Neural Activity in Primate Caudate Nucleus Associated With Pro- and Antisaccades

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Ford KA, Everling S. Neural activity in primate caudate nucleus associated with pro- and antisaccades. J Neurophysiol 102: 2334–2341, 2009. First published August 19, 2009; doi:10.1152/jn.00125.2009. The basal ganglia (BG) play a central role in movement and it has been demonstrated that the discharge rate of neurons in these structures are modulated by the behavioral context of a given task. Here we used the antisaccade task, in which a saccade toward a flashed visual stimulus must be inhibited in favor of a saccade to the opposite location, to investigate the role of the caudate nucleus, a major input structure of the BG, in flexible behavior. In this study, we recorded extracellular neuronal activity while monkeys performed pro- and antisaccade trials. We identified two populations of neurons: those that preferred contralateral saccades (CSNs) and those that preferred ipsilateral saccades (ISNs). CSNs increased their firing rates for prosaccades, but not for antisaccades, and ISNs increased their firing rates for antisaccades, but not for prosaccades. We propose a model in which CSNs project to the direct BG pathway, facilitating saccades, and ISNs project to the indirect pathway, suppressing saccades. This model suggests one possible mechanism by which these neuronal populations could be modulating activity in the superior colliculus.

INTRODUCTION

The flexible control of behavior requires the appropriate mapping between a stimulus and a response. This mapping puts a given action into a behavioral context, informing us of how to properly respond to the complex demands of our environment. Often, this process is complicated by the necessity of inhibiting an automatic response in favor of a voluntary goal-directed behavior. The antisaccade task (Hallett 1978) requires subjects to inhibit a saccade to a flashed visual stimulus and, instead, to generate a saccade away from it. It has been widely used to investigate behavioral flexibility and response suppression in both humans (Fischer and Weber 1992; Hallett 1978; Hallett and Adams 1980) and monkeys (Amador et al. 1998; Everling et al. 1998, 1999; Schlag-Rey et al. 1997).

A number of clinical populations show deficits on antisaccade task performance including patients with schizophrenia (Broerse et al. 2001), attention-deficit hyperactivity disorder (ADHD) (Munoz et al. 2003), Tourette’s syndrome (LeVasseur et al. 2001), Parkinson’s disease (Briand et al. 1999), and Huntington’s disease (Lasker et al. 1987). Notably, both Parkinson’s and Huntington’s diseases indicate involvement of the basal ganglia (BG) (Purdon et al. 1994; Sharp and Ross 1996). In fact, work by Rivaud-Pechoux and colleagues (2007) examining Parkinson’s patients has provided further evidence the BG may participate in the generation of antisaccades in randomized trial presentations. Evidence shows that BG neurons are strongly modulated by the behavioral context of a given task (Apicella et al. 1992; Hikosaka and Wurtz 1983a; Hikosaka et al. 1989b,c; Lau and Glimcher 2007; Lauwereyns et al. 2002; Yoshida and Tanaka 2008). Further evidence of the valuable role of the BG in the flexible control of behavior comes from functional magnetic resonance imaging studies in both humans (Rauenackers et al. 2002, 2006) and monkeys (Ford et al. 2009). These studies have revealed higher activation in the BG during antisaccades compared with prosaccades. Despite this evidence, the role of the BG in reflexive saccade inhibition remains difficult to interpret, as human lesions in the striatum and thalamus do not appear to influence antisaccade error rates (Condy et al. 2004). The caudate nucleus (CN) is a major input structure in the BG. Neurons in the CN project both directly to the substantia nigra pars reticulata (SNr) and indirectly to the SNr via the globus pallidus external (GPe) and subthalamic nucleus (STN) (Hikosaka et al. 2000; Uuter and Basso 2008). Evidence shows that the SNr, an output nucleus of the BG, plays an important role in saccade initiation by releasing the superior colliculus from tonic inhibition immediately prior to saccade initiation (Wurtz and Hikosaka 1986). Therefore the CN represents a vital node in BG processing and is ideally situated to influence the flexible control of behavior through both direct and indirect projections to the SNr. Indeed, neurons in the CN show activity that is time locked to voluntary saccades (Hikosaka et al. 1989a, 2000), and inactivation of the CN causes disorders of voluntary saccades (Kori et al. 1995). In this study, we examined the role of the CN in the antisaccade task by recording extracellular neuronal activity while monkeys performed randomly interleaved pro- and antisaccade trials.

