues. Establishing correlations between activity and behavioral performance is critical for building a convincing link between neural activity and attention, but previous single-unit studies in the SC and FEF have lacked such data.

**Distinguishing attention-related activity from visual responses to a cue**

A difficulty in isolating attention-related activity is that the visual onset of the cue itself can evoke activity. Ignashchenkova et al. (2004) examined the role of the SC in attention using a task in which a discrimination target was preceded by a valid cue (a spot of light) in the cell’s receptive field or by no cue. SC visual activity was higher when the target was preceded by a cue, suggesting an attention-related modulation. However, like the earlier results of Robinson and Kertzman (1995), these data do not clearly differentiate visual responses to the cue from cognitive activity related to attention because they compare trials in which a cue preceded the target in the RF with trials in which no stimulus preceded the target. Indeed, both studies found that in a second condition, when the attention cue was presented outside the cell’s RF while the target remained inside the RF, no increase in SC activity was observed. The authors interpreted this as evidence that the SC shows attention-related activity only for spatially-precise cues, but it could also indicate that the increased response in the original experiment was visual in nature, related to cue onset rather than attention.

Our task avoids this ambiguity by balancing the visual stimulation at cued and uncued locations. For reflexive attention, rather than using single cue and target stimuli, we use a cue array of isoluminant stimuli containing a color oddball and a target array of isoluminant achromatic target and distractor stimuli (see Fig. 17 for details). For top-down attention shifts, we use a probability manipulation that does not involve any immediate sensory cues. In both cases, the low-level visual events at cued and uncued locations are identical from the viewpoint of the SC or FEF, which are not normally color-selective (Marrocco and Li 1977; Mohler et al. 1973; Schall et al. 1995; McPeek and Keller 2002). Thus, our tasks effectively separate attention-related activity from purely visual responses.

**Background to Aim 2: Investigating the causal role of the SC and FEF in attention**

Recording studies can only reveal correlations between activity and attention. Causal experiments are needed for a direct test of how changes in activity affect attention. Microstimulation in the SC and FEF has been shown to shift spatial attention (Moore and Fallah 2001; Müller et al. 2005; Cavanaugh and Wurtz 2004), but stimulation studies cannot reveal whether SC or FEF activity is necessary for an attention shift. For example, microstimulation may cause a phosphene or other sensory transient that acts as a cue to shift attention, or it may cause preparation of a saccade, which indirectly affects attention. It is also unclear whether stimulation-evoked attention shifts engage reflexive or top-down attention (or both).

Rather than transiently imposing a new pattern of activity, temporary inactivation decreases the naturally-evolving level of activity in a relatively tonic manner, allowing one to test whether this reduced activity causes a deficit. Inactivation also has the advantage that its direct effects are limited to the inactivated region, in contrast to microstimulation, which can cause antidromic activation of brain areas providing input to the stimulated area. Thus, inactivation is better suited for dissecting the functional differences between anatomically-connected areas like the SC and FEF (Sommer and Wurtz 1998, 2001).

A necessary role for the FEF in covert attention was revealed by Wardak et al. (2006), who studied the effects of unilateral FEF inactivation on feature and conjunction search tasks. They found that reaction times to detect a target among distractors were longer after FEF inactivation, consistent with a role of the FEF in covert attention. In Aim 2, we will use inactivation to expand on this finding. First, we will use reflexive and top-down tasks to distinguish inactivation effects on these two modes of attention. Second, Wardak et al. made multiple injections in each session to inactivate a large area of the FEF, and found spatially diffuse effects. Our inactivations are more focal, and in preliminary data, we see spatially-restricted effects. Third, we will directly compare the effects of SC and FEF inactivation in the same tasks and same individual animals. Finally, our paradigm measures contrast thresholds to rigorously determine whether deficits in performance are truly due to attentional, rather than visual, deficits.

A causal effect of SC inactivation on attention is suggested by the results of Lomber and colleagues (e.g., Lomber and Payne 1996; Payne et al. 1996), who found that in cats, inactivation of the SC (like inactivation of suprasylvian cortex) reduces orienting responses to stimuli in the contralateral hemifield,
resulting in neglect-like symptoms. These studies indicate a causal role for the SC in orienting, but investigated overt orienting responses rather than covert attention, presumably due to the difficulty of measuring attention in cats. McPeek and Keller (2004) found after SC inactivation, monkeys tended to make errors in target selection for saccades, resulting in movements to distractors rather than to a target in the inactivated field. This finding is suggestive, but, again, involves overt rather than covert orienting.

The effects on covert attention of superficial SC inactivation were investigated by Robinson and Kertzman (1995) using a speeded detection task with valid and invalid cues. They found that inactivation slowed performance overall in the task, but did not find a significant change in the effectiveness of the attentional cues, suggesting that SC inactivation does not affect attention. Our proposed experiments improve on this study by using a difficult discrimination task that shows more robust cueing effects. Indeed, attention effects may be weak or inconsistent if the behavioral task is too easy (e.g., Nakayama and Joseph 1998). Furthermore, we will target intermediate- and deeper-layer sites in the SC, where inactivation causes target selection errors (McPeek and Keller 2004) and microstimulation affects attention (Cavanaugh and Wurtz 2004; Müller et al. 2005), rather than the superficial layers targeted by Robinson and Kertzman (1995). Finally, we will use a performance measure which does not depend on reaction time, eliminating a potential confound with motor preparation.

Background to Aim 3: Mechanisms for perceptual improvement by attention

In addition to investigating the signals controlling attention, we must also consider the means by which attention affects perception. Intuitively, we may think of attention as a “spotlight” that increases responses to attended stimuli. However, models of attention have postulated several different mechanisms for perceptual improvement by attention, including external noise exclusion, reduction of uncertainty in the decision process, and enhancement of the attended signal. External noise exclusion refers a change in spatial or feature tuning that filters out irrelevant (noise) stimuli, including lateral masking from distractors and masking from noise stimuli presented at the target location (e.g., Baldassi and Burr 2000; Lu and Dosher 2000; Shiu and Pashler 1994). Single-unit studies in visual cortical areas have found that attention effectively constrains visual receptive fields around the attended location (Moran and Desimone 1985), reducing the influence of distractors, and this change in spatial tuning could be considered a form of external noise exclusion. Reduction of uncertainty refers to the fact that when the target location is uncertain, sensory information from several locations must be evaluated to make a decision. Cueing reduces this statistical uncertainty, improving performance by decreasing noise in a decision stage (e.g., Palmer 1994; Verghese and Stone 1995; Morgan et al. 1998; Eckstein et al. 2002).

Signal enhancement describes an increase in sensory gain for stimuli at an attended location. Evidence for signal enhancement has been found in both psychophysics (e.g., Lu and Dosher 1998; Luck et al. 1996; Cameron et al. 2002) and single-unit studies (e.g., McAdams and Maunsell 1999; Reynolds et al. 2000; Martinez-Trujillo and Treue 2002). Two different enhancement mechanisms have been proposed: contrast gain and response gain. Contrast gain produces an effect equivalent to an increase in the contrast of the attended stimulus, resulting in a leftward shift of the contrast response function (see Fig. 2, left panel). Response gain is a multiplicative increase in response across all contrasts (Fig. 2, right panel). Contrast gain reduces threshold (C50 in Fig. 2) without affecting the saturating portion of the contrast response function. On the other hand, response gain produces its strongest effects at high contrasts, resulting in an increase in the upper asymptotic response (Rmax in Fig. 2) to high-contrast stimuli. Recording studies have found evidence for both contrast gain (e.g., Reynolds et al. 2000; Treue and Martinez-Trujillo 1999), and response gain (e.g., Williford and Maunsell 2006; McAdams and Reid 2005), and psychophysical experiments have also found evidence for both components (Morrone et al. 2004; Carrasco et al. 2004; Huang and Dobkins 2005).

Do reflexive and top-down attention recruit different mechanisms of attention? Lu and Dosher (2000) concluded while both types of attention improve external noise...
exclusion, reflexive attention provides stronger signal enhancement than top-down attention. Ling and Carrasco (2006) developed a paradigm which distinguishes between contrast gain and response gain, and found that reflexive attention enhances the signal with a combination of contrast and response gain, while top-down attention provides only contrast gain. These results add weight to the suggestion that reflexive and top-down attention are governed by different neural mechanisms, and open the possibility that different sources of control signals, such as the SC and FEF, could engage different mechanisms. Aim 3 will test whether SC or FEF inactivation affects the signal enhancement mechanism of attention, and if so, will determine whether inactivation of either structure preferentially affects contrast gain or response gain.

Clinical relevance

Disorders of attention can arise from a variety of sources, including stroke, traumatic brain injury, Parkinson’s disease, autism, and ADHD. Unilateral neglect, which is a severe form of attention deficit, is estimated to occur in 25-30% of all stroke-afflicted individuals (Corbetta et al. 2005; Pedersen et al. 1997; Appelros et al. 2002). Deficits in attention can seriously impair visual and cognitive functioning, interfere with activities of daily life, and have been associated with greater risk of accident and injury (Webster et al. 1994).

We currently have a very limited understanding of the basic mechanisms and brain areas involved in controlling visual attention. The bulk of research in this area has gone into understanding the role of parietal cortex, due to the fact that right parietal injury often causes neglect. Much less is known about the roles of frontal cortex and the midbrain, despite the fact that injury to these areas in humans also affects attention (e.g., Hellman and Valenstein 1972; Housain and Kennard 1996; Posner et al. 1982; Weddell 2004; Sapir et al. 1999; Sereno et al. 2006; Posner et al. 1985). This study will illuminate the roles of frontal cortex (FEF) and the midbrain (SC) in the control of visuo-spatial attention. In particular, our inactivation experiments may provide new insights for understanding the acute effects of injury to these parts of the brain (e.g., Rushmore et al. 2006). Gaining an understanding of the basic neural mechanisms that control attention is essential for progress in treating disorders of attention.

C. Progress Report/Preliminary Studies

The period covered is 3.25 years, from August 1, 2003 through October 31, 2006. This time included the set-up of our newly-established primate neurophysiology lab, which is now fully operational, and the recruitment and training of key lab personnel.

Although study section recommended 5 years of funding for the previous grant period, the duration was administratively cut to four years. For this reason, we postponed work on Aim 2 of the original project to concentrate on Aims 1 and 3. Progress on Aim 1, investigating the roles of the SC and FEF in saccade target selection, is described below in items 1-8. Progress on Aim 3, initiating the investigation into whether these areas are involved in spatial attention, is described in item 9 and in the subsequent preliminary studies section. In this continuation, we have focused the proposal on attention. Concentrating our efforts on attention makes the project more cohesive and will accelerate progress on this less well-understood aspect of SC and FEF function.

Completed, published projects supported or partially supported by the previous grant:

1. A causal role for the SC in saccade target selection. Earlier studies used correlational data to suggest that the SC is involved in saccade target selection. We directly tested this idea by temporarily inactivating the SC and testing saccadic performance with targets presented alone or in a search task with distractors. When a single target was presented in the inactivated field, monkeys showed minor motor impairments: smaller saccade amplitudes, lower velocities, longer latencies. If the SC were not involved in saccade target selection, the impairments in the search task should be similar. However, we found that in search, when the target is in the inactivated field, monkeys often make saccades directed to distractors rather than to the target. Control experiments showed that the deficit is not due to low-level visual or motor impairments. The results, published in Nature Neuroscience, provided the first evidence for a causal role of the SC in saccade target selection by showing that manipulation of SC activity changes the choice of saccade goals (McPeek and Keller 2004).

