Context-Dependent Effects of Substantia Nigra Stimulation on Eye Movements

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INTRODUCTION

Saccadic eye movements rapidly realign the center of gaze to objects of interest. Voluntary saccades arise from eye fields of the prefrontal cortex (FEF) (Bruce and Goldberg 1985; Bruce et al. 1985; Schall 1997). FEF neurons have direct access to the superior colliculus (SC), a midbrain structure considered part of the final common pathway for voluntary and reflexive saccades (Moschovakis et al. 1996; Sommer and Wurtz 1998, 2000; Sparks 1986; Sparks and Hartwich-Young 1989). The FEF also has projections to structures in the brain stem involved in saccades (Stanton et al. 1988). Thus the coordinated activity of the FEF–SC and the FEF–brain stem pathways underlies the generation of voluntary saccadic eye movements (Hanes and Wurtz 2001; Schiller 1998; Schiller et al. 1979, 1980).

A lesser-understood pathway arising from FEF courses through the basal ganglia (BG). Indeed, most of the cerebral cortex projects to the input nuclei of the BG, the caudate and putamen (collectively the striatum) (Alexander et al. 1986; Nambu et al. 2002; Parthasarathy et al. 1992; Selemon and Goldman-Rakic 1985). For saccades, the inputs from FEF and dorsomedial frontal cortex (DMFC) or supplementary eye fields (SEFs) provide the principal input (Parthasarathy et al. 1992). One of the two output nuclei of the BG is the substantia nigra pars reticulata (SNr). Our current understanding of the role of the BG in eye movement control proposes that the command to initiate a voluntary saccade arises first in FEF neurons. Caudate neurons receiving FEF input are activated by the cortical drive. These caudate neurons in turn are directly connected to the SNr (Hikosaka and Sakamoto 1986; Hikosaka et al. 1989a,b,c, 1993) and contain γ-aminobutyric acid (GABA) (Gerfen 1985; Gerfen and Wilson 1996). Therefore the discharge of caudate neurons results in a decrease in the discharge of SNr neurons. SNr neurons are tonically active, contain GABA (Chevalier and Deniau 1990; Chevalier et al. 1981a, 1984, 1985; Hikosaka and Wurtz 1983a,d), and have direct projections to the SC. Thus decreasing SNr neuronal activity reduces inhibition of SC neurons, resulting in a robust discharge of action potentials (APs) in SC neurons signaling a command to initiate a saccade (Hikosaka and Wurtz 1983d; Hikosaka et al. 2000). A schematic of the activity profiles of the caudate, SNr, and SC during a saccade is shown in Fig. 1A.

The results of the original recording experiments in the monkey SNr suggested that SNr neurons were involved preferentially in saccades guided by particular behavioral contexts such as memory (Hikosaka and Wurtz 1983c). Consistent with this hypothesis, injection of muscimol, a GABA agonist, into the SNr produces deficits in memory-guided eye movements (Hikosaka and Wurtz 1985). More recent electrophysiological recordings suggest that SNr neuronal activity is not specific for memory-guided saccades (Bayer et al. 2002). Therefore there remain a number of questions regarding the precise nature of the role of SNr in saccadic eye movements.

In this report we describe the results of experiments in which we explore the role of the SNr in saccadic eye movement control using electrical stimulation. Our goal was twofold.
First, in light of the previous muscimol results (Hikosaka and Wurtz 1985) indicating a preferential effect on memory-guided eye movements, we asked whether electrical stimulation of the SNr would also have a preferential effect on memory-guided eye movements. A demonstration of this using a second technique would provide additional evidence that the BG are principally concerned with nonvisually guided movements. Furthermore, with electrical stimulation we have control over the precise timing of the manipulation and therefore can present stimulation specifically during the preparation and initiation of the movement. Second, electrical stimulation of brain regions [deep brain stimulation (DBS)] is an increasingly popular treatment option for patients with neurological disease who are refractory to traditional medical therapies. Yet, neither is the precise mechanism of action of DBS known, nor are consistent results obtained from experiments assessing second-order effects of DBS on target structures (Anderson et al. 2003; Hashimoto et al. 2001, 2003). How the effects of DBS translate to changes in behavior are even less well understood (Anderson et al. 2003; Ashby et al. 1999; Dostrovsky et al. 2000; Hashimoto et al. 2003; Larsy et al. 2003; Perlmutter and Mink 2006). In light of the well-established relationship of SC neuronal activity and eye movements (Moschovakis et al. 1996; Sparks et al. 2004; Wurtz et al. 2000) and the fact that the SC is a major target of the BG (Gerfen and Wilson 1996; Gerfen et al. 1982), we reasoned that establishing the efficacy of electrical stimulation of SNr on eye movements might lead to insights into the mechanism of action of DBS as well as provide a framework for future experiments exploring the role of DBS on behavior.

Here we show that electrical stimulation of the SNr influences the generation of saccadic eye movements. The directions and amplitudes of saccade vectors were altered with stimulation. The changes in saccade vectors occurred primarily during memory-guided saccades. We also found that electrical stimulation both decreased saccade latency and increased saccade latency. Finally, electrical stimulation of the SNr decreased the likelihood of both contralateral and ipsilateral memory-guided saccades. Visually guided saccade probability was unaltered. We conclude from these results that electrical stimulation of the SNr influences saccades in a manner consistent with an activation of SNr outputs. The pattern of effects of SNr stimulation on saccades indicates that the SNr has widespread effects on the SC. This is consistent with an alteration in the pattern of activity across the map of saccadic eye movements bilaterally. Preliminary reports of this work were previously published (Day et al. 2005; Liu et al. 2003).