METHODS

Data were obtained from two male rhesus monkeys (Macaca mulatta, 10 and 8 kg). All training, surgical, and experimental procedures described were in accordance with the Canadian Council of Animal Care policy on the use of laboratory animals and approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care. Standard surgical procedures were used to prepare both animals for chronic electrophysiological recordings (Demouza and Everling 2004).

Extracellular neuronal activity was recorded from the body of the right caudate nucleus (CN) in both monkey G and monkey C. Anatomical images obtained using magnetic resonance imaging were used to guide the placement of recording chambers. A computer-controlled multielectrode microdrive (NAN drive; Plexon, Dallas, TX) was used to drive arrays of one to four dura-penetrating tungsten
microelectrodes (#UE-WLGDSMNNIE; FHC, Bowdoin, ME). Electrodes were advanced until the activity of one or more neurons in the CN could be isolated. To ensure an unbiased sampling of caudate activity, all well-isolated neurons were recorded. Monkeys performed the experimental paradigm (see following text) while neural activity was recorded and neurons were not prescreened for task-related responses. Monkeys performed, on average, 120 ± 5 correct trials (range 29–229) for each recorded neuron. Neural activity was amplified, filtered, and digitized and waveforms were stored for off-line waveform sorting applying principal-component analysis (Off-line Sorter; Plexon).

The presentation of stimuli, control of the behavioral paradigm, and delivery of rewards were performed by two Pentium PCs running a real-time data acquisition system for conducting neurophysiological and behavioral experiments (CORTEX; http://www.cortex.salk.edu). Eye position was monitored at 500 Hz using infrared video-based eye tracking (Eyelink II; SR Research, Toronto, ON). Monkeys were first trained to perform a simple saccade task consisting of a central stimulus that was followed by the appearance of a peripheral target 8° to the left or right of the central fixation point. Once performance on this task reached 85% accuracy, training for antisaccades began. To train the monkeys to perform antisaccades the central fixation point was presented as one of two colors, red or green. The red central fixation point indicated a prosaccade trial and only one peripheral target was presented. On antisaccade training trials the initial task was color matching the central green fixation point with the peripheral green target 8° to the left or right and ignoring the red distracter target at the diametrically opposite peripheral location. These trials were randomly interleaved with prosaccade trials and the colors were counterbalanced for the second animal. After a sufficient level of performance was achieved (>85%), the circumference and luminance of the antisaccade target stimulus were decreased until the visual target was removed.

Monkeys performed randomly interleaved prosaccade and antisaccade trials, as shown in Fig. 1A. Trials began with the presentation of a central fixation point on a black background, the color of which conveyed the instruction to generate either a pro- or antisaccade on stimulus presentation (monkey C, green filled circle, 0.2° for antisaccades).

![Fig. 1. A: visual presentation of stimuli for prosaccades (top) and antisaccades (bottom). Monkeys performed randomly interleaved prosaccades and antisaccades trials, with equal probability of left or right peripheral stimulus presentations. Monkeys were trained to look toward the peripheral stimulus on prosaccade trials and to look away from the peripheral stimulus on antisaccade trials. B: distribution of saccadic reaction times combined across both monkeys for correct (filled bars) and error (unfilled bars) prosaccades (top) and antisaccades (bottom). The numbers of saccades are plotted on the y-axis, whereas saccadic reaction times are plotted on the x-axis.]
cade trials, red filled circle, 0.2° for prosaccade trials; monkey G, green filled circle, 0.2° for prosaccade trials, red filled circle, 0.2° for antisaccade trials). Monkeys were given 2,000 ms to acquire the central fixation point. They were required to maintain fixation within a 2 × 2° window for 700 ms, at which point a peripheral stimulus (red filled circle, 0.2°) was flashed. The location of the flashed stimulus was pseudorandomly chosen with equal probability: either 8° to the left or 8° to the right of the central fixation point. Monkeys were trained to look toward the peripheral stimulus (within a window of 5 × 5° surrounding the stimulus) on prosaccade trials and to look away from the peripheral stimulus to its mirror position in the opposite hemifield on antisaccade trials, within 500 ms of stimulus presentation, for a liquid reward. Trials in which the monkey either broke fixation prematurely or failed to execute the correct response were aborted and no reward was given.