2. Activity in the SC related to centrally- and peripherally-cued saccade target selection. SC activity was studied while monkeys performed two saccade target selection tasks. In pop-out search, animals made saccades to an odd-colored target among distractors. In the choice response task, a central cue signaled which stimulus was the saccade target. Most SC neurons showed activity correlated with target selection in both tasks. In search, SC activity evolves over time to signal the target location. In the choice response task, cells show a transient increase in activity for the onset of the foveal cue, even though it lies outside their
response fields. Subsequent target-selective activity indicates the location of the instructed target. These results support the idea that the SC is involved in saccade target selection in both pop-out and non-popout selection tasks (Keller, Lee, and McPeek 2005).

3. **Effect of distractors on saccade trajectories in a target selection task.** This study examined how saccade trajectories are affected by distractors in a pop-out search task. Monkeys made saccades to an odd-colored target, and the location and color of the target and number of distractors were changed randomly trial by trial. Saccade latencies and the proportion of saccades to distractors decreased as the number of homogenous distractors increased. Furthermore, saccades showed more dispersion in initial direction, curvature, and averaging when distractors were present. As the number of distractors increased, latency, dispersion, and curvature decreased. We conclude that this trajectory variability results from incomplete or inaccurate specification of the target when competing stimuli are present, and that a smaller number of widely-spread distractors results in greater variability due greater difficulty of target selection (Arai, McPeek, and Keller 2004).

4. **Competition during target selection in the SC results in saccade curvature.** We examined the neural correlates of saccade curvature in the SC during a pop-out search task. We found that saccades which ended near the target but curved toward a distractor were accompanied by increased pre-saccadic activity of SC neurons coding the distractor site. To determine whether this activity is causally related to curvature, monkeys made saccades to visual stimuli without distractors, and we stimulated sites in the SC corresponding to distractor sites in search. Stimulation was sub-threshold for evoking saccades, but when its temporal structure mimicked the activity recorded for curved saccades, the subsequent movements to the visual target showed curvature toward the stimulation site. These results support the hypothesis that the increased saccade curvature observed in search arises from rivalry between target and distractor goals, consistent with the idea that the SC is involved in the competitive neural interactions underlying saccade target selection (McPeek and Keller 2003).

5. **The role of distractor-related activity in FEF for saccade curvature in a target selection task.** Based on work in the SC, we speculated that saccade curvature arises when a movement is initiated before competition between the target and distractor goals has been resolved. To test this hypothesis, we recorded FEF activity for curved and straight saccades in search. In contrast to the SC, activity in FEF is normally poorly correlated with saccade dynamics. However, the FEF, like the SC, is involved in target selection. Thus, if curvature is due to incomplete target selection, we expect to see its neural correlates in FEF. We found that saccades that curve toward a distractor are accompanied by an increase in peri-saccadic FEF activity at the distractor location, and saccades that curve away are accompanied by a decrease in activity. To establish that this activity is causally related to curvature, we applied sub-threshold microstimulation to FEF sites before saccades to single targets. When the stimulation train resembled the activity recorded for curved saccades, resulting movements curved toward the stimulation site. These findings support the idea that saccade curvature results from incomplete suppression of distractor-related activity throughout the network of areas involved in target selection (McPeek 2006).

Manuscripts in preparation supported by the previous grant:

6. **Comparison of the effects of SC and FEF inactivation on saccade target selection.** Earlier studies have indicated an important role for the FEF in saccade target selection. We investigated the consequences of temporary chemical inactivation of the FEF to determine whether this area plays a necessary role in target selection. We found that FEF inactivation results in target-selection deficits in a search task, similar to what we observed for SC inactivation. Control experiments ruled out simple visual or motor explanations of this target-selection deficit. Although the effects of SC and FEF inactivation were similar in most respects, performance after SC inactivation grew worse with more homogenous distractors, while the opposite trend was seen for FEF inactivation. Overall, the results indicate that both the SC and FEF play causal roles in target selection, but suggest that the selective mechanisms in the SC depend more strongly on inhibition among nearby stimuli. These results have been reported in abstract form (McPeek 2004), and a manuscript is in preparation.

7. **Effects of FEF inactivation on top-down selection of saccade targets.** Our earlier work demonstrated that FEF inactivation impairs saccade target selection in pop-out search (McPeek 2004). Here, we tested the effects of FEF inactivation in a target selection task in which the goal is indicated by the shape of a foveal cue. After FEF inactivation, performance in this top-down selection task was disrupted when the target was in the
inactivated field, even though the instructional cue was located outside the inactivated field. This indicates that FEF inactivation decreases the probability that a target in the inactivated field will be selected as a saccade goal, regardless of whether selection is based on properties of the target or on an abstract cue located elsewhere. Data collection for these experiments is complete, and analysis is nearing completion.

8. Latency of visual responses in the SC and FEF. Comparing visual response latencies in different brain areas provides valuable information constraining the pathways through which visual information could flow. We found that distinct sub-populations of SC neurons, defined both by depth and response properties, differ in their latencies to register the onset of visual stimuli. Superficial phasic visual neurons have the shortest onset latencies, followed by tonic visual neurons, located approximately 0.5-1 mm deeper. Onset latencies of intermediate-layer visuo-motor burst neurons form a later latency distribution that is almost non-overlapping with the visual neurons. Deeper-layer buildup neurons have the longest and most variable latencies, which are virtually indistinguishable from those of FEF neurons. Data collection and analysis for these experiments is complete, and a manuscript is in preparation.

9. Psychophysical studies of exogenous and endogenous attention in monkeys. We studied attentional visual search in monkeys by training them to maintain fixation and discriminate the orientation of a target embedded in distractors. When the target color differed from the distractors (pop-out condition), performance was independent of the number of distractors. When target and distractor colors were identical, performance was worse with more distractors. We also found that performance improved when a spatial cue was presented before target onset, and that this cueing effect followed a transient time-course. Performance could also be influenced by manipulating the probability of the target appearing at a given location (Lee and McPeek 2006). These experiments are finished, and a manuscript is nearing submission.

Preliminary Studies for Aim 1: SC and FEF activity related to reflexive and top-down attention shifts

1.1. SC activity related to reflexive attention

We have recorded preliminary data from SC neurons during our reflexive attention task (described in detail in Section D, Fig. 17) with valid cues. Responses were compared when attention was cued into the cell's response field (RF) vs. out of the RF. An example cell is shown in Figure 3. At time zero, a cue array consisting of one red cue among five green non-cue stimuli is presented for 100 ms. The red and green stimuli are isoluminant and identical except for color. In each trial, either the cue or a non-cue stimulus appears in the cell's RF, and attention is reflexively drawn to the odd-colored cue (e.g., Constantinidis and Steinmetz 2005). After the cue lead time (667 ms in this example), a second array consisting of the target and distractors is briefly presented, followed by a high-contrast masking stimulus (not shown). The target is a Landolt-C stimulus with a vertically-aligned gap and the distractors are Landolt-C stimuli with horizontally-aligned gaps (see Fig. 3). Monkeys report the target orientation (up or down) by making a saccade to one of two choice stimuli, which are located above and below fixation. Discrimination of the target is better at the cued location and worse at an uncued location, when compared with a neutral condition in which all elements of the cue array are the same color (Lee and McPeek 2006).

The earliest phase of the cell's activity does not depend on whether attention is cued into or out of its RF: in both cases, we see a brief transient increase due to the onset of a red or green stimulus in the RF. Soon
after, activity increases when attention is cued into the RF. During the 667 ms cue lead time before the target array is presented, activity gradually declines, but is consistently greater when attention is cued into the RF than when it is cued out. After the cue lead time, the onset of the target array produces another transient activity increase. About 400 ms later, the fixation point is extinguished, instructing the monkey to report the perceived orientation of the target by making a saccade to one of two choice stimuli. There is no change in activity during this response period because the choice stimuli are located outside the cell’s response field.

To understand how SC and FEF activity correlates with attention, we measured mean activity during a period from 70 ms before to 30 ms after target array onset (gray box in Fig. 3). We call this the “pre-target period,” because it encompasses SC activity just before the visual response to the target array. The justification for choosing this period is that perception of a briefly-presented target is improved when attention is focused at the target location. If we hypothesize that SC cells participate in the moment-to-moment control of attention, then SC activity near the time of target onset, immediately before target-related signals arrive, should be related to the level of attention in the cell’s RF. For this cell, we see significantly greater pre-target activity when attention is cued into the RF than out (t-test: P < 0.001). As shown in Figure 4, this difference is highly significant across the population of SC cells we have recorded (paired t-test: P < 0.001).

This attention-related activity cannot be explained as a simple preference for red cue stimuli over green ones: it continues to be seen when the colors of the cue and non-cue stimuli are reversed, such that attention is drawn to a green cue among red stimuli. We also explicitly test the color selectivity of each cell by measuring activity for single red and green stimuli randomly presented in the RF.

Relating SC activity to perceptual performance in the reflexive attention task

If the SC is involved in attention, then a failure of attention (resulting in more errors in the discrimination task) should accompanied by lower SC activity. For cells with a sufficient number of trials, we compared pre-target activity for correct vs. incorrect trials when attention was cued into the cell’s RF. Activity was significantly lower across the population of cells, although the magnitude of this decline was modest. One problem with this analysis is that there could be reasons for an incorrect response besides a failure of attention, such as a failure to associate the target orientation with the corresponding choice stimulus or a failure to remember an intended response during the delay between the target and the signal to respond. Better data could be obtained if we directly manipulated the level of reflexive attention.

To accomplish this, we took advantage of the transient time-course of reflexive attention, described in the Background and shown in Figure 1. If SC activity is related to attention, then activity should change over time consistent with this behaviorally-measured time-course. To test this, we varied the cue lead time. In preliminary experiments, we tested cue lead times of 293 ms, 667 ms, and 1000 ms in separate blocks. These times were chosen to place the earliest cue-lead time just beyond the peak of the attentional benefit curve, and the other times further along the function’s downward slope (Fig. 1). Neural and behavioral data for an example cell are shown in Figures 5-6. As expected, discrimination performance declines for longer cue-lead times (Fig. 6, upper; likelihood ratio test, P < 0.05). Pre-target activity shows a corresponding decline for trials in which attention is cued to the cell’s RF (Fig. 6, lower, red trace; linear contrast, P < 0.001). When

Figure 5. Activity of one SC neuron for 3 different cue lead time conditions. Time zero corresponds to onset of target array. Dashed vertical line (not shown in lower panel) marks onset of 100-ms duration cue array, occurring 293 ms, 667 ms, or 1000 ms before target array in upper, middle, and lower panels, respectively. Red solid and green dashed traces denote attention in vs. out of RF, respectively. Gray area shows 100-ms pre-target interval used in analyses.

Figure 6. Upper panel: Discrimination performance for three different cue lead times. Lower panel: Mean pre-target activity of SC neuron in the same trials. Red trace: attention cued into RF; Green dashed: attention cued out of RF.
Principal Investigator/Program Director (Last, First, Middle): McPeek, Robert M

attention is cued away from the RF, there is little change in activity across the three cue lead times (dashed green trace; $P = 0.83$). These results are consistent with a correlation between SC activity and the level of attention directed to the cell’s RF.

1.2. FEF activity related to reflexive attention shifts

In separate testing sessions, we have recorded preliminary data from FEF neurons in the same individual animals performing the same reflexive attention task used for the SC recordings. The activity of an example FEF visuo-movement cell is shown in Figure 7, for three different cue-lead times. The activity of this cell is strongly modulated according to whether attention is cued into the cell’s RF or out of the RF. Furthermore, across the different cue-lead times, the pre-target activity of the cell shows a decline consistent with the transient time-course of reflexive attention.