METHODS

General behavioral procedures

We used a real-time experimental data acquisition and visual stimulus generation system (Tempo and VideoSync; Reflective Computing) to create the behavioral paradigms and acquire eye position and neuronal data. Monkeys were trained to sit in a custom-designed primate chair with head fixed during the experimental session (typically 3–5 h). Visual stimuli were rear-projected onto a tangent screen at 51-cm distance using a DLP projector (LP335, InFocus) with a native resolution of 1,024 × 768 and operating at 60 Hz. The background luminance was 0.28 cd/m². The visual stimulus presentation was controlled by VideoSync software (Reflective Computing) running on a dedicated PC with a 1,024 × 768 VGA video controller (Computer Boards). The PC was a slave device to the PC used for experimental control and data acquisition. Because there is an inherent time limitation in DLP projectors (both the vertical refresh rate and the vertical sync pulse) one photocell was placed on the top left corner of the screen and a second photocell was placed on the lower right corner of the screen. Both photocells sent signals (a TTL pulse) to the experimental PC within 1 ms, providing an accurate measure of stimulus onset and offset.
Behavioral task and electrical stimulation

Monkeys performed memory-guided and visually guided delayed saccades (Fig. 2). In the delayed-saccade task, initially a centrally located visual spot appears and monkeys are required to fixate this spot. After a random time of 500–1,500 ms, a peripheral spot appears somewhere in the visual field. After another delay (800–1,200 ms), the fixation spot is removed and this serves as a cue for the monkeys to make a saccade to the visual spot located in the periphery (Fig. 2B).

The memory version of this task (Hikosaka and Wurtz 1983c) is identical except that the spot located in the visual field appears only transiently (200 ms). Monkeys must remember the location and make a saccade to that memorized location when the central spot disappears (Fig. 2A). Memory-guided and visually guided saccade trials were randomly interleaved. The target for the saccade appeared at one of eight possible locations throughout the visual field (Fig. 2D). The location of the target was also randomly interleaved. When monkeys performed a trial correctly they received a drop of water as reward. Stimulation often interfered with the ability to produce accurate saccades. To avoid having monkeys no longer participate in the task we imposed a less-restrictive criterion for accepting eye movements as correct for the stimulation trials compared with the nonstimulation trials. By 500 ms after the cue to make a saccade appeared, the eye position had to be within 3° square around the target for nonstimulated trials. For stimulated trials the eye position had to be within 10° square around the target position.

Finally, electrical stimulation of the SNr was introduced at the time the fixation point was removed and continued for 400 ms. The parameters of stimulation were 35–60 µA and 150 µs pulse width. The train of electrical stimulation occurred on randomly interleaved trials. In separate blocks of trials, we used three different frequencies of stimulation: 75, 125, and 300 Hz. We used 300 Hz at 61 sites in three monkeys, 75 Hz at 21 sites in two monkeys, and 125 Hz at 11 sites in two monkeys. All three frequencies overlapped at 11 sites. We had clinically relevant parameters in mind when selecting these. For clinical use, typically between 100- and 130-Hz stimulation is used in the subthalamic nucleus (Ashby et al. 1999). A Grass S88 dual-output square-wave–pulse generator provided the input driving two PSIU6s (photoelectric stimulus isolation unit). These units in turn, each produced one phase of a biphasic pulse with constant current. For safety, these units are optically isolated (Grass Technologies, AstroMed). The pulses in the stimulation trains were current balanced to minimize tissue damage. To ensure accurate current intensities, we measured the current before and after stimulation experiments on an oscilloscope using a 10 Ω resistor in series with the stimulating electrode.

Surgical procedures for electrophysiology

For electrophysiological recording of single neurons and monitoring eye movements, cylinders and eye movement measuring devices were implanted in three rhesus monkeys (Macaca mulatta) using procedures described previously (Li and Basso 2005). Anesthesia was induced initially with an intramuscular injection of ketamine (5.0–15.0 mg/kg). Atropine (0.5 mg/kg) was provided to minimize salivation. Monkeys were intubated and maintained at a general anesthetic level with isoflurane. A subconjunctival coil was implanted (Judge et al. 1980). A plastic head holder for restraint and two cylinders for subsequent microelectrode recording were mounted on top of the exposed skull and secured with titanium screws and dental acrylic. Plastic hardware allowed subsequent magnetic resonance (MR) images to be obtained with minimal artifact. For access to the SNr, the two recording cylinders were placed stereotaxically (A10 L5) and angled mediodlaterally approximately 40–45° depending on the angle of the SNr as determined by presurgical MR images. An antibiotic (cefadroxil, 25 mg/kg) was given 1 day before the operation and every day for a minimum of 4 days after the operation. Analgesia was provided by the administration of buprenorphine (0.01–0.03 mg/kg) and flunixin (1–2 mg/kg) for 48 h postsurgically as needed. Monkeys recovered for 1–2 wk before behavioral and physiological recording commenced. All experimental protocols were approved by the University of Wisconsin–Madison Institutional Animal Care and Use Committee and complied with or exceeded standards set by the Public Health Service policy on the humane care and use of laboratory animals.
Neuronal classification