Data analysis

Task-related neurons were identified as those CN neurons that exhibited perisaccadic responses. Saccade onset was defined as the time when the radial eye velocity exceeded 30°/s. The times for each saccade onset were checked by an experimenter and incorrectly marked trials were removed. We found that saccades with reaction times of <110 ms had a nearly 50% chance of going in the right direction (see Fig. 1B). We therefore excluded all trials with reaction times of <110 ms from any analysis. For each neuron, we performed two-way ANOVA with the factors saccade direction (ipsilateral or contralateral to recording site) and task (prosaccade, antisaccade) on the activity in an interval of 200 ms centered at saccade onset (perisaccade window). For each trial, we measured baseline activity in a 200-ms window ending at peripheral stimulus onset. This baseline was subtracted from perisaccade activity.

We defined each neuron’s preferred saccade direction as the direction that was associated with the largest change in discharge rate for either pro- or antisaccades, thus yielding two populations of neurons: contralateral saccade neurons (CSNs) and ipsilateral saccade neurons (ISNs).

Next, we compared the activity of these saccade-related neurons between ipsilateral and contralateral saccades aligned on both stimulus presentation and saccade onset. This allowed us to look for preparatory differences aligned on stimulus presentation and saccade-related differences aligned on saccade onset. To determine whether presaccade activity differed between ipsilateral and contralateral saccades, we compared the mean activity levels over the period from 100 to 20 ms prior to saccade onset for both CSNs and ISNs. This window was chosen based on evidence that stimulating in the CN induces eye movements with latencies not <20 ms (Kitama et al. 1991).

All analyses were based on raw spike rate data. To construct peristimulus and perisaccade histograms, data were smoothed by convolving each recorded spike with an asymmetric function that resembled a postsynaptic potential (Thompson et al. 1996).

RESULTS

Extracellular activity was recorded from a total of 222 neurons in the body of the right CN of two monkeys (84 in monkey G; 138 in monkey C). A large majority of neurons in the CN are medium-spiny projection neurons (Kita 1993), whereas interneurons comprise only 5–7% of the entire striatal neuronal population (Kita 1994; Phelps et al. 1985). Based on their discharge characteristics (Hikosaka et al. 1989a) we assume that the majority of the neurons in this study are medium-spiny projection neurons.

To identify task-related neurons, we performed a two-way ANOVA with the factors saccade direction and task on the neural activity in an interval of 200 ms centered at saccade onset (see METHODS). We identified 13 neurons that showed a...
main effect of task, 22 neurons that showed a main effect of saccade direction, 5 neurons that showed both main effects, and 28 neurons that showed an interaction \((P < 0.05)\). In all, 68 of 222 (31%) neurons showed some form of selectivity (saccade direction, task, or interaction) (26% in monkey G; 33% in monkey C).

For the remainder of this report, we focus on those neurons that increased their discharge rate around the time of the saccade, in the interval of 200 ms centered at saccade onset (41/68, 60%). Figure 2 shows example responses. We defined each neuron’s preferred saccade direction as the direction associated with the largest increase in discharge rate for either pro- or antisaccades. The majority of neurons showed greater responses when the saccade was directed to the field contralateral to the recording site (27/41, 65.9% preferred contralateral saccades, CSN; 14/41, 34.1% preferred ipsilateral saccades, ISN). Figure 3A shows the location of all neurons recorded and Fig. 3B shows the distribution of neurons identified as CSNs and ISNs. We did not find any difference in the distribution of CSNs and ISNs.

