Context-Dependent Effects of Substantia Nigra Stimulation on Eye Movements

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Basso MA, Liu P. Context-dependent effects of substantia nigra stimulation on eye movements. J Neurophysiol 97: 4129 – 4142, 2007. First published March 28, 2007; doi:10.1152/jn.00094.2007. In a series of now classic experiments, an output structure of the basal ganglia (BG)—the substantia nigra pars reticulata (SNR)—was shown to be involved in the generation of saccades made in particular behavioral contexts, such as when memory was required for guidance. Recent electrophysiological experiments, however, call this original hypothesis into question. Here we test the hypothesis that the SNR is involved preferentially in nonvisually guided saccades using electrical stimulation. Monkeys performed visually guided and memory-guided saccades to locations throughout the visual field. On 50% of the trials, electrical stimulation of the SNR occurred. Stimulation of the SNR altered the direction, amplitude, latency, and probability of saccades. Visually guided saccades tended to be rotated toward the field contralateral to the side of stimulation, whereas memory-guided saccades tended to be rotated toward the hemifield ipsilateral to the side of stimulation. Overall, the changes in saccade vector direction were larger for memory-guided than for visually guided saccades. Both memory- and visually guided saccades were hypometric during stimulation trials, but the stimulation preferentially affected the length of memory-guided saccades. Electrical stimulation of the SNR produced decreases in visually guided saccades bilaterally. In contrast, memory-guided saccades often had increases in saccade latency bilaterally. Finally, we found approximately 10% reduction in the probability of memory-guided saccades bilaterally. Visually guided saccade probability was unaltered. Taken together the results are consistent with the hypothesis that SNR primarily influences nonvisually guided saccades. The pattern of stimulation effects suggests that SNR influence is widespread, altering the pattern of activity bilaterally across the superior colliculus map of saccades.

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; Handel and Glimcher 2000) and may be involved in target selection for saccades (Basso and Wurtz 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.
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 $1,024 \times 768$ 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 $1,024 \times 768$ 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. 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 (photolecetric 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 mediotlaterally 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. 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 dB, 180 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 $50^\circ$/s and $5,000^\circ$/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 MΩ 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 MathWorks) 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).
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 were 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 quantified 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.
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 (θstim - θnonstim). 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 + (ρstim - ρnonstim)/ρnonstim, where ρ 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
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 saccades were virtually unchanged in length and rotation, whereas memory-guided sac-
Distributions of length changes are shown in Figs. 5 and 6. Angular deviations of the eye during memory-guided saccades were larger at 9.44° (Fig. 7, A and E) than for visually guided saccades at 5.54° (Fig. 7, 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 ipsilateral \( P < 0.02 \) 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 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 ipsilateral 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 ipsilateral \( P < 0.02 \) memory-guided saccades. Qualitatively similar results were obtained using 75 and 125 Hz (Fig. 8, E and F).

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The current model of the role of SNr in the monkey saccadic system emphasizes the control of contralateral saccades. How-
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 irreplaceable 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-
cording studies. It is unlikely that differences in the rate of reward can explain the differences we obtained with electrical stimulation because our monkeys were given rewards virtually regardless (the electronic windows were increased on stimulation trials) of their performance to encourage continued participation in the task. Rather, our results are consistent with the role of the BG in behavior more generally (Mishkin and Petri 1984). When salient sensory information is present, the BG are less critical for movement (Glickstein and Stein 1991; Morris et al. 1996). In contrast, when sensory information is absent or provides no new information, the BG are critical. For saccades, this may result simply because generating the burst in SC neurons (Moschovakis et al. 1988a,b, 1996; Ozen et al. 2004) is more dependent on decreases in SNr activity when no cortical (Helmski and Segraves 2003; Paré and Wurtz 1997, 2001; Sommer and Wurtz 2000) or cerebellar (May et al. 1990) drive is present.

The idea that the burst in SC neurons is influenced differently by the loss of SNr inhibition when an excitatory drive is present is suggested by the subtle differences in saccade vector rotation in visually guided versus memory-guided saccades (Figs. 5–7). The difference in efficacy of stimulation with and without a visual drive (presumably excitatory) suggests a hypothesis for the type of inhibition arising from the SNr to the SC. We suggest that the inhibition arising from the SNr acts as a shunting inhibition (Borg-Graham et al. 1998), decreasing the efficacy of other excitatory inputs to SC neurons. Rather than producing a hyperpolarizing “hole” in one region of the SC, the inhibition from the SNr may act to sculpt the SC population activity determining where a saccade will be made. Future experiments in vivo and in vitro, altering SNr activity, and recording from SC populations can address these important issues.

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 excitatory postsynaptic potentials recorded in SC neurons with stimulation of the SNr (Karabelas and Moschovakis 1985). We think this interpretation is unlikely for two reasons. First, we routinely increased the stimulation current ≥80 μA, to purposefully activate the internal capsule fibers. As confirmation of the current spread into the capsule, 80 μA usually 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 disinhibit 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 monkey 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; Harting and Van Lieshout 1991; Harting et al. 1988; Huerta et al. 1991; Jayaraman et al. 1977; Jiang et al. 2003; Mana and Chevalier 2001). Furthermore, dorsally located neurons in the SNr tend to project more rostrally in the SC compared with ventrally located SNr neurons that tend to project more caudally in SC (Gerfen et al. 1982). These anatomical results suggest that further inquiry into the topographical organization of the SNr–SC pathway in the behaving monkey would be a worthwhile effort.

A recent study of eye movements in PD patients indicates that the BG dysfunction associated with this disease impairs voluntary movement initiation but also impairs the ability to suppress reflexively driven saccades. This lesser-appreciated symptom was determined by measuring an increase in saccadic intrusions (errors) during the delay period of a delayed antisaccade task (Amador et al. 2006; Briand et al. 1999). Although controversial, according to classical models of BG dysfunction in PD the net effect of reduced dopamine tone is to 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 stimulation 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 experimentally 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
electrical stimulation clinically as a treatment for certain neurological diseases was to produce a temporary lesion (Benabid 2003). Because the neurosurgical treatment of certain diseases involved making lesions electrolytically, Benabid (2003) reasoned that he could produce a temporary lesion by chronically stimulating the nucleus rather than permanently damaging it. Since then, there has been a wealth of investigation aimed at understanding how DBS alters neural tissue (e.g., Anderson et al. 2003; Dostrovsky et al. 2000; Hashimoto et al. 2003; Larsy et al. 2003; McIntyre et al. 2004; Montgomery and Baker 2000). However, comparatively less effort is aimed at understanding how the effects of stimulation translate to behavior. Based on the results reported here, we believe that future experiments using the SNr–SC pathway and eye movements should provide a powerful model system in which to explore the contribution of SNr activity to saccade control, downstream effects of DBS on the discharge properties of SC neurons, and also the mechanism of DBS on behavior.

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