Monkeys performed the delayed-saccade task to eight targets located throughout the visual field (Fig. 2D). SNr neurons recorded during the performance of a visually guided and/or memory-guided delayed-saccade task were classified as visual, saccade, or visual-delay saccade neurons. We measured 200 ms of baseline discharge rate while monkeys fixated and before a visual stimulus appeared. We then measured the first 200 ms of neuronal discharge after the onset of the visual stimulus. The delay interval was defined as the discharge rate occurring 600–800 ms after the target spot appeared. The saccade interval was defined as the discharge rate occurring 50 ms before to 50 ms after the onset of the saccade. SNr neurons were classified as visual if they contained statistically significant differences \( P < 0.05 \) in discharge rate during the visual interval compared with baseline. Saccade neurons were defined as those showing a significant difference in discharge rate during the saccade interval compared with baseline \( P < 0.05 \). Visual-delay–saccade neurons were classified as those with statistically significant discharge rates during all three intervals compared with baseline \( P < 0.05 \). Some visual and saccade neurons also had significant modulation during at least one other interval. For these cases we classified neurons according to which interval was maximally modulated as determined by visual inspection. Because SNr neurons often have a mixture of response types, we believe that this or any classification scheme does not separate SNr neuron types. Rather, SNr neurons are best described as representing a continuum of response types (Basso and Wurtz 2002; Handel and Glimcher 1999, 2000; Hikosaka and Wurtz 1983a,b,c). Of the 61 neurons in our sample, 15 of 61 (26%) were classified as visual neurons; 31 of 61 (51%) had a reduced level of activity compared with the baseline during all three intervals of the delayed-saccade task and were classified as visual-delay–saccade neurons; 12 of 61 (20%) of our sample were classified as saccade neurons because of their reduced level of discharge during the saccade interval (see METHODS). Three of 61 (3%) were classified qualitatively because of an insufficient number of trials for statistical classification. We did not encounter neurons with decreases in activity only during the delay period. Of the three classified qualitatively, one had an increase in activity at the time the visual stimulus appeared and was considered a visual neuron. Figure 3 shows two schematics of sections through the midbrain of a rhesus monkey (Mikula et al. 2007). The black squares represent locations from which electrode tracts were reconstructed from four hemispheres of two monkeys (eight in one monkey and 22 in the second). The other 31 sites come from a monkey that is...

Data acquisition

Using the magnetic induction technique (Robinson and Fuchs 1969) (Riverbend Instruments), voltage signals proportional to horizontal and vertical components of eye position were filtered (eight-pole Bessel, \(-3 \text{ dB}, 180 \text{ Hz}\)), digitized at 16-bit resolution and sampled at 1 kHz (Measurement Computing; CIO-DAS1602/16). The data were saved for off-line analysis using an interactive computer program designed to display and measure eye position and calculate eye velocity. We used an automated procedure to define saccadic eye movements by applying velocity and acceleration criteria of 50°/s and 5,000°/s\(^2\), respectively. Adequacy of the algorithm was corrected if necessary, on a trial-by-trial basis by the experimenter. Single neurons were recorded with tungsten microelectrodes (FHC) with impedances between 0.1 and 1.0 M\(\Omega\) measured at 1 kHz. Electrodes were aimed at the SNr through stainless steel guide tubes held in place by a plastic grid secured to the cylinder (Crist et al. 1988). Action potential waveforms were identified with a window discriminator (Bak Electronics) that returned a TTL pulse for each waveform that met voltage and time criteria. The TTL pulses were sent to a digital counter (National Instruments; PC-TIO-10) and were stored with a 1-ms resolution. Once an SNr neuron was isolated and characterized in the delayed-saccade task, using the same electrode, electrical stimulation commenced.

Data analysis

All statistical analyses were performed using Matlab (The Math-Works) or Oriana (Kovach Computing Services) for circular statistics. All linear data were first tested for normality using the Lilliefors test. If they passed this test of normality, we then made comparisons using parametric ANOVA or \( t \)-test (modified Bonferroni methods). If the data failed to pass normality tests, nonparametric ANOVA (Kruskal–Wallis) or Wilcoxon rank-sum tests were used. To test differences between cumulative density functions we used a two-tailed Kolmogorov–Smirnov test (Keppel 1991).

RESULTS

We stimulated at 61 sites in six SNrs of three monkeys. At each site we documented the response profile of the neuron recorded before introducing electrical stimulation. We observed response profiles similar to those previously reported by us and others (Basso and Wurtz 2002; Basso et al. 2005; Bayer et al. 2002; Handel and Glimcher 1999, 2000; Hikosaka and Wurtz 1983a,b,c). Of the 61 neurons in our sample, 15 of 61 (26%) were classified as visual neurons; 31 of 61 (51%) had a reduced level of activity compared with the baseline during all three intervals of the delayed-saccade task and were classified as visual-delay–saccade neurons; 12 of 61 (20%) of our sample were classified as saccade neurons because of their reduced level of discharge during the saccade interval (see METHODS). Three of 61 (3%) were classified qualitatively because of an insufficient number of trials for statistical classification. We did not encounter neurons with decreases in activity only during the delay period. Of the three classified qualitatively, one had an increase in activity at the time the visual stimulus appeared and was considered a visual neuron. Figure 3 shows two schematics of sections through the midbrain of a rhesus monkey (Mikula et al. 2007). The black squares represent locations from which electrode tracts were reconstructed from four hemispheres of two monkeys (eight in one monkey and 22 in the second). The other 31 sites come from a monkey that is...
still participating in experiments, although the electrode path targeting the SNr was confirmed using MR imaging.