Figure 2A shows an example of the activity of a neuron that was more active for contralateral than for ipsilateral saccades. This CSN exhibited a phasic increase in discharge rate for contralateral saccades that peaked after saccade onset and a brief pause in activity after the onset of ipsilateral saccades. This neuron also had a higher level of activity prior to the saccade for contralateral prosaccades compared with that for ipsilateral prosaccades but did not exhibit any different levels of presaccade activity between ipsilateral and contralateral antisaccades. Figure 2B illustrates the activity of a neuron that was classified as an ISN. This ISN had no change in activity for contralateral versus ipsilateral prosaccades, but increased its firing rate for ipsilateral antisaccades. This neuron also had a higher level of presaccade activity for ipsilateral antisaccades compared with that for contralateral antisaccades.

We then compared the population activity of these saccade-related neurons between ipsilateral and contralateral saccades. Figure 4, A and B shows the activity of the population of CSNs aligned on stimulus presentation and saccade onset, respectively. To determine whether the stimulus-related activity differed between ipsilateral and contralateral saccades, we compared the mean activity levels in the period 50–150 ms after stimulus onset for the population of contralateral neurons (Fig. 4A). The mean (±SD) discharge rate of CSNs was 16.7 ± 3.4 spikes/s before contralateral prosaccades and 14.5 ± 3.6 spikes/s before ipsilateral prosaccades. This difference was significant \(t\)-test, \(P < 0.01\). No differences were found between contralateral (17.2 ± 3.0 spikes/s) and ipsilateral antisaccades (18.7 ± 3.1 spikes/s) \(t\)-test, \(P = 0.21\). To determine whether the presaccade activity differed between ipsilateral and contralateral saccades, we compared the mean activity levels in the period from 100 to 20 ms prior to saccade onset for the population of contralateral neurons (Fig. 4B). The mean (±SD) discharge rate of CSNs was 17.6 ± 3.3 spikes/s before contralateral prosaccades and 14.9 ± 2.7 spikes/s before ipsilateral prosaccades, a difference that was significant \(t\)-test, \(P < 0.05\). No differences were found between contralateral (16.7 ± 2.6 spikes/s) and ipsilateral antisaccades (16.6 ± 2.8 spikes/s) \(t\)-test, \(P = 0.86\).

Figure 4, C and D shows the activity of the population of ISNs aligned on stimulus presentation and saccade onset. Again we compared the mean activity levels in the period 50 to 150 ms after stimulus onset for the population of ipsilateral neurons (Fig. 4C). The mean (±SD) discharge rate of ISNs was 15.2 ± 3.9 spikes/s before contralateral prosaccades and 14.6 ± 3.1 spikes/s before ipsilateral prosaccades, a difference that was not significant \(t\)-test, \(P < 0.68\). In addition, no differences were found between contralateral (17.9 ± 5.6 spikes/s) and ipsilateral antisaccades (15.6 ± 4.1 spikes/s) \(t\)-test, \(P = 0.22\). We also compared the mean activity levels in the period from 100 to 20 ms prior to saccade onset for the population of ipsilateral neurons (Fig. 4D). These neurons were more active prior to ipsilateral antisaccades (17.9 ± 3.1 spikes/s) than to contralateral antisaccades (12.1 ± 2.5 spikes/s) \(t\)-test, \(P < 0.02\). The presaccade activity of these neurons was also significantly higher for ipsilateral antisaccades compared with that for ipsilateral prosaccades \(t\)-test, \(P < 0.05\). The presaccade activity levels of these neurons did not differ for ipsilateral (15.5 ± 2.8 spikes/s) versus contralateral prosaccades (13.5 ± 3.7 spikes/s) \(t\)-test, \(P = 0.39\). In addition, we examined the prestimulus or preparatory activity (±200 to 0 ms prior to stimulus onset). We found that during this preparatory period there was no difference in the mean activity of ISNs between pro- and antisaccades. The mean discharge rate (±SD) was 15.5 ± 3.5 spikes/s for prosaccades and 16.2 ± 4.1 spikes/s for antisaccades \(t\)-test, \(P = 0.21\). CSNs did show differences in preparatory activity between pro- and antisaccades. The mean discharge rate (±SD) was
14.9 ± 3.0 spikes/s for prosaccades and 16.9 ± 3.1 spikes/s for antisaccades (t-test, P < 0.01).