1.3. FEF activity related to top-down attention shifts

We have also collected preliminary data examining the neural correlates of top-down attention shifts in the FEF. In these experiments, the array of targets and distractors is not preceded by any explicit sensory cues for attention (e.g., Fig. 16). Instead, attention is cued by varying, across different blocks, the probability that the target will appear at a given location in the target array. In agreement with earlier work (e.g., Ciaramitaro et al. 2001), we have collected extensive behavioral data showing that monkeys can use this information to shift top-down attention, resulting in perceptual improvement (Lee and McPeek 2006; see Fig. 8). Since target location probability is learned over time, when we switch the probability, we first conduct a block of training trials. From experience, we have found that learning of a new set of probabilities in our task reaches asymptote after 20-40 training trials. Here, we analyze data from blocks of test trials which immediately follow the training blocks.

An example FEF cell is shown in Figure 9. For this cell, we collected data in two conditions: in one condition, the target appeared in the cell’s RF in 75% of trials and in an opposite hemifield location in 25% of trials; in the other condition, the probabilities were reversed. The cell’s activity prior to the target array was higher when the probable target location was in the RF vs. in the opposite hemifield (t-test on pre-target activity: $P < 0.001$). Importantly, this activity difference cannot be visual in nature, because it is present well before the onset of the target when the animal is simply fixating and awaiting the target array. However, it is related to the locus of attention, as measured by perceptual performance, which is better at the more probable target location.

1.4. SC activity related to top-down attention shifts

Preliminary SC results are very different from the FEF results. We have recorded 88 SC cells in the same animals performing the same top-down attention task, and have not found a single SC neuron with activity related to top-down attention, even among cells with robust activity for reflexive attention. A typical cell is shown in Figure 10. This cell is essentially non-responsive during the pre-target period, regardless of the target location probability. Thus, while activity in the SC and FEF is similar for reflexive shifts of attention, we see very different patterns for top-down attention cued by location probability. This dissociation provides strong evidence for different neural mechanisms underlying reflexive and top-down attention shifts.
Preliminary Studies for Aim 2: Do the SC and FEF play necessary roles in reflexive and top-down attention shifts?

2.1 Effects of FEF inactivation on reflexive attention

We conducted preliminary experiments to determine whether the FEF plays a necessary causal role in reflexive shifts of attention. We tested reflexive attention immediately before inactivation, during muscimol inactivation of FEF, and after recovery from inactivation (the next day).

Mapping the extent of the inactivation effect across the visual field

To assay the spatial extent of the inactivation effect, we first tested performance in a memory-saccade task (see Section D for details). Memory saccades are very sensitive to FEF inactivation (Dias and Segraves 1999; Sommer and Tehovnik 1997), and we measured the endpoint error of memory saccades to the six discrimination target positions used in the attention task as well to the locations of the two choice stimuli. The upper panel of Figure 11 shows mean endpoint error as a function of target position, for a typical site. In order to compare data across sites, we normalized target position according to the field location represented by the injection site (Fig. 11, lower panel). Thus, we designated the 0° position as the center of the region coded by the injection site. Positions further to the right on the abscissa correspond to locations shifted progressively further in direction from the center of the inactivated region. The first three positions are contralateral to the injected FEF, while the second three are ipsilateral. After FEF inactivation, we see deficits in memory saccades at the location corresponding to the injection site, and progressively smaller effects at other contralateral locations. For ipsilateral locations, we see no change in performance, and nor do we see an effect for vertical saccades to the choice stimuli (far right of Fig. 11), which are represented bilaterally. Injection sites were chose to avoid effects on vertical saccades.

Effects of FEF inactivation on reflexive attention

To determine the effects of inactivation on reflexive attention shifts, we measured pre-injection, post-injection, and recovery performance in the reflexive cueing task described above (and in detail in Section D, Fig. 17). In 67% of trials, the color-oddity cue is valid, drawing attention to the future location of the target. In the other 33% of trials, the cue is invalid, drawing attention to a location diametrically opposite the target location. In some preliminary experiments, we measured percent correct discrimination of the target at each target location. In other experiments, we measured the contrast threshold needed to discriminate the target with 75% accuracy at each target location using separate interleaved staircases for each location and each cue type (valid vs. invalid). We found a similar pattern of results using either performance measure.

Results averaged across 10 sites measuring percent correct are shown in Figure 12. Target location has been normalized as for the memory saccade task. For valid cues (left panel), after FEF inactivation there is a clear drop in performance at center of the injection site and at the neighboring location (0° and 45° contra; likelihood ratio test: P < 0.01). At more distant locations, there is no change in performance. For the randomly-interleaved invalid trials (right panel), attention is drawn away from the target location. Because the target array is presented very briefly, there is little time to locate the target and re-orient attention away from the invalid cue toward the target. Thus, performance in this invalid condition is
worse than in the valid condition, and likely represents the performance that can be attained without significant focal attention at the target location (e.g., Lee et al. 1999). Importantly, however, performance with invalid cues is significantly better than chance level (P < 0.01). Moreover, there is no deficit with invalid cues after FEF inactivation, showing that when attention is already withdrawn from the target location, FEF inactivation does not produce an additional impairment in perception. The lack of a deficit here indicates that the deficit seen for the valid condition cannot be due to a low-level visual impairment, such as a decrease in contrast sensitivity, because a low-level problem should affect perception regardless of where attention is cued. Instead, we see a deficit only in the valid condition, when attention should have been drawn to the inactivated location. This is consistent with an impairment in shifting attention into the inactivated field.

A final point of interest is the change in performance in the invalid cue condition when the cue is presented in the inactivated field and the target is presented in the opposite location (far right data point of Fig. 12). Here, we see a small, but significant, improvement in performance after inactivation (P < 0.05). This is consistent with the hypothesis that FEF inactivation reduces the ability of peripheral cues to shift attention into the inactivated field. In this case, the cue is misleading, and consequently, by impeding an attention shift away from the target, inactivation improves performance.

2.2 Effects of SC inactivation on reflexive attention shifts

We have collected similar preliminary data with SC inactivation, using the same task in the same individual animals. Here, we estimated the spatial extent of the SC inactivation by measuring the peak velocity of memory-saccades to various locations (not shown). In previous work we have found that peak velocity is a sensitive measure of SC inactivation (McPeek and Keller 2004). Figure 13 shows data pooled across 2 injection sites in the reflexive attention task. The results parallel what we found for the FEF: when a valid cue appears in the inactivated field, discrimination performance is worse after SC inactivation, suggesting that the cue is less effective in summoning attention. With invalid cues, we see no change in performance at the inactivated site, confirming that the deficit in the valid condition is not due to a low-level visual impairment, which would affect performance regardless of where attention is cued. Finally, we see a slight improvement in performance when an invalid cue is presented in the inactivated field, further supporting the interpretation that SC inactivation renders peripheral cues in the inactivated field less effective in summoning attention.
2.3 Effects of SC inactivation on top-down attention shifts

We have also collected preliminary data investigating the effects of SC inactivation on top-down shifts of attention. Based on the recording experiments, we predict that SC inactivation will not cause a deficit in the top-down task, while FEF inactivation will. In these pilot experiments, the basic attention task without peripheral cues was used, but there were only two potential target locations, one at the location represented by the injection site, and the other at the diametrically opposite location in the other hemifield. Five distractors were always presented as before.

Top-down attention was cued by manipulating target probability as in the recording experiments. Using probabilities of 75% in one location and 25% in the other location, psychophysical results show that monkeys perform better at the more probable target location, while performance at the less probable location is similar to performance when all six target locations are equally probable (e.g., Fig. 8). After a probability switch, 40 training trials are conducted to familiarize monkeys with the new probabilities. In these experiments, we measured percent correct target discrimination.

Figure 14 shows preliminary results averaged across two SC inactivation sites. If inactivation affects top-down attention, then we expect performance to decline from pre-injection to post-injection when the probable target location is in the inactivated field. At the same time, we should see either no change in performance for the opposite location (or possibly a slight improvement when the opposite location is improbable). In Figure 14, left panel, we see that for targets in the inactivated field, there is little or no change in performance after SC inactivation, indicating that inactivation does not impair a top-down shift of attention into the inactivated field. In Figure 14, right panel, we see that performance in the intact hemifield is also unaffected by SC inactivation. These results support the hypothesis, based on the recording data, that the SC does not play a significant role in top-down shifts of attention.

Preliminary Studies for Aim 3: Do the SC and FEF engage different mechanisms of attention?

To test broadly for an attentional deficit resulting from SC or FEF inactivation, Aim 2 uses a task in which performance could be affected by deficits in any of several attention mechanisms. The presence of distractors and masking stimuli means that inactivation effects found in Aim 2 could involve deficits in external noise exclusion or uncertainty reduction, as well as in signal enhancement.

To specifically probe the effects of SC and FEF inactivation on the signal enhancement mechanism of attention, we minimized spatial uncertainty and external noise using a paradigm developed by Ling and Carrasco (2006). We modified our basic discrimination task, such that the target was randomly presented in one of two isoeccentric locations left or right of fixation. To obtain psychometric functions, target contrast was randomly selected trial-to-trial from 9 levels. Importantly, even at the lowest contrast levels, the target was always well above detection threshold, so there was little uncertainty about target location. External noise was minimized by presenting the discrimination target without distractors or masks. To distinguish contrast gain and response gain components of signal enhancement, asymptotic performance (at high contrasts) must be below ceiling so that changes in R_max can be measured (see Fig. 2). Consequently, the target size was set so that performance at the highest contrast was below ceiling. Ceiling was determined in a separate block that measured performance at each location with a large, high-contrast Landolt-C target.

Reflexive attention was cued by presenting a 0.5° black square abutting one of the target locations for 53 ms at a cue lead time of 150 ms. The target was presented for 50 ms. The cue was valid in 67% of trials and invalid in 33%. In separate blocks, top-down attention was cued by manipulating target location probability, as in previous experiments. The location probabilities used were 67% and 33%.

Results are shown in Figure 15. For both reflexive and top-down attention shifts, performance was better with valid cues, indicating that attention enhances the target signal in both tasks, consistent with human results (Ling and Carrasco 2006). To distinguish contrast gain from response gain, we fit the Naka-Rushton contrast response model (Albrecht and Hamilton 1982; see Section D for details) to the psychometric functions in order to compare the R_max and C50 parameters. We also directly compared the two models by modifying the Naka-Rushton function to produce a contrast gain model, a response gain model, and a mixed model by...
adding up to two attentional parameters which changed contrast gain and response gain, respectively (see Fig. 2 and Section D; Ling and Carrasco 2006; Martinez-Trujillo and Treue 2002). Parameter estimates from the original Naka-Rushton fits to the invalid cue data were used for these modified functions, and only the attentional parameter(s) were allowed to vary to fit the valid condition data.

For the reflexive attention task, the response gain model fit the data better than the contrast gain model (permutation test: P < 0.02), and the mixed model was superior to response gain alone (likelihood ratio test: P < 0.05). For the top-down task, the contrast gain model provided a better fit than the response gain model (permutation test: P < 0.05), and the mixed model was no better than the contrast gain model in predicting the cueing effects (likelihood ratio test: P > .1). These results, similar to those reported in humans by Ling and Carrasco (2006), indicate that reflexive attention engages both the contrast gain and response gain components of signal enhancement, while top-down attention engages only contrast gain.