One possible outcome of electrical stimulation effects on SNr and eye movements is shown schematically in Fig. 1, B–E. When electrical stimulation is introduced in cortex it is believed that the stimulus train mimics a train of APs activating the underlying neuronal tissue (Bruce and Goldberg 1985; Salzman et al. 1990; Schiller and Stryker 1972); thus we reasoned that trains of electrical stimulation to the SNr may mimic the occurrence of APs in SNr (Fig. 1B). Because SNr neurons are tonically active and often pause for saccades, electrical stimulation might introduce APs during a time when none would normally occur. As a result of the direct projections of SNr to SC, electrical stimulation of the SNr should influence the activity of SC neurons (Fig. 1C). If the influence of the SNr is on memory-guided rather than visually guided saccades preferentially, then the influence of electrical stimulation should be maximal for memory-guided saccades (cf. Fig. 1, D and E).

To test the hypothesis that manipulation of SNr neuronal activity would preferentially influence nonvisually guided eye movements, we presented trains of electrical stimulation to the SNr during performance of delayed-saccade tasks (Fig. 2). Memory-guided and visually guided trials were randomly interleaved and electrical stimulation of the SNr occurred on 50% of all trials (randomly) at the time when the cue was provided to make a saccade (Fig. 2, E and F). The train lasted for 400 ms. An example of the effects of SNr stimulation is shown for one site in Fig. 4. The neuron recorded at this site was classified as a visual-delay–saccade neuron because of the reduced activity compared with baseline for all intervals of the delayed-saccade task (Fig. 4, A and B).

Introduction of electrical stimulation of the SNr at this site influenced both the direction and the amplitude of the saccades made. In this example, the effect of SNr stimulation, however, was evident only during performance of saccades guided by memory. Visually guided saccades were not influenced by the stimulation (cf. Fig. 4, C and D). In the example shown in Fig. 4, memory-guided saccades made to the contralateral hemifield were mostly affected and ipsilateral saccades were slightly affected. Saccades made to the upper contralateral hemifield were often curved downward and contralaterally. Note also that there are fewer saccades made to the contralateral upward location. Furthermore, the endpoints of saccades made to the lower contralateral hemifield were often displaced upward relative to the endpoints of the saccades made in the nonstimulation trials. In some ipsilateral trials, notably for the direction along the horizontal meridian, saccades were also less likely to occur with stimulation than without stimulation (Fig. 4D, rightward horizontal location; cf. cyan and black lines). This example illustrates the general observations we made with electrical stimulation of the SNr. We subsequently quantify three main effects across our sample of 61 stimulation sites. First, we show that the direction and length of saccade vectors are altered with SNr stimulation. Second, we show that electrical stimulation of the SNr alters the latency of saccades. Finally, we show that the frequency of saccade occurrence to ipsilateral and contralateral targets is subtly but statistically significantly altered with SNr electrical stimulation.

**FIG. 4.** SNr stimulation alters memory-guided eye movements. A: recording from an SNr neuron during performance of the delayed-saccade task. Saccades were directed to a target located in the hemifield contralateral to the recorded neuron. B: saccades were directed to the target located in the hemifield ipsilateral to the recorded neuron. Each tick is an action potential and each row of ticks is a trial. Spike density function (s = 12 ms) is superimposed on the raster. Lines below the rasters are horizontal eye position (H eye) and vertical eye position (V eye). Arrows indicate the alignment of the traces. In each panel, the left trace is aligned on the onset of the target and the right trace is aligned on the saccade onset. C: XY position traces in nonstimulation (black) and stimulation (cyan) trials. D: same as in C for memory-guided saccades. Neuron recorded at this site was classified as a visual-delay–saccade neuron. Note that there is an asymmetry in saccade gain for ipsilateral and contralateral saccades for this monkey. We believe this likely arises from the damage resulting from repeated penetrations.

**Direction and amplitude of saccades**

We measured the direction and the length of the vector for each saccadic eye movement made to each target location. We then computed the average direction (angle in degrees, $\theta$) and the average length (amplitude in degrees, $\rho$) of the saccades made to each target location. This was done for both the stimulation and the nonstimulation trials.

Figure 5A provides a visual comparison of the average saccade vectors made in the memory-guided saccade condition taken from the example case shown in Fig. 4D. This illustration shows that the vertical saccades were rotated downward, contralateral upward oblique saccades were rotated downward, and some saccades were rotated upward (contralateral downward oblique saccades). With respect to the site of stimulation, some saccades were rotated ipsilaterally and some were rotated contralaterally. It also shows that some vectors became longer (hypermetric) and some became shorter (hypometric) with stimulation of the SNr. This observation suggests that the influence of SNr stimulation is not all or none on saccades but has a varying and broad influence on the map of saccades.
within the SC. To assess the influence of SNr stimulation across the stimulation sites and across the saccade target locations, we computed the angular difference and the length difference between the saccade vectors made in the stimulation and nonstimulation trials. For the angular difference we subtracted the stimulation from the nonstimulation angle ($\theta_{\text{stim}} - \theta_{\text{nonstim}}$). We reflected the data such that negative values indicate contralateral rotations (with respect to the side of stimulation) and positive values indicate ipsilateral rotations (with respect to the side of stimulation) of the saccade vector. For the amplitude data, we computed a ratio of the vector lengths in the stimulation and the nonstimulation conditions using the equation: $1 + (\rho_{\text{stim}} - \rho_{\text{nonstim}})/\rho_{\text{nonstim}}$ where $\rho$ is the length of the vector. We did this for both visually guided and memory-guided trials. We then plotted the vector differences in polar coordinates for the visually guided (Fig. 5B) and memory-guided (Fig. 5C) trials for 60 stimulation sites (see caption).

Figure 5, B and C plots the average vector rotations and amplitudes for each target location for 60 stimulation sites. Vectors pointing to 0 (upward in Fig. 5, B and C) indicate that there was no change in the direction of the saccade vector for saccades made with and without electrical stimulation of the SNr. Vectors pointing toward 180 (downward in Fig. 5, B and C) indicate that the saccade vector was rotated into the opposite hemifield with stimulation of the SNr. Clockwise rotations indicate that the saccade was rotated toward the ipsilateral direction (with respect to the side of stimulation), whereas counterclockwise rotations indicate that the saccade was rotated toward the contralateral direction with respect to the stimulation site in the SNr. A vector length of 1 in this plot indicates that the amplitude of the saccade was unchanged with SNr stimulation, whereas vectors with tips <1 indicate the saccade was hypometric and vectors with tips >1 indicate the saccade was hypermetric with electrical stimulation of the SNr.

Analysis of the 60 stimulation sites revealed two important findings. First, whereas visually guided saccades were influenced by SNr stimulation, the effect of SNr stimulation on saccades was more profound for memory-guided saccades (cf. Fig. 5, C and B). The changes seen in saccades were composed of saccade vector rotations and changes in saccade vector length. Visually guided saccades, if altered, tended to be rotated contralaterally or away from the side of stimulation. Memory-guided saccades were rotated contralaterally, but also ipsilaterally. For visually guided saccades changes in saccade amplitude were rare, but evident, whereas for memory-guided saccades SNr stimulation produced mainly hypometria (vector tips <1) but also hypermetria (vector tips >1).

To quantitatively confirm these results across our sample of stimulation sites, we also plotted the distribution of angular and amplitude differences measured with and without stimulation in the visually guided and memory-guided trials in linear form (Fig. 6). The average amplitude ratio of visually guided saccades in the stimulation trials compared with the nonstimulation trials across all stimulation sites was 0.98 (Fig. 6A). This value, very close to 1, indicates that visually guided saccades, overall, were scarcely affected in length by SNr stimulation. In contrast, memory-guided saccades had an average normalized length ratio of 0.91 (Fig. 6B), indicating a 9% reduction, on average, in saccade length with SNr stimulation. A comparison of the two distributions indicated that this difference between

![Figure 5](http://jn.physiology.org/)

**Fig. 5.** SNr stimulation alters the direction and amplitude of saccadic eye movements. A: in memory-guided saccades, at one stimulation site (the same as shown in Fig. 3), saccades made to each target location are drawn as vector arrows. Each black arrow is an average of ≥10 saccades. Outer circle is saccade amplitude with stimulation relative to saccade amplitude without stimulation (see text for computation). Cyan arrows are the average saccade vector (≥10 trials) to a single target location with stimulation of the SNr. Note that the asymmetry in saccade gain is also evident in this plot. B: angular difference between the saccade vector measured with and without stimulation (see text) is plotted in polar coordinates for visually guided saccades for ≥8 target locations for 60 SNr stimulation sites in 3 monkeys. One site is omitted because the stimulation saccade amplitudes were largely hypermetric (outliers) and would have distorted scaling of the plot. C: difference in the angle of the saccade vector with and without SNr stimulation is plotted in polar coordinates for the same 60 stimulation sites during memory-guided trials. Arrows pointing at 0° indicate no difference between the stimulated and nonstimulation vector. Arrows pointing at 180° indicate that the saccade produced with stimulation was deflected to the opposite hemifield. Counterclockwise rotations (negative values) indicate that the saccade was rotated contralaterally with stimulation, whereas clockwise rotations (positive values) indicate that the saccade vector was rotated ipsilaterally with stimulation.
memory-guided and visually guided saccades was statistically reliable (see Fig. 6, A and B; \( P < 0.001 \)).

We plotted the distribution of differences in saccade direction with and without SNr stimulation and found that the angular difference of vectors for memory-guided saccades (Fig. 6D) was broader than the distribution of angular differences for visually guided saccades (Fig. 6C). The mean angular difference for visually guided saccades was 6.65° (SD = 50.97), whereas the mean angular difference for memory-guided saccades was 21.87° (SD = 90.30). Note that to compute the means we used the absolute value of the angular differences, which does not provide directional information. To compare these distributions statistically, we used the nonparametric Wilcoxon signed-rank test and found that, indeed, the difference between visually guided and memory-guided stimulation distributions was statistically significant (\( P < 0.001 \)). Importantly, we also compared the distributions of vector lengths and angles for the nonstimulation condition. If the distribution of amplitudes and angles differed in the nonstimulation condition we could not be certain that the differences obtained in the stimulation condition resulted from stimulation or from the preexisting differences in memory-guided and visually guided saccades (White et al. 1994). We performed the F-test on the distribution of amplitudes in the nonstimulation condition for visually guided and memory-guided eye movements and found no significant differences across the sample of sites (\( P = 0.212 \)). Likewise, for the distribution of angles in the two conditions without stimulation, there was no significant difference using the F-test (\( P = 0.138 \)). Thus we conclude that for both saccade direction and saccade amplitude the influence of SNr stimulation was greater for memory-guided saccades than for visually guided saccades and this difference was not dependent on prestimulation differences in saccade characteristics.