If SC and FEF inactivation have no effects in this task, but cause deficits in Aim 2, then it would imply that inactivation selectively affects other attentional mechanisms, such as external noise exclusion or uncertainty mechanisms, without affecting signal enhancement. On the other hand, if inactivation impairs signal enhancement, we will determine whether SC and FEF inactivation have different effects on contrast gain and response gain. The fact that response gain is observed for reflexive, but not top-down, attention, suggests that the SC may provide the signal that selectively engages response gain, while the FEF provides a signal related to contrast gain for both modes of attention shifts.

In earlier work, we tested the effects of SC and FEF inactivation on contrast thresholds (McPeek and Keller 2004; McPeek 2004), but these experiments used randomized target locations with no attentional cues. Thus, effects of inactivation on the attentional signal enhancement mechanism would not have been seen. However, these experiments established that SC and FEF inactivation do not produce a low-level visual (non-attentional) impairment in contrast sensitivity.

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D. Research Design and Methods

General Methods. Rhesus monkeys (Macaca mulatta) implanted with a scleral eye coil, recording chambers to access the SC and FEF, and a head restraint, are used as experimental subjects. During experiments, the monkeys are seated in a primate chair with their heads restrained, in a dim, sound-attenuated room. Operant techniques with liquid rewards are used. Eye position is monitored with a magnetic coil system (CNC Systems) and sampled at 1 kHz. Experimental control, data acquisition, and presentation of visual displays are carried out by a custom real-time Matlab program on a Macintosh G4 computer using the Psychophysics Toolbox (Brainard 1997; Pelli 1997). Visual stimuli are presented on a 29-inch color CRT in synchronization with the monitor's vertical refresh. The monitor has a spatial resolution of 800 by 600 pixels and a non-interlaced refresh rate of 75 Hz. The monitor is positioned 35 cm in front of the monkey and allows stimuli to be presented in a field of view of approximately ± 30° along the horizontal meridian and ± 28° along the vertical meridian. Accurate control of stimulus contrast is achieved using a video card with 10-bit resolution for each color channel, and calibrating the monitor with a Minolta CS-100 spectrophotometer.

Neural recording and electrical microstimulation. Isolated neurons are recorded using commercial tungsten microelectrodes (FHC, Inc.) with impedances ranging from 0.8 – 2.5 M ohms at 1 kHz, lowered into the brain by hydraulic microdrives or by the Plexon NAN multi-drive. A 16-channel Plexon MAP system amplifies and bandpass filters the signals, and identifies action potentials of 1-4 individual neurons from each electrode. Local field potentials (0.7 Hz-300 Hz) are digitized at 1 kHz, and bandpass-filtered spike waveforms are digitized at 40 kHz and saved to disk. Spike occurrences are registered with a resolution of 1 kHz, and are stored with the behavioral measurements. Spike-sorting is verified offline using the Plexon Offline Analysis software. Bipolar, constant-current electrical microstimulation pulses (0.25 ms pulse width) are delivered using
a computer-controlled optical stimulus isolation unit (FHC) connected between the stimulating electrode and guide tube. Our standard pulse train frequency is 400 Hz in the SC and 300 Hz in the FEF.

**Microinjections.** Injections sites are chosen based on the presence of saccade-related activity and the ability to evoke saccades with low threshold (< 50 μA) stimulation. We deliver microinjections of muscimol (FEF: 1 μl, 5 μg/μl; SC: 0.5-1 μl, 0.5-1 μg/μl) using a custom-built "injectrode" which combines an injection cannula with a microelectrode for recording and microstimulating at the injection site. The design is modified from Chen et al. (2001), and has a smaller diameter than most commercially-available devices, reducing the impact on neural tissue and allowing the injectrode to pass through a 23 gauge guide tube. The cannula is connected by Teflon tubing to a Hamilton syringe driven by a digital infusion pump (Harvard Instruments). The system is filled with sterile saline and back-filled with the injection agent. A small bubble separates the injection agent from the saline, and movement of the bubble is measured to determine the injected volume. Injections are made at a rate of 500 nl/min. "Dummy" injections of saline, matched in volume to the inactivating agents, are made at a few sites to verify that the observed effects are due to chemical inactivation of neurons. Muscimol is a GABA<sub>A</sub> agonist which inhibits cell activity but does not affect fibers of passage.

**Behavioral tasks.**

**Memory saccade task.** During an initial interval of 450-650 ms, monkeys fixate a fixation point in the central position. At the end of this interval, a target stimulus is presented at a peripheral location while the fixation point remains illuminated. After 150 ms, the target is removed and the monkeys continue fixating for an additional 500-700 ms. At the end of this "memory" period, the fixation point disappears and monkeys are rewarded for making a saccade, within 70-500 ms, to the location where the target stimulus had been presented. The target is a red or green disc at a contrast of 74% on a homogenous background of 14 cd/m<sup>2</sup>. Target size is scaled according to the cortical magnification factor to keep its salience constant across different eccentricities (Rovamo and Virsu 1979). At an eccentricity of 15°, the target subtends 2°.

**Basic discrimination task.** The task is illustrated in Figure 16. After monkeys fixate a central fixation point for 1.2-2 sec, a "target array," consisting of one target and five distractors, is presented for 80-133 ms (depending on the monkey's proficiency in the task). The target is a Landolt-C with the gap aligned either upwards or downwards, and the distractors are identical to the target except for the horizontal orientations of their gaps. In each trial, the target randomly appears at one of six isoecentric locations, arranged such that one location lies at the center of the RF of the cell being recorded (or site being inactivated). The target and distractors are achromatic (67% contrast). After presentation of the target array, high-contrast random-dot masking stimuli (99% contrast) are shown at all six locations for 240 ms. After a delay of 333-533 ms, the target is removed and monkeys report the target orientation by making a saccade to one of two choice stimuli. After incorrect responses, the screen goes blank and there is a 0.5-2 s time-out before the next trial. If a saccade is made elsewhere or made too early, the trial is immediately aborted. The size of the target array stimuli in this task (and in the subsequent tasks) is scaled according to the cortical magnification factor (Rovamo and Virsu 1979). At an eccentricity of 10°, the target and distractors typically subend 2.5°, although size could be changed depending on the monkey's level of proficiency. Monkeys are trained for 6-8 months using different array and choice stimuli positions until they are proficient and their performance is stable.

**Reflexive cueing task.** See Figure 17. Trials proceed as in the basic task, but after an initial fixation duration of 0.8-1.1 sec, a cue array is presented, consisting of six boxes centered at the six possible target locations. Five of the boxes in the cue array (the non-cue stimuli) are identical. The sixth (the cue) is isoluminant with the others (76% contrast), but differs in color. The diameter of the cue and non-cue stimuli is adjusted to be slightly larger than the size of the target. In the valid condition, the odd-colored cue is presented
at the target location. In the invalid condition, the odd-colored cue is presented at the location diametrically opposite the target location. After 100 ms, the cue array is removed. A delay follows, which depends on the cue lead time (defined as the interval from the onset of the cue array to the onset of the target array). Cue lead time is varied in separate blocks from 0-1000 ms. With a 0 ms cue lead time, the cue array and target array are presented simultaneously. After the delay, the target array is presented and the trial proceeds as described for the basic task. Training in this task occurs after training in the basic task, and cueing effects are evident from the first day of training. Stable performance is obtained after a few weeks.

Top-down cueing task. Trials are identical to the basic task (Fig. 16), except that top-down attention is cued by manipulating the probability of the target appearing at a given location. The properties of the target array are unchanged, but we use only two target locations, one in the center of the RF of the recorded cell (or injection site) and the other in the opposite hemifield at the diametrically opposite position. Distractors are presented at the 5 non-target positions in each trial. The probability of the target appearing in one of the two locations is manipulated in different blocks of trials, typically using 0%, 25%, 33%, 50%, 67%, 75%, or 100% probability in a block. When the target appears at the likely position, it is considered a valid-cue trial, and when the target appears at the unlikely position, it is considered an invalid-cue trial. The 50% condition is considered a neutral condition. Target location probability is held stable for 80-100 trials. The first 40 trials after each change in probability are discarded. In preliminary testing, we have found that learning of a new target location probability reaches asymptote in fewer than 40 trials. Training in this task occurs after training in the reflexive cue task. Cueing effects are seen in the first day of training, and stable performance is obtained after a few weeks.

Data analysis. Unless indicated otherwise, all analyses consider only correct trials. Offline data analysis is performed in Matlab using custom software. Saccades are detected using velocity and acceleration criteria, and trial are visually inspected to verify correct marking of saccades. To generate continuous spike-density functions, recorded neural events are convolved with a gaussian kernel (sigma = 4-10 ms; Richmond and Optican 1987).

1. Measuring RFs and classifying neurons. Upon isolating a cell, we will first determine the position and size of its RF using the memory saccade task. Since the SC and FEF contain several different sub-populations of cells, we will classify the cell by its responses in the memory-saccade task, which dissociates visual, delay period, and saccade-related activity. The presence or absence of significant changes in activity during these epochs will allow us to identify each neuron as phasic visual, tonic visual, phasic visuo-movement, tonic visuo-movement, or purely movement-related (e.g., McPeek and Keller 2002 - see appendix; McPeek 2006).

2. ROC analysis of neural activity. Signal detection theory (Green and Swets 1966) is used to quantitatively analyze discrimination at the single-neuron level (e.g., Bradley et al. 1987; Britten et al. 1992; Thompson et al. 1996; McPeek and Keller 2002 - see appendix). We compare the activity of each neuron when attention is cued into the cell's response field vs. to the opposite location with ROC analysis. The area under the ROC curve, which we will refer to as "ROC area," provides an unbiased estimate of the separation between the distribution of firing rates when the cue is in the response field vs. the opposite field. When ROC area is 0.5, the two distributions are completely overlapping. ROC area increases or decreases to a maximum of 1.0 or minimum 0, which indicate non-overlapping distributions. Thus, ROC area will describe how well a neuron's discharge discriminates whether attention is cued into or out of its RF. If we assume that for each neuron, there is a similar "anti-neuron" (Britten et al. 1992) which discharges for attention shifts to the opposite location, the brain could theoretically weigh the outputs from the neuron and anti-neuron to determine which location has been attentionally selected. Two specific analyses using this technique are described below.

3. Onset of differential activity related to attention. To determine the earliest time at which an SC neuron discriminates the cued location, based on ROC analysis.

Figure 17. Reflexive attention cueing task. Trial sequence is similar to the basic task, but a cue array precedes the target array. Attention is reflexively drawn to the odd-colored cue.

Figure 18. Determination of earliest time at which an SC neuron discriminates the cued location, based on ROC analysis.
earliest time when neurons discriminate the cued location ("discrimination time"), we will use a method developed by Thompson et al. (1996) and Sato et al. (2001) to study saccade target selection (also see appendix: McPeek and Keller 2002). Trials are aligned on the presentation of the cue array. ROC curves are constructed at each millisecond using the spike densities of in-field and opposite-field trials (see Fig. 18). Over time, as the neuron begins to discriminate the cued location, ROC area shifts away from 0.5. To extract discrimination time, we will adopt criteria used by Sato et al. (2001), who defined it as the earliest time at which ROC area surpasses the 0.75 level, and remains above this level for at least 10 ms of the subsequent 15 ms. In preliminary analyses, results are not critically dependent on these parameters.