**Stimulation with 75 and 125 Hz**

In 21 sites from two monkeys we introduced 75-Hz trains of stimulation to the SNr and in 14 sites from two monkeys we introduced 125-Hz stimulation trains. All other parameters were the same as those described for the 300-Hz condition. For 11 of the total 35 sites all three frequencies of stimulation occurred.

The effects of lowering the stimulation frequency on saccades showed the same trend of influencing memory-guided saccades more than visually guided saccades as did the 300-Hz stimulation (Fig. 7, A and B), although the effects were slightly more subtle. Visually guided saccades were virtually unchanged in length and rotation, whereas memory-guided sac-
The distributions of length changes are shown in E shown for visually guided saccades in and length deviations of saccade vectors occurring with SNr stimulation are included; 11 of these sites overlap with those shown in Figs. 5 and 6. Angular C and 6, A–D. In all, 35 stimulation sites from the same 3 monkeys are included; 11 of these sites overlap with those shown in Figs. 5 and 6. Angular and length deviations of saccade vectors occurring with SNR stimulation are shown for visually guided saccades in A and memory-guided saccades in B. Distributions of changes in angles are shown in E and F, whereas the distributions of length changes are shown in C and D.

Saccade latency

The influence of SNR stimulation on saccade latency is shown in Fig. 8. Electrical stimulation of the SNR influenced the latency of both memory-guided and visually guided saccades. The same effects on saccade latency were obtained for all three stimulation parameters, although the effects of 75 and 125 Hz were less profound (Fig. 8, A–D, 300 Hz; Fig. 8, E–H, 75 and 125 Hz). Furthermore, the stimulation influenced saccade latency bilaterally. For memory-guided saccades, latency was reduced on stimulated trials compared with nonstimulation trials (Fig. 8, A and E). By approximately 200 ms, saccade latencies were increased for contralaterally directed memory-guided saccades (Fig. 8, A and E). A similar trend was evident for ipsilaterial saccades (Fig. 8, B and F). Ipsilateral saccades had a reduced latency ≤200 ms and a prolonged saccade latency >200 ms. We confirmed this observation statistically using the Kruskal–Wallis ANOVA and determined that saccade latency was significantly different in stimulation trials compared with controls for contralateral (P < 0.001) and ipsilaterial (P < 0.02) memory-guided saccades. Qualitatively similar results were obtained using 75 and 125 Hz (Fig. 8, E and F).

For visually guided saccades we found that saccade latency was also affected bilaterally (Fig. 8, C, D, G, and H). Electrical stimulation of the SNR reduced the latency of all visually guided saccades. Interestingly, in our monkeys, the latency of visually guided saccades was most often <200 ms.

Frequency of saccades

The current conceptual model of the SNR in eye movements predicts that increasing SNR neuronal activity should enhance inhibition of saccade-related SC neurons and decrease the occurrence of saccades. Consistent with this, we found that electrical stimulation of the SNR reduced the occurrence of saccades (Fig. 9), although this effect was not always as profound as we expected. In fact, the suppression of saccades with stimulation often subsided with repeated experiments in the same monkey. We saw this phenomenon in all three monkeys. To quantify the influence of SNR stimulation on saccade occurrence, we counted the number of saccades made to each target in the stimulation and control conditions. We then summed the number of saccades made in the stimulation and nonstimulation conditions across contralateral and ipsilateral directions. These sums were then divided by the number of saccades made in the nonstimulation condition. Multiplying this ratio by 100 yields the percentage of saccades made. The nonstimulation condition in both memory-guided and visually guided trials had a percentage of 100 (Fig. 9, A and B, black bars). Despite the reduced magnitude of the effect of stimulation with repeated experiments, we found that the occurrence of contralateral saccadic eye movements was reduced by roughly 6% on trials with electrical stimulation of the SNR across all three monkeys and all 61 stimulation sites. Importantly, this reduction was seen only for memory-guided trials (Fig. 9A; contralateral memory: *P = 0.03; visual: P = 0.81).

The current model of the role of SNR in the monkey saccadic system emphasizes the control of contralateral saccades. How-

FIG. 7. SNR stimulation at 75 and 125 Hz also influences memory-guided saccades. Arrangement of this figure is the same as that shown in Figs. 5, B and C and 6, A–D. In all, 35 stimulation sites from the same 3 monkeys are included; 11 of these sites overlap with those shown in Figs. 5 and 6. Angular and length deviations of saccade vectors occurring with SNR stimulation are shown for visually guided saccades in A and memory-guided saccades in B. Distributions of changes in angles are shown in E and F, whereas the distributions of length changes are shown in C and D.
ever, with electrical stimulation, we also found that the occurrence of ipsilaterally directed saccades was reduced. For ipsilateral saccades, the reduction in occurrence was approximately 12% and statistically reliable (Fig. 9B; ipsilateral memory: \( P < 0.001 \)). Like contralateral saccades, the reduction in ipsilateral saccades was evident only in the memory-guided trials (Fig. 9B; ipsilateral visual: \( P = 0.67 \)). Indeed, the difference in the reduction of contralateral and ipsilateral saccades was also significantly different (88 vs. 12%, \( P = 0.02 \)). This result suggests that there may be differences between the influence of the SNr on the two SCs (Jiang et al. 2003).

In summary, we found that electrical stimulation of the SNr influenced the generation of saccades. Electrical stimulation altered the vector of the saccade produced during SNr stimulation. Frequently saccades were shorter in length and the direction of the saccade vector was rotated in stimulation trials compared with control trials. The alteration in saccade vector was also more prominent for saccades made to targets guided by memory than those with visual stimuli present to guide them. We found that SNr stimulation influenced saccade latency. In general, visually guided saccades tended to be <200 ms without stimulation and had an even shorter latency with stimulation of the SNr. Memory-guided saccades showed both a reduced latency and an increase in latency with SNr stimulation. These results suggest two influences of SNr stimulation on saccade latency. Finally, we found that the occurrence of saccades was altered with SNr stimulation. When stimulation was applied, the likelihood of producing a saccade was reduced slightly. Reduction in the occurrence of saccades was found bilaterally and was more evident in memory-guided trials than in visually guided trials.

**Discussion**

The experiments described in this report were designed with two goals in mind. First, recent electrophysiological evidence...
calls into question the precise nature of the role of the SNr in eye movements (Basso and Wurtz 2002; Basso et al. 2005; Bayer et al. 2002; Handel and Glimcher 1999, 2000; Sato and Hikosaka 2002). Second, a number of neurological disorders are being treated successfully with electrical stimulation of BG nuclei (Houeto et al. 2005; Larsy et al. 2003; Mayberg et al. 2005). This treatment is used despite our rather poor understanding of the mechanism underlying the effects of electrical stimulation of inhibitory neuronal structures or its relationship to normal behavior. Therefore to begin exploring these two issues, we presented electrical stimulation to the SNr, one of two inhibitory outputs of the BG, immediately before and during the generation of saccadic eye movements. We measured eye movements with and without stimulation in visually guided and memory-guided delayed saccade tasks.

We found that electrical stimulation of the SNr influenced memory-guided saccades more profoundly than visually guided saccades. We found a reduction in the occurrence of saccades made to the contralateral and the ipsilateral hemifields, only in the memory-guided task. The frequency of visually guided saccades did not change with stimulation. Stimulation of the SNr altered the characteristics of saccades. The length of the saccade vector as well as the direction of the saccade vector was changed. Often stimulation produced hypometria and sometimes produced hypermetria. Stimulation also often rotated the saccade direction. Again, this result was observed most often for memory-guided saccades. Finally, electrical stimulation of the SNr influenced saccade latency. SNr stimulation both reduced saccade latency and increased saccade latency. Later, we discuss and interpret our results within the context of previous work. We first discuss the suppression of saccades, then we discuss the changes in saccade vector and latency.

**SNr stimulation suppressed saccades bilaterally**

Current schemes of the role of the SNr in saccades in monkeys rely on the well-known and strong projection from the SNr to the ipsilateral SC (Beckstead et al. 1981; Graybiel 1978; Harting and Van Lieshout 1991; Harting et al. 1988; Hikosaka and Wurtz 1983d; Huerta et al. 1991). A crossed SNr–SC pathway was recently studied in the anesthetized cat (Jiang et al. 2003). Single-pulse orthodromic, antidromic, and anatomical methods demonstrate that the crossed projection is GABAergic and inhibits SC activity, similar to the uncrossed pathway (Chevalier and Deniau 1990; Chevalier et al. 1981b; Hikosaka and Wurtz 1983d). Our stimulation results revealing a suppression of saccades bilaterally are consistent with this observation and indicate that the monkey SNr may inhibit both SCs. Furthermore, because injection of muscimol into the SNr to inhibit SNr output results in increases in saccades (Hikosaka and Wurtz 1985) and electrical stimulation shown here results in decreases in saccades, we propose that although other effects are possible, SNr stimulation influences behavior through activation of the inhibitory output of the SNr targeting both SCs.

In the anesthetized cat, the neurons constituting the uncrossed pathway are tonically active and pause for the presentation of moving visual stimuli. In contrast, the crossed pathway neurons are transiently active at the onset of a moving visual stimulus. Based on their data, the authors concluded that the crossed pathway suppressed unwanted movements whereas the uncrossed pathway simultaneously released a desired orienting movement (Jiang et al. 2003). In our sample of SNr sites, some neurons had gradual increases in activity during the delay period of the delayed-saccade task like those reported previously (Sato and Hikosaka 2002), but we did not often see transient increases in SNr neuronal activity with visual stimuli. Further, we did not explore the responses of our sample to moving visual stimuli, so a direct comparison with the results obtained in cats is not possible. Finally, because we used trains of electrical stimuli rather than single pulses, we think it is unlikely that we would selectively alter the behavior of individual neurons, precluding a comparison of the effects of stimulation on different neuronal response types. Further work will be required to determine whether a similar, functional organization of the crossed and uncrossed pathway appears in monkeys as it does in cats.