4. Level and statistical significance of differential activity. In addition to analyzing the time-course of discrimination, we will also measure how well neurons discriminate the attended location during the pre-target period (70 ms before to 30 ms after target onset). The justification for choosing this period is that perception of a briefly-presented target is improved when attention is focused at the target location. If we hypothesize that these neurons participate in the moment-to-moment control of attention, then the time around target onset should be critical. By ending the analysis window before visual responses to the target begin, we avoid mixing target-onset related activity with attention-related activity in our analyses. We will measure the number of spikes in each trial during this pre-target period and construct a single ROC curve based on activity for trials in which attention is cued into vs. opposite the RF. The degree to which the cell's activity during this time distinguishes the cued location will be measured as the area under this ROC curve. Significance of this value (relative to the 0.5 baseline) will be evaluated using a permutation test (Britten et al. 1996). We will measure ROC area, rather than the difference in activity or a t-test ratio, because we want to relate the level of neural discrimination to performance. Comparing ROC area with performance is natural, because both are expressed as probability measures: ROC area represents the probability that the neuron correctly discriminates the attended location, while percent correct indicates the probability that the task is performed correctly.

5. Determination of visual latencies using Poisson spike train analysis. For some analyses, we will identify bursts of visual activity in neurons using a Poisson spike train analysis described in Hanes et al. (1995), as modified by Thompson et al. (1996). This analysis is applied to spike trains from individual trials to identify the first period during which more spikes occurred than expected based on a Poisson random process. The onset time is recorded, and the modal onset is computed for each cell (Thompson et al. 1996) to estimate the latency for the cell to register the onset of a visual stimulus in its RF.

6. Measurement of contrast thresholds using a staircase. In some experiments, we will use a staircase procedure to measure contrast thresholds. The threshold for 75% correct performance is obtained by decreasing contrast after three correct responses and increasing it after one incorrect response. Contrast steps, range, and starting point are determined in preliminary testing. Threshold will be estimated for each target location in each cueing condition from the reversals of the respective staircase (Falmagne 1985). Error estimates for thresholds will be generated using bootstrap procedures applied to the reversals, and thresholds in different conditions of individual experiments will be compared using permutation tests applied to the reversals (Effron and Tibshirani 1994).

7. Naka-Rushton Contrast response function. Psychometric functions are fit via maximum-likelihood to the Naka-Rushton contrast response model (e.g., Fig. 15; Albrecht and Hamilton 1982):

\[
\text{resp} = \frac{\text{R}_{\text{max}} \cdot C^n}{C^n + C_{50}^n} + M.
\]

where resp is % correct performance, C is contrast level, C50 is contrast at half saturating response (threshold), Rmax is the asymptotic level at which the function saturates, n is the slope of the function, and M is performance at the lowest contrast.

For the fits, threshold (C50), slope (n), and asymptote (Rmax) are allowed to vary.

8. Modified contrast response functions. To examine contrast gain and response gain components of attentional enhancement, we modified the Naka-Rushton function by adding an attention parameter: N (Martinez-Trujillo and Treue 2002). Values for the Rmax, C50, and n parameters are obtained by fitting the invalid cue data to the original Naka-Rushton function. Effects of attention are measured by fixing these parameters and allowing only the attentional parameter (N) to vary to fit the valid cue data. A mixed model incorporates two attention parameters (Ling and Carrasco 2006): N1, controlling response gain and N2, controlling contrast gain. Likelihood ratio tests are used to compare the mixed model to the individual models.

Response gain model: \( \text{resp} = N_1 \cdot \frac{\text{R}_{\text{max}} \cdot C^n}{C^n + C_{50}^n} + M \)

Contrast gain model: \( \text{resp} = \frac{\text{R}_{\text{max}} \cdot N \cdot C^n}{N \cdot C^n + C_{50}^n} + M \)

Mixed model: \( \text{resp} = N_1 \cdot \frac{\text{R}_{\text{max}} \cdot N_2 \cdot C^n}{N_2 \cdot C^n + C_{50}^n} + M \)
Methods specific to Aim 1: Do the SC and FEF contain signals appropriate for controlling reflexive and top-down attention shifts?

**Rationale:** This aim will determine whether the SC and FEF contain neural signals appropriate for controlling reflexive and top-down attention shifts, and will determine how these signals differ in the two structures. If neural signals control attention, then they should fit the following criteria: (1) they should occur after cueing and prior to target onset; (2) they should be correlated with the level of attention in the RF; and (3) they should not critically depend on the specific physical features of the cue stimuli. We will also determine the extent to which attention-related and saccade-related activity is segregated by determining whether the same cells carry both types of signals.

**Experiment 1.1: SC and FEF activity related to reflexive attention shifts**

Isolated SC or FEF neurons will be recorded in a variety of conditions using the reflexive attention task (Fig. 17). Our standard task will use valid cues with a cue lead time of 293 ms, but other conditions will also be used, as described below.

1. **Latency of visual and attention-related activity.** If a neuron is involved in controlling attention, then it should discriminate the cued location prior to the onset of the discrimination target. In preliminary experiments, we found that initial responses to the cue array did not discriminate cued from uncued locations, but a slightly later phase of activity did (e.g., Fig. 3). We will estimate when each neuron discriminates the cued location ("discrimination time," see Data Analysis #3 and Fig. 18) to determine if it occurs before target onset. We will also compare discrimination times in the SC and FEF, and among different classes of cells in each area. Determining which structure first signals the cued location will provide insight into how information related to reflexive attention flows between these areas. We will also determine the latency of each cell to visual onsets in its RF (Data Analysis #5). Comparing visual latency with discrimination time will allow us to estimate the additional processing time for color-oddity information to reach the cell.

2. **Correlation of activity with attention and behavioral performance.** If the SC and FEF are involved in controlling attention, then cell activity should correlate with the level of attention in the RF. We will parametrically manipulate attention by taking advantage of the transient time-course of reflexive attention (e.g., Mackeben and Nakayama 1989). Cells will be tested with valid cues at cue lead times of 0 ms, 150 ms, 293 ms, 667 ms, and 1 sec in separate blocks. We expect that behavioral performance will follow the pattern found in the preliminary data (Fig. 1; Lee and McPeek 2006), indicating a systematic change in the level of attention for different cue lead times.

   For each cue lead time condition, we will quantify how well each neuron discriminates the cued location, based on its pre-target activity (Data Analysis #4). If neural activity is correlated with the level of attention in the RF, then we expect ROC area to increase monotonically with increasing discrimination performance. To measure the relationship between neural discrimination and performance, we will plot performance vs. ROC area across different cue lead times (e.g., Fig. 19). If there is a systematic relationship, it will be quantified with a linear (or non-linear) fit. The fit parameters (e.g., slope) will provide an index of how well neural activity predicts performance. The distribution of slopes for the SC and FEF will be compared to determine how strongly each area is related to reflexive attention.

3. **Robustness of cue-related activity.** If activity is related to attention, then it should not depend critically on the physical features of the cue array. Moreover, the above analyses assume that SC and FEF cells are non-selective for the colors of the cue and non-cue stimuli. We will test color selectivity in every cell by comparing responses in the memory-saccade task for randomly-intermixed red and green targets having colors and luminances identical to those of the cue array stimuli in the attention task. Differences in activity for red vs. green stimuli will be quantified and significance assessed (Data Analysis #4). In a subset of cells, we will also record in the attention task while changing the cue array in different blocks: (1) We will systematically reverse the colors of the cue and non-cue stimuli in alternate blocks; (2) we will change the duration of the cue array so that it appears and stays on during presentation of the target array, or make its duration very short (40 ms); (3) we will present an array of six identical non-cue stimuli during initial fixation, and change the colors of all but one in order to cue attention to the (color oddball) unchanged stimulus. Psychophysical studies suggest that reflexive attention behaves similarly in each of these conditions (e.g., Nakayama and Mackeben 1989). If SC and FEF
activity is related to attention, then we should see pre-target activity consistent with the monkeys' attentional performance in each of these conditions (Data Analysis #4). Ideally, ROC area and performance in each condition would fall along the cell's function relating performance and ROC area (e.g., Fig. 18) derived from Analysis #2 of this Experiment.

4. Activity for neutral cues and at uncued locations. Does activity decline when attention is cued away from a cell's RF? Behavioral data indicate that, relative to a neutral condition, monkeys perform better when attention is validly cued to the target location and worse when attention is invalidly cued away from the target (e.g., Lee and McPeek 2006; Golla et al. 2005). This suggests that attention is not only focused at the cued location, it is also removed from uncued locations. This analysis will determine the extent to which cueing leads to a decrease in activity for cells coding uncued sites. We will measure activity in blocks of trials in which a valid cue is presented in 67% of trials, and a neutral cue (all cue array stimuli the same color) in 33% of trials. To keep the number of trials tractable, we will not include an invalid cue condition for most cells, bearing in mind that valid-cue trials in which attention is cued away from the cell's RF will give essentially the same information about neural discharge. In a few cells, however, we will use cueing conditions of 60% valid, 20% invalid, and 20% neutral so that behavioral performance in each cue condition can be measured in parallel with recordings.

We will compare each cell's firing rate in the neutral condition with its activity when attention is cued either toward or away from the RF. If neurons reflect a removal of attention from uncued locations, then we expect their activity to decline, relative to the neutral condition. The latency of this decline (Data Analysis #3) and the significance of the decline during the pre-target period (Data Analysis #4) will be determined.

6. Neural responses to the target array. How are sensory responses in the SC and FEF affected by attention? We will examine the visual activity elicited by the target array, to determine the extent to which this visual response is modulated by attention, by comparing mean discharge 75-175 ms after target array onset when attention is validly cued into vs. out of the RF (Data Analysis #4).

7. Overlap of saccade- and attention-related activity. Are attention- and saccade-related signals present in the same cells or are they segregated in different sub-populations of cells? The correlation of each cell with attention is measured in Exp. 1.1, Analysis #2. The effects of attention on sensory responses are measured for each cell in Exp. 1.1, Analysis #6. We will compare the distribution of these two measurements for visual vs. movement-related cell classes in the SC and FEF to determine which cell classes could be controlling attention, and which cell classes show the effects of attention in their sensory responses.

Experiment 1.2: SC and FEF activity for top-down attention shifts

We will use the top-down task to examine whether neural activity in the SC and FEF is appropriate for controlling top-down attention. We will examine (1) the timing of attention signals to determine whether they are appropriate, and (2) the correlation of activity with performance at different levels of attention. Here, target location probability serves as the cue to control attention. The assumption is that proportionally more attention is allocated to the more probable location (e.g., Posner et al. 1980; Ciaramitaro et al. 2001), as confirmed in preliminary results (Fig. 8). When referring to each probability condition, we will indicate the probability of the target appearing in the cell's RF (i.e., "the 75% condition" means that the target appears in the RF in 75% of trials and outside the RF in 25%). For ROC analyses, neuron/anti-neuron pairs for attention cued in vs. out of the RF are generated from the activity of the neuron in appropriate matched pairs of probability conditions. The 100% condition, which corresponds to attention cued into the RF, and the 0% condition, which corresponds to attention cued out of the RF, form one matched pair. Another matched pair is formed by the 75% and 25% conditions, which correspond to a weaker level of attention in the RF vs. out of the RF, respectively.

1. Timing of attention-related activity. Is the timing of top-down attention signals appropriate? We will determine whether discrimination of the cued location occurs before target onset (Data Analysis #3), which would be required if the signals controlled attention.

Interestingly, our preliminary data in the top-down task show a build-up in FEF activity shortly before target onset (Fig. 9). This suggests that monkeys shift attention in anticipation of the target array. To investigate this anticipatory timing, for some cells we will test monkeys using two different delays between fixation and target array. First, we will test in the 100% and 0% probability conditions with the delay fixed at 800 ms. Then, we will re-test both probability conditions using a longer 1500 ms delay. We expect that monkeys will learn the delay along with the probabilities in the training blocks. We will compare discrimination time in the two delay conditions (Data Analysis #3). If monkeys use timing information for top-down attention shifts, then we expect neural discrimination of the probable target location to occur earlier in blocks with short delays and later in blocks with long delays.

2. Correlation of activity with top-down attention and performance. If the SC or FEF are involved in
controlling top-down attention, then we predict a correlation between activity and the level of attention in the RF. The level of attention directed to the RF is parametrically manipulated using different probability conditions. We will measure the reliability and significance of neural discrimination of the attended location during the pre-target period (Data Analysis #4) using activity from matched pairs of probability conditions (see above). We expect to see greater ROC area in the higher probability condition, commensurate with better performance for targets in the RF. The relationship between performance and ROC area will be plotted and analyzed as in Exp. 1.1, Analysis #2 (Fig. 19).

3. **Neutral and invalid cue activity.** Does activity decline when top-down attention is cued away from a cell's RF? This analysis is analogous to Exp. 1.1, Analysis #4. We will compare activity in an improbable condition (0% or 25%) with activity in a neutral condition (50%). If neurons reflect a removal of attention from the improbable location, then we expect activity to decline, relative to the neutral condition. The latency (Data Analysis #3) and significance of this decline during the pre-target period (Data Analysis #4) will be measured.

4. **Visual responses to the target array.** Are visual responses to the target array modulated by top-down attention? This analysis is similar to Analysis #6 in Experiment 1.1. We will compare mean activity 75-175 ms after target array onset for the 100% and 0% conditions, corresponding to attention in the RF vs. attention out of the RF, and will estimate the extent to which the cell's visual response to the target array is modulated by attention (Data Analysis #4).

5. **Overlap of reflexive and top-down attention-related activity.** Are reflexive and top-down attention signals present in the same cells or in different subsets? This analysis builds on Analysis #7 in Exp. 1.1, which measures, for each cell, the correlation of pre-target activity with reflexive attention and the modulation of visual responses by reflexive attention. Here, we will obtain corresponding measures from Exp. 1.2, Analyses #2 and #4. We will compare the distribution of these measures for the two modes of attention in cells of different classes in the SC and FEF. This will reveal whether signals for both modes of attention are present in the same cells, or are segregated.

For cells having both top-down and reflexive activity, we will compare the relationship between ROC area and performance across the two modes of attention. To do this, we will create a plot, similar to Figure 19, that superimposes ROC area vs. performance for both modes of attention. If the two data sets overlap, it would suggest that a given level of neural discrimination is associated with a particular level of attention, regardless of whether it is cued reflexively or top-down. This would argue against the idea of a strict anatomical dissociation between reflexive and top-down attention in the studied brain region.

**Potential difficulties for Aim 1**

**Data collection.** Comparing results between the SC and FEF and among cell classes in each structure will require recording many neurons. To reduce the amount of data that must be collected, cells which clearly don't have attention-related responses will be tested in a limited set of conditions. Whenever possible, we will record in each structure on alternate days to minimize differences in animals' performance for data from the two structures.

**Data analysis.** In this proposal, we have relied on ROC analysis to construct functions relating activity to performance. However, it is possible that better results will be obtained using some other measure of neural modulation, such as the difference in activity for attention in the RF vs. out. Along similar lines, we have selected specific analysis times based on theoretical assumptions that may turn out the be incorrect. This study is among the first to relate SC and FEF activity to attentional performance. Therefore, we will be flexible in our analysis methods and will consider alternatives to these specific methods when indicated by the data.

**Interpretation of cue-related activity.** In interpreting these experiments, we speculate that reflexive or top-down cue-related activity represents a salience signal that shifts attention to the cued location, and modulates processing in visual areas via feedback connections. However, on the basis of these data alone, several other interpretations are possible. Since attention increases the gain of sensory signals, it is possible that increased SC or FEF activity at attended locations is an effect of attention on sensory signals, rather than a signal that controls attention. We try to differentiate these two types of modulation by separately considering pre-target activity and responses to target onset. Indeed, pre-target activity in our task is measured during a period in which cells are not responding to a stimulus in their RFs, suggesting an increase in this activity is not simply an amplification of sensory signals. However, an increase in spontaneous firing rate could also be an effect of attention (e.g., Luck et al. 1997; Reynolds et al. 2000). Another alternative is that changes in activity could be unrelated to attention, and simply be due to covert planning of a saccade to the cued location. While recording evidence cannot rule this out, saccades to cued locations are rare in our task, and when they occur the trial is immediately aborted. Indeed, the only rewarded saccades are those to the choice stimuli, which lie outside the RFs of the recorded cells. Moreover, saccade planning could not explain why attention-related
activity would be correlated with performance in the attention task, and why this activity would follow the characteristic time-course of reflexive attention.

However, the bottom line is that recording data cannot definitively determine whether activity is causally involved in controlling attention. Aim 2 addresses the crucial issue of causality using temporary inactivation. The value of the recording experiments lies in their ability to measure cell-specific activity with high temporal resolution, to reveal how attention-related activity unfolds over time and how it is related to performance, to determine whether reflexive and top-down attention shifts engage the same or different populations of cells, and to determine whether cells with attention-related activity are also active for saccades.

Methods specific to Aim 2: Do the SC and FEF play necessary roles in reflexive and top-down attention shifts?

**Rationale:** These experiments will investigate whether the SC and FEF play essential roles in reflexive and top-down attention by determining how performance is affected by temporary focal inactivation of either structure. We will compare results from each area to determine how they differ in their contributions to attention. If preliminary results are borne out, we expect to find a dissociation between the SC and FEF for top-down shifts, providing strong physiological evidence that these two modes attention are processed, at least in part, via different neural pathways, and providing insight into the functional differences between the SC and FEF.

In this aim, we use a task in which performance could be affected by deficits in any of several attention mechanisms, including external noise exclusion, reduction of uncertainty, and signal enhancement. This allows us to test broadly for an attention deficit arising from SC or FEF inactivation. More fine-grained testing of specific attention mechanisms will be conducted in Aim 3.

**Experiment 2.1: Effects of SC or FEF inactivation on reflexive attention**

**Attention task.** We will measure contrast thresholds in the reflexive attention task (Fig. 17) with 67% valid and 33% invalid cues using a staircase procedure. Invalid cues are always presented at the location diametrically opposite the target location. Valid and invalid cues will be randomly intermixed within each block of trials. Preliminary results show robust cueing effects under these conditions (e.g., Figs. 12, 13). Thresholds will be collected for five or six target locations across the visual field using randomly interleaved staircases, one for each combination of location and cue type (valid vs. invalid).

The use of contrast thresholds and a staircase has two advantages over a percent correct measurement for inactivation experiments: first, if we measured percent correct, monkeys would be rewarded less frequently for target locations where performance is worse, potentially causing a shift in the animals' strategy. With the staircase, as each staircase converges on threshold, the probability of obtaining a reward will be the same (75%) for all locations and conditions. Second, in preliminary experiments we have found contrast-threshold data to be more stable than percent correct data.

**Mapping spatial extent of inactivation effect.** To measure the extent of the inactivation effect across the visual field, we will measure saccadic performance in the memory-saccade task. In a separate block of trials, memory saccades will be made to the target locations and to the choice stimulus locations. For FEF inactivation, we will measure the change in saccade endpoint error for each location pre- and post-injection, and after recovery. For SC inactivation, we will measure peak saccade velocity. We have found that memory saccades are quite sensitive to inactivation of either the SC or the FEF, with the largest effects on saccade endpoint for FEF inactivation, and on saccade velocity for SC inactivation (McPeek 2004).

The purpose of measuring memory-saccade performance is two-fold: first, it will independently assay the efficacy of the injection and reveal its spatial extent through its effects on saccades to the locations used in the attention task. Second, it will allow us to verify that saccades to the choice stimuli are unimpaired. If we detected an impairment in saccades to the choice stimuli, we would abort the experiment and exclude the data.

**Inactivation procedure.** After the injectrode has reached the injection site, and the region of the visual field that it represents has been identified by recording and stimulation, we will collect pre-injection data in the memory-saccade and attention tasks. Following this, muscimol will be infused (see Inactivation Methods). During inactivation, the tasks will be repeated. Recovery data is collected on the following day.

**Analysis:** For individual inactivation experiments, we will compare pre- vs. post-injection thresholds and pre- vs. recovery thresholds at each location using a permutation test (Data Analysis #6), with a Bonferroni correction. Pre- vs. post-comparisons will reveal the effects of inactivation. Pre- vs. recovery comparisons will provide an indicator of normal variability across testing sessions. Across sites, we will use linear contrasts (Rosenthal et al. 2000), which are similar to t-tests, to individually test at each location the significance of the statistical hypothesis that the pre- and recovery thresholds are equal, and the post-injection threshold is worse.
Contrasts will be Bonferroni-corrected. We will use two-tailed tests to estimate the significance of increases or decreases in performance.

**Expected results:** Our prediction is that deficits in attention will spatially coincide with movement deficits observed for memory saccades. Preliminary experiments suggest that after inactivation, reflexive cues presented at the injection site are less effective in drawing attention to the cued location (Figs. 12, 13). If this is true, then we expect worse performance in the valid cue condition when the cue and target are presented in the inactivated part of the field, and better performance when an invalid cue is presented in the inactivated field and the target is presented in the opposite position.

In earlier work, we directly tested contrast sensitivity across the visual field to establish that inactivation of the SC or FEF in monkeys does not cause a low-level visual impairment (McPeek and Keller 2004; McPeek 2004). Since this point is critical for interpreting the current experiments, we will reinforce it with an additional analysis of the reflexive attention data. To test for the presence of a visual deficit, we will compare thresholds when the discrimination target is presented in the inactivated field, and attention is cued to the opposite side by an invalid cue. If the decrease in performance that we see at the inactivated location with valid cues is due to a low-level visual impairment, then we should see a similar deficit when the target is in the inactivated field and attention is cued away (invalid condition). In preliminary data, we see no change in performance in this condition (Figs. 12, 13). This would indicate that the results are not due to a low-level visual deficit in the inactivated field, because a low-level deficit should reduce performance regardless of where attention is cued. The lack of a deficit in the invalid condition for targets in the inactivated field would imply that when attention has already been withdrawn from the target location, inactivation of the SC or FEF does not impose an additional penalty on performance.

**Experiment 2.2: Effects of SC or FEF inactivation on top-down attention**

**Procedure and analysis:** This experiment will assess the effects of SC or FEF inactivation on top-down shifts of attention. We will follow the same procedure as in Experiment 2.1, except that the top-down attention task will be used. We will test in the 75/25% and 25/75% probability conditions, which produce robust top-down cueing effects in preliminary experiments (Figs. 8, 14). The two probability conditions will be tested in separate blocks preceded by blocks of 40 training trials. Contrast thresholds will be measured (Data Analysis #6) at the two target locations in each probability condition, using interleaved staircases. Thresholds will be compared pre- and post-injection, and after recovery, both in individual experiments and across injection sites, as in Experiment 2.1.

**Expected results:** Based on the recording experiments, our hypothesis is that FEF inactivation will affect top-down attention, but SC inactivation will not. If inactivation causes a deficit in shifting top-down attention into the inactivated field, then we expect performance to decline for targets in the inactivated field when it is the probable target location (valid condition). On the other hand, we expect little or no deficit for targets in the inactivated field when it is the improbable target location (invalid condition). In this condition, attention will presumably have been shifted to the probable location in the intact field. Indeed, in the normal animal, the brief duration of the target array prevents re-orienting toward a target presented in the improbable location, leading to a level of performance similar to what is seen with invalid cues in the reflexive task (Lee and McPeek 2006). Thus, we do not expect inactivation to cause a significant deficit when the improbable location is in the inactivated field. On the other hand, when the probable location is in the inactivated field but the target is presented in the intact hemifield (invalid condition), an impairment in shifting attention into the inactivated field might cause an improvement in performance, as for the invalid condition in Experiment 2.1 (e.g., far right data point of Figs. 12, 13). Comparing the pre-injection and recovery thresholds will give an indication of the stability of the data.