**SNr stimulation alters saccade direction and amplitude**

Based on previous work showing that reducing SNr activity with muscimol, the GABA agonist, results in irrepressible saccades, we expected that electrical stimulation would activate SNr neuronal output pathways and result in a profound suppression of saccades. That we did not see a complete suppression of eye movements may indicate that stimulation had effects other than that shown in Fig. 1B. For example, activation of presynaptic input fibers arising from the caudate nucleus might be activated along with the SNr output fibers. If this happened, we might expect the electrical stimulation to inactivate the SNr by increasing local inhibition. Thus the effects of SNr stimulation may reflect a mixture of activation and inactivation of SNr activity. Based on our finding that saccades were suppressed, we think that although there may be mixed local effects of stimulation, the effects on behavior result from activation of the output pathway. That the suppression was not often as profound as we anticipated may reflect these mixed effects or may reflect a depletion of neurotransmitter resulting from long stimulation trains. Interestingly, the initial experiments often did show profound effects on saccade occurrence that waned with repeated experiments. Similar reductions in the efficacy of stimulation were reported in dorsal premotor cortex (Churchland and Shenoy 2007). Therefore another possibility is that the reduced effects and decline in efficacy of SNr stimulation result from active compensation or tissue damage. Sorting this out will require further experiments.

Nevertheless, across the sample of 61 sites, SNr stimulation did alter saccades when they were produced. Both the direction and the amplitude of the saccade vector were altered. The alteration in vectors was more prominent for memory-guided saccades than for visually guided saccades. This is consistent with the hypothesis that the SNr plays a preferential role in nonvisually guided movements (Hikosaka and Wurtz 1983c; Wichmann and Kliem 2004). However, in contrast to the previous recording results, other recordings of SNr neurons during saccades showed little difference between the activity of SNr neurons during performance of visually guided and memory-guided saccades (Bayer et al. 2002). One possibility for this difference, as suggested by the latter authors (Bayer et al. 2002), is that the rate of reward for visually guided versus memory-guided eye movements differed between the two re-
posingly activate the internal capsule fibers. As confirmation 

experiments. Second, given the stimulation parameters we used

ms and increases in latency

of the SNr–SC pathway in monkeys. The prediction based on 

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saccade latency. SNr stimulation also influenced both visually 

SNr stimulation alters saccade latency

SNr stimulation both reduced saccade latency and prolonged 
saccade latency. SNr stimulation also influenced both visually 
guided and memory-guided saccade latency. In our minds, the 
results of stimulation on saccade latency are the most difficult 
to understand with what is currently known about the function 
of the SNr–SC pathway in monkeys. The prediction based on 
current knowledge is that electrical stimulation of the SNr 
should activate an inhibitory drive to the SC and increase 
saccade latency. However, we saw decreases in latency <200 
ms and increases in latency ≥200 ms. One possibility is that 
current spread from the SNr to the adjacent internal capsule 
activating excitatory inputs to the SC arising from cortex. This 
was suggested as an explanation for the monosynaptic excita-
tory postsynaptic potentials recorded in SC neurons with stim-
ulation of the SNr (Karabelas and Moschovakis 1985). We 
think this interpretation is unlikely for two reasons. First, we 

usual evoked shoulder twitches. We then reduced the current intensity for the experiments. Second, given the stimulation parameters we used (60 μA, 300 Hz) we would expect that driving excitatory input to the SC directly would evoke saccades with latencies shorter than what we generally observed (Bruce and Goldberg 1985; Robinson 1972; Robinson and Fuchs 1969). Therefore as we

suggested earlier the electrical stimulation may initially disin-
hbit the SC and then subsequently inhibit the SC. One way this 
may come about is if inhibitory caudate fibers projecting to the 
SNr (Hikosaka et al. 1993) are activated, initially resulting in a

suppression of SNr activity. Because our trains were long 
(400 ms), GABA may be depleted, thus allowing SNr neurons 
to recover from inhibition and then inhibit SC neurons.

A second possibility is that the SNr neurons project to GABAergic interneurons that are known to exist in the SC (Behan and Kime 1996; Behan et al. 2002). A projection to interneurons was not demonstrated directly (Bickford and Hall 1992), although anatomical experiments in rat, cat, and mon-
key suggest that the pathway from the SNr to the SC has more nuances than appreciated by the model developed from the 

monkey experiments (Beckstead 1983; Beckstead et al. 1981; 

Deniau and Thierry 1997; Harting et al. 1988). There are at 
least three pathways from the SNr to the SC. The first is 
uncrossed, arises from the lateral SNr, and projects to the 
superficial layers of the SC and the dorsal intermediate layers 
of the SC. The second is uncrossed, arises from the medial 
SNr, and projects to the lower intermediate layers of the SC 
and the deep layers of the SC. The third is crossed and 
terminates in the contralateral SC (Gerfen et al. 1982; 

increase the inhibitory output of the BG (Albin et al. 1995). 
Thus the decreased latency reported here may be interpreted as 
evidence that the stimulation acts to inactivate the SNr, but also 
may indicate that electrical stimulation mimics the PD state 
and increases the inhibitory output. We propose that the stim-
ulation increases the inhibitory output of the BG and produces 
an inability to suppress a reflexive saccade. Indeed, that BG are 
involved in reflex suppression is well documented (Basso and 
Evinger 1996; Basso et al. 1993, 1996; Mink and Thatch 
1991a,b). Thus consistent with the recent work in humans 
(Amador et al. 2006; Briand et al. 2001), our stimulation 
experiments suggest that the BG may play a role in both 
reflexive and nonvisually guided, voluntary eye movements.

Clinical implications for DBS

The technique of electrical stimulation is used experiment-
ally to mimic naturally occurring neuronal signals (Bruce et al. 
1985; Hanks et al. 2006; Salzman et al. 1990; Schiller and 
Stryker 1972). In contrast, the original rationale for using
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