**Potential difficulties for Aim 2**

**Visual effects.** It is important to distinguish attentional effects of inactivation from purely visual effects. Our task accomplishes this by measuring discrimination performance in the inactivated field in the invalid condition. It should be noted that the invalid condition in our task is very different from the invalid cue condition in the classic Posner paradigm (e.g., Posner 1980). In Posner’s task, subjects have unlimited time to detect the onset of a supra-threshold target, and reaction times in the invalid condition measure the ability to re-orient attention away from an invalid cue. In our task, the target is presented very briefly with distractors, and the task is designed so that the target disappears and is masked before subjects can locate it and re-orient attention away from an invalid cue. Thus, invalid-cue performance in our task represents the performance that can be attained without significant focal attention at the target location (e.g., Lee et al. 1999; Golla et al 2004).

If the decline in performance after inactivation is due to a low-level visual deficit, then the deficit should...
also be seen with invalid cues, because a low-level deficit should should be present regardless of where attention is cued. If we do find evidence of a visual deficit, it might prove interesting for understanding how SC or FEF contributes to vision, but it would create difficulties in teasing apart attention deficits and visual deficits. On strategy would be to compare the extent of the deficit for targets in the inactivated field with valid vs. invalid cues. The latter could give an estimate of the deficit from visual causes and the former, an estimate of the combined deficit.

However, we think this outcome is unlikely because in earlier work, we directly tested contrast sensitivity across the visual field in the absence of attentional cues, and established that inactivation of the SC or FEF in monkeys does not cause a low-level visual impairment (McPeek and Keller 2004; McPeek 2004). Moreover, preliminary data from the experiments proposed here show no change in performance for targets in the inactivated field in the invalid cue condition, indicating the absence of a visual deficit.

Motor effects. Our task requires monkeys to saccade to two choice stimuli. If inactivation affected saccades to one or both choice stimuli, it would cause a drop in performance. We deal with this potential problem in several ways: first, we have some flexibility in where the choice stimuli are positioned, and we will position them away from the region represented by the inactivation site. (Note that choice stimulus location is held constant for the pre-, post- and recovery phases of each individual experiment.) Second, since the choice stimuli are located near the vertical meridian, we will avoid injection sites that represent near vertical visual field locations. This is also important because locations near vertical are bilaterally represented, so unilateral inactivation of a vertical site would be expected to be less effective, and therefore, might lead to a spurious negative result. Third, we will explicitly test execution of saccades to the choice stimuli using the memory saccade task, which is very sensitive to inactivation. If we did find evidence of impairments for these saccades, then the data from that session would be discarded. Finally, we note deficits in saccades to the choice stimuli would reduce performance for all cue and target locations. Thus, it is unlikely that a motor deficit could produce results that would be mistaken for a spatially-localized attention deficit like those seen in preliminary results (Figs. 12, 13).

Order effects. In the top-down attention task (Exp. 2.2), valid and invalid trials at each location will come from different blocks. This could prove troublesome if performance varies widely across blocks during the course of the experiment. In preliminary experiments, this has not proved to be a problem (e.g., Fig. 14), but we will interleave blocks of the different probability conditions throughout testing and compare performance in blocks with the same probability taken at different points during the session to gauge variability. If necessary, order effects could be minimized by counterbalancing the order of the probability conditions across sites.

Frustration effects. A common worry in inactivation studies is that if performance worsens, the animal may become frustrated, leading to a downward spiral. We effectively avoid this problem through the use of a staircase procedure, which ensures that performance stabilizes near 75% correct in all conditions.

Methods specific to Aim 3: Do the SC and FEF engage different mechanisms of attention?

Rationale: The primary goal of this aim is to determine whether SC and/or FEF inactivation affects the signal enhancement mechanism of attention. We will isolate the signal enhancement mechanism using a task developed by Ling and Carrasco (2006) which minimizes external noise and spatial uncertainty. If SC or FEF inactivation does not affect signal enhancement, it will indicate that deficits in attention observed in Aim 2 are likely due to failures of other mechanisms, such as external noise exclusion or uncertainty reduction, and would indicate that the SC and/or FEF are necessary for engaging some mechanisms of attention, but not for signal enhancement.

The task also distinguishes the contrast gain and response gain components of signal enhancement. Thus, if inactivation affects signal enhancement, we will determine whether SC or FEF inactivation preferentially affects contrast gain or response gain. In particular, the hypothesized selective involvement of the SC in reflexive attention suggests that it may provide the signal that engages response gain, which is present for reflexive attention shifts, but absent from top-down shifts (e.g., Fig. 15), while the FEF may engage contrast gain, which is present for both modes of attention.

Experiment 3.1: Effects of SC and FEF inactivation on the signal enhancement mechanism of attention

Task and procedure: As in the earlier experiments, the task is to report the orientation of a vertically-aligned Landolt-C target. Monkeys fixate, and the target is presented for a duration of 53 ms at one of two isoeccentric positions in opposite hemifields. Target location is randomized trial to trial, and a peripheral cue appears 150 ms before the target for a duration of 53 ms. The cue is located adjacent to one of the two target locations, and validly cues the target location in 67% of trials. In the other 33%, it invalidly cues the opposite
location. To reduce possible lateral masking noise, the cue will consist of a single black square at the cued location, rather than a cue array. The size of the cue is 0.5° at an eccentricity of 10°, and is scaled according to the cortical magnification factor (Rovamo and Virsu 1979).

The contrast of the Landolt-C target is randomly varied trial-to-trial from 10% to 70% in seven log-spaced steps to obtain psychometric functions of percent correct performance vs. contrast for valid and invalid cues. In a separate block of saccade trials, we verify that monkeys can reliably detect and saccade to the Landolt-C target in either location at the lowest contrast tested. This ensures that the target is always above detection threshold, minimizing target location uncertainty. External noise is minimized by presenting the target without distractors or masks. The size of the target is the same in every trial, and is selected in preliminary experiments such that, at the highest contrasts, percent correct discrimination performance asymptotes at a level that is below the monkey’s absolute performance ceiling for the task. Keeping asymptotic performance below ceiling is important for revealing the signature of response gain, which is an increase in $R_{\text{max}}$ (see Fig. 2). Rather than assuming that 100% correct performance is the monkey’s ceiling, we will empirically determine ceiling in a separate block of trials that measures performance at each location with a large, high-contrast Landolt-C target. In other respects, the experiments will follow the procedures used in Aim 2, Experiment 2.1.

**Analysis:** Psychometric functions relating contrast and percent correct performance will be constructed for each condition and location, and the Naka-Rushton contrast response model will be fit (Data Analysis #7). To test for differences between conditions, we will use a nested hypothesis test, which tests separate fits for each condition vs. one fit for both conditions collapsed together (Mood et al. 1974). For individual sites, significance of differences between the C50 and $R_{\text{max}}$ parameters (see Data Analysis #7, Fig. 2) will be evaluated using a permutation test (Good 2000). Across sites, significance will be tested using t-tests.

Modified Naka-Rushton functions will be used to evaluate the presence or absence of contrast gain, response gain, or both (see Data Analysis #8). The fitted attention parameter(s) from the models will provide estimates of the magnitude of contrast gain and/or response gain in each condition. Likelihood ratio tests will be used to determine whether the mixed model is superior to the individual models.

**Expected results:** Pre-injection, we expect reflexive cues to engage both response gain (higher $R_{\text{max}}$) and contrast gain (lower C50), consistent with the preliminary data (Fig. 15). Post-injection, we expect a deficit in response gain after SC inactivation and response gain after FEF inactivation for valid cues in the inactivated field. This would indicate that the two areas engage different components of the signal enhancement mechanism, both of which contribute to the improvement in performance with reflexive attention.

In the invalid condition, we expect to see no change in performance, pre-injection vs. post-injection, for targets in the inactivated field, due to the fact that attention is already removed from the target by the invalid cue (similar to the preliminary results for Aim 2). In the ipsilateral field, we may observe an improvement in performance in the invalid cue condition, as seen in the preliminary results for Aim 2. For we expect this improvement to involve response gain for SC inactivation and contrast gain for FEF inactivation.

**Experiment 3.2: Effects of SC and FEF inactivation on signal enhancement mechanisms of top-down attention**

**Task and procedure:** These experiments will proceed as in Experiment 3.1, except that visual cues are not presented. Top-down attention is manipulated by setting the probability of the target appearing at each location to either 67% or 33% in separate blocks preceded by training trials.

**Analysis:** The analysis is performed as in Experiment 3.1. The probable target location corresponds to the valid condition, and the improbable location corresponds to the invalid condition.

**Expected Results:** In the intact animal, we expect to find that top-down attention changes contrast gain, but not response gain. If the FEF engages contrast gain, then we expect FEF inactivation to reduce contrast gain in this task for validly-cued targets in the inactivated field. Inactivation might also improve contrast gain for invalidly-cued targets in the ipsilateral field, similar to the improvements seen in Aim 2. If the SC is not involved in top-down attention, then SC inactivation should have no effect.

**Potential difficulties for Aim 3**

Potential problems here are similar to those in Aim 2. Potential visual and motor effects will be dealt with in the same fashion as in that aim. One new potential problem is that too much data might be needed to collect the psychometric functions. Preliminary studies without inactivation suggest that this will not be a problem (e.g., Fig. 15), but if it is, we will use an alternative strategy. Although we prefer to obtain full psychometric functions due to the additional information provided, we could achieve our goals with fewer trials if we (1) tested at a few high contrast levels to determine $R_{\text{max}}$ (asymptotic performance); and then (2) used a staircase to determine C50 (threshold). This alternative approach could also be used if monkeys showed...
evidence frustration after inactivation, because it involves testing (1) in the easiest conditions (high contrast); and (2) with a staircase, where reward frequency is held roughly constant.

**Negative result.** If we obtain deficits in Aim 2, but no deficits here, it will indicate that inactivation does not impair signal enhancement, but it will not tell us which other attention mechanisms are impaired. However, such a result would also be quite interesting in itself, because it would provide the first evidence for a dissociation in the neural substrates controlling different mechanisms of attention. Furthermore, we could perform follow-up experiments to test other mechanisms. For example, external noise exclusion could be tested by adding varying amounts of masking noise to the target (e.g., Lu and Dosher 1998). We have highlighted the signal enhancement mechanism in this proposal simply because of our novel hypothesis that the SC and FEF could separately control different components of it, not from a lack of interest in testing other mechanisms of attention.

**Timetable:**

Five years of support will be required to complete the project. Training of monkeys for these experiments is a lengthy process, so we will overlap training of new monkeys with experiments in trained animals. Animals can only be used in a limited number of inactivation experiments, so six different monkeys will be required to complete Aims 2 and 3. Two of these monkeys will also participate in Aim 1, along with one additional monkey devoted to Aim 1, resulting in a grand total of seven animals.

**Year 1:** Recording studies in Aim 1 will be performed in two monkeys.

**Year 2:** Recording experiments in an additional animal will be completed, along with data analysis. The results will be submitted for publication. Training of animals for Aim 2 will begin.

**Year 3:** Training of animals for Aim 2 will be completed and the Aim 2 inactivation studies will begin.

**Year 4:** Completion of Aim 2. The results will be analyzed and submitted for publication. Training of monkeys for Aim 3 will begin.

**Year 5:** Inactivation studies in Aim 3 are completed, the results are analyzed, and the findings are submitted for publication.

**E. Human Subjects:** Not applicable.

**F. Vertebrate Animals:**

1. All experimental protocols are approved by the Institutional Animal Care and Use Committee at The Smith-Kettlewell Eye Research Institute and comply with the guidelines of the Public Health Service policy on Humane Care and Use of Laboratory Animals. A total of seven male rhesus monkeys (*Macaca mulatta*) will be used for these experiments over the five-year project period.

2. We require nonhuman primates for our experiments because the attentional capabilities of lower animals are limited. While rhesus monkeys do not have as much flexibility in attention as humans, they possess enough to make them a good model for simple attentional control. Moreover, it has been shown that the basic visual, attentional, and oculomotor systems in the rhesus monkey are similar to those of humans. Anatomical, and more recently, functional MRI studies have shown that similar neural structures and functions are present in eye movement and attentional control regions of the brains of both species. Therefore, much of what we learn about functional brain organization for eye movements and vision can be directly applied to understanding human eye movement and visual disorders. In addition, data on the anatomical coordinates of brainstem and cortical structures has been accumulated in the rhesus monkey for over 20 years. It is also our experience that monkeys of this particular species perform better on the training paradigms than other species of macaques.

We have restricted the number of animals used to be the minimum number that is consistent with obtaining statistically reliable experimental results that can be published in high quality scientific journals. Our every effort will be spent attempting to reduce the number of animals used in these experiments without compromising the health or psychological well being of the animals. Aim 1 will require the use of three animals.
for recording experiments. Aim 2 will require three animals for combined inactivation experiments. Aim 3 will require two animals for inactivation experiments.

3. Monkeys will be housed under his supervision. These facilities are operated under the supervision of a consulting veterinarian. The animal care staff under his supervision provide daily care and observation of the animals. We generally order two monkeys of similar age and sex at a time so that they can be paired in a double cage with a retractable door that opens into a shared playroom area between the two cages. If animals are socially compatible, they are allowed to spend time in the playroom together.

4. Aseptic surgical procedures will be carried out in a dedicated surgical suite to implant recording chambers, an eye coil, and a head restraint device. A dedicated, licensed Anesthetist induces anesthesia, and monitors and records physiological variables. Monkeys will be allowed free access to water, but no food the night prior to scheduled surgery. On the morning of surgery, the animal will be given an anticholinergic drug (atropine sulfate, 0.08 mg/kg, i.m.) to prevent excess salivation during the surgery. One-half hour later, the animal will be sedated with ketamine hydrochloride (12 mg/kg) given i.m. Monkeys will be endotracheally intubated and anesthetized with gas (Isofluorane) to effect. Depth of anesthesia and condition of the animal will be monitored throughout the surgery by a dedicated anesthesia technician. An intravenous catheter will be inserted in a leg vein and a continuous slow-rate IV drip of lactated Ringer's solution will be given, at a baseline level of 10 ml/kg/hr, adjusted to compensate for additional fluid loss, if necessary. A prophylactic antibiotic dose will be given approximately 30 minutes prior to the first skin incision, and at least once intraoperatively. Throughout the surgery, core body temperature, heart rate and respiration will be continuously monitored. The animal will rest on a heating pad connected through a feedback circuit to a rectal thermometer, to prevent hypothermia. Surgical procedures are as follows. Eye coil: a small coil of Teflon insulated, stainless steel wire is placed under the conjunctiva of one eye and, typically, sutured to the sclera. The conjunctiva is sutured and the eye coil lead wires routed out of the orbit and under the skin to the top of the head, where they are soldered to an electrical connector, embedded in an acrylic matrix, and secured to the skull by screws. Recording chambers: the animal will be placed in a stereotaxic frame, and the head scrubbed and draped. The skull will be exposed with a midline incision and cleaned of periosteum. Holes will be located stereotaxically and trephined into the skull, taking care to avoid damage to the dura mater. The chambers will then be cemented over the openings using bone cement or dental acrylic that is secured to the skull by bone screws. The chambers will be filled with sterile saline and capped with tight-fitting caps. Head restraint: animal will be placed in a stereotaxic frame, and the head scrubbed and draped. Part of the skull will be exposed, and mounting holes located and drilled with a surgical drill. A lightweight head fixation device will be affixed with bone screws, and the scalp closed to approximate the implant, minimizing the risk of post-operative infection. The device will mate with a matching post attached to the primate chair to restrain the head of the animal during experiments. The animal is returned to its home cage after waking from the anesthesia and is allowed to recover fully from the effects of surgery before behavioral training starts. During the period of post-surgical recovery, the animal is monitored closely and analgesics are used according to the recommendations of the consulting veterinarian (typically buprenex, 0.01-0.02 mg/kg, i.m. for 2-4 days, or longer if signs of pain or distress are observed).

After recovery, the animal is trained to climb voluntarily out of its cage into a Lexan primate chair. This is done by supplying the animal with rewards of fruit and juice. The chair has a perch with an adjustable height on which the animal sits. The animal is transported to the investigator's laboratory inside the closed chair and the chair is placed in the experimental setup in the laboratory. The animal is trained by the delivery of water or fruit juice rewards to perform behavioral tasks. Animals are typically scheduled for training sessions for five weekdays during which time they are allowed to work until satiated each day. Fluid intake is monitored and maintained according to guidelines established by the IACUC. Animals are allowed free access to water in their cages over the weekend. Daily records are kept of the animal's weight, and if an animal drops below 90% of its pre-surgical weight, it is removed from liquid restriction and the training is halted until its weight again rises above 90%.

When the animal has been fully trained, the experiments begin. Neurons are recorded with sterile microelectrodes advanced through the chambers implanted on the head. Recording sessions last 3 to 4 hours and are usually conducted 5 days a week. After each session the animal is returned to its home cage. In injection experiments, in place of the microelectrode, we insert a small sterile metal cannula of similar size as the microelectrode. This cannula allows minute (500 – 1250 nanoliter) injections of a chemical (muscimol, 0.5 – 5 ug/ul) which temporarily inactivate neurons in a small area of neural tissue. The inactivation effect is painless and monkeys show no outward signs that they are even aware that an injection has been made. The effects of injection wear off within 6-8 hours. During the experimental sessions, the animals' heads are restrained.
through the use of an implanted socket which is held in a matching rod attached to the primate chair. We do not believe that the head restraint produces undue discomfort from our experience of observing animals in the restraint. They continue to train steadily for the period of time that they are in the restraint and can even fall asleep briefly as they sit in the darkened room between blocks of trials. Neural recordings or inactivation experiments are typically continued for a period of 6-8 months depending on the data obtained and the site being studied.

5. At the conclusion of experiments in each animal, focal electrolytic marking lesions are made at sites of interest (20 μA anodal current for 30 sec). Animals are sedated with ketamine (12 mg/kg) and then administered a lethal overdose of Nembutal (100 mg/kg, i.v.), consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. They are then perfused through the heart with 1 liter of cold 0.1% buffered saline rinse followed by 3 liters of cold 10% buffered formalin fixative using a peristaltic pump. The head is removed and placed in the same fixative for two weeks with the skull and dura opened. Tissue blocks are removed under stereotaxic control and cryoprotected through a graded series of phosphate buffered sucrose solutions before 50 μm sections are cut on a freezing microtome to allow localization of tracks and micro-anatomical landmarks.

G. Select Agent Research: Not applicable.

H. Literature Cited:


Thompson KG and Bichot NP. A visual salience map in the primate frontal eye field. Prog Brain Res147: 251-262, 2005.


I. Multiple PI Leadership Plan: Not applicable.

J. Consortium/Contractual Arrangements: Not applicable.

K. Resource Sharing: Not applicable.
L. Consultants: Not applicable.
CHECKLIST

TYPE OF APPLICATION (Check all that apply.)

☐ NEW application. (This application is being submitted to the PHS for the first time.)

☐ REVISION/RESUBMISSION of application number:__________________________

(This application replaces a prior unfunded version of a new, competing continuation/renewal, or supplemental/revision application.)

☐ COMPETING CONTINUATION/RENEWAL of grant number: R01EY014885-04

(This application is to extend a funded grant beyond its current project period.)

☐ SUPPLEMENT/REVISION to grant number:__________________________

(This application is for additional funds to supplement a currently funded grant.)

☐ CHANGE of principal investigator/program director.

☐ CHANGE of Grantee Institution. Name of former institution:__________________________

☐ FOREIGN application ☐ Domestic Grant with foreign involvement

List Country(ies) Involved:

1. PROGRAM INCOME (See instructions.)

All applications must indicate whether program income is anticipated during the period(s) for which grant support is requested. If program income is anticipated, use the format below to reflect the amount and source(s).

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</tr>
</tbody>
</table>

2. ASSURANCES/CERTIFICATIONS (See instructions.)

In signing the application Face Page, the authorized organizational representative agrees to comply with the following policies, assurances and/or certifications when applicable. Descriptions of individual assurances/certifications are provided in Part III. If unable to certify compliance, where applicable, provide an explanation and place it after this page.

- Human Subjects Research
- Research Using Human Embryonic Stem Cells
- Research on Transplantation of Human Fetal Tissue
- Women and Minority Inclusion Policy
- Inclusion of Children Policy
- Vertebrate Animals-
- Debarment and Suspension
- Drug-Free Workplace (applicable to new [Type I] or revised/resubmission [Type I] applications only)
- Lobbying
- Non-Delinquency on Federal Debt
- Research Misconduct
- Civil Rights (Form HHS 441 or HHS 690)
- Handicapped Individuals (Form HHS 641 or HHS 690)
- Sex Discrimination (Form HHS 639-A or HHS 690)
- Age Discrimination (Form HHS 680 or HHS 690)
- Recombinant DNA Research, Including Human Gene Transfer Research
- Financial Conflict of Interest
- Smoke Free Workplace
- Prohibited Research
- Select Agent Research
- PI Assurance

3. FACILITIES AND ADMINISTRATIVE COSTS (F&A)/ INDIRECT COSTS. See specific instructions.

☐ DHHS Agreement dated: 07/18/06 ☐ No Facilities And Administrative Costs Requested.

☐ DHHS Agreement being negotiated with __________________________ Regional Office.

☐ No DHHS Agreement, but rate established with __________________________ Date __________________________

CALCULATION* (The entire grant application, including the Checklist, will be reproduced and provided to peer reviewers as confidential information.)

| a. Initial budget period: | Amount of base $182,210 x Rate applied 76.30 % = F&A costs $139,026 |
| b. 02 year | Amount of base $228,776 x Rate applied 76.30 % = F&A costs $174,556 |
| c. 03 year | Amount of base $256,806 x Rate applied 76.30 % = F&A costs $195,943 |
| d. 04 year | Amount of base $199,106 x Rate applied 76.30 % = F&A costs $151,918 |
| e. 05 year | Amount of base $205,079 x Rate applied 76.30 % = F&A costs $156,475 |

TOTAL F&A Costs $817,919

*Check appropriate box(es):

☐ Salary and wages base ☐ Modified total direct cost base ☐ Other base (Explain)

Explanation (Attach separate sheet, if necessary):

salary, wage and benefit base

DHHS Agreement dated: 07/18/06

No DHHS Agreement, but rate established with __________________________

Date __________________________

TOTAL F&A Costs $817,919

*Check appropriate box(es):

☐ Salary and wages base ☐ Modified total direct cost base ☐ Other base (Explain)

Explanation (Attach separate sheet, if necessary):

salary, wage and benefit base