Thus our results show stimulus-related activity differed for CSNs across saccade directions for prosaccades but not for antisaccades. No differences were found in stimulus-related activity for ISNs. In contrast, the presaccade activity of CSNs was different across the saccade directions for prosaccades but not for antisaccades, whereas the presaccade activity of ISNs differed between the saccade directions for antisaccades but not prosaccades.

**DISCUSSION**

In this study, we examined the role of the caudate nucleus in antisaccade task performance by recording extracellular neuronal activity while monkeys performed both pro- and antisaccade trials. We identified two populations of neurons: those that preferred contralateral saccades (CSNs) and those that preferred ipsilateral saccades (ISNs). Consistent with previous findings, many neurons exhibited their peak discharges very close to or after saccade onset—thus too late to be involved in triggering of the saccade (Hikosaka et al. 1989a; Lau and Glimcher 2007; Watanabe et al. 2003). Many neurons also showed presaccadic activity (Hikosaka et al. 1989a; Lau and Glimcher 2007; Watanabe et al. 2003). CSN and ISN populations showed distinctive response patterns, with CSNs increasing their firing rates for prosaccades, but not for antisaccades, and ISNs increasing their firing rates for antisaccades, but not for prosaccades.

What potential functional roles might CSNs and ISNs be playing in pro- and antisaccade task performance? Moreover,
how can we explain the discharge patterns of these two populations of neurons? Evidence suggests activation of the direct pathway through the BG facilitates movement (Gerfen et al. 1990; Hikosaka 1989) and may therefore mediate saccade initiation. Excitation of neurons in the caudate nucleus inhibits tonically active substantia nigra pars reticulata (SNr) neurons, in turn disinhibiting the superior colliculus (SC) (Hikosaka and Wurtz 1983b). Once the SC is released from inhibition, SC saccade-related neurons can discharge, ultimately producing the desired saccade. We hypothesize that the CSN population projects via the direct BG pathway. Figure 5 shows how the pathway through the BG might operate. We have demonstrated that CSNs in the CN have increased presaccade activity for contralateral prosaccades and decreased presaccade activity for ipsilateral prosaccades (Fig. 5, caudate, blue arrows), but no change for antisaccades (Fig. 5, caudate, red bar). We hypothesize that these neurons project through the direct pathway and inhibit the tonically active neurons in the SNr. This in turn leads to decreased activity in the ipsilateral SC on prosaccade trials (Fig. 5, left SC, blue arrow) and increased activity in the contralateral SC on prosaccade trials (Fig. 5, right SC, blue arrow) (Everling et al. 1999), leading to the initiation of prosaccade.

By contrast, the indirect BG pathway inhibits movement (Gerfen et al. 1990). This pathway may mediate saccade suppression by either maintaining or increasing tonic inhibition of the SC. Excitation of neurons in the CN inhibits activity in the globus pallidus external segment (GPe). Because GPe neurons are inhibitory (Smith and Bolam 1990), their inhibition leads to an increase in activity in the subthalamic nucleus (STN). Activation of the STN excites SNr neurons via an excitatory projection from STN to SNr (Hammond et al. 1978; Nakanishi et al. 1987); thus the SNr increases tonic inhibition on neurons in the SC, thereby suppressing saccade initiation. We hypothesize that ISNs project via this indirect BG pathway (Fig. 5). We have shown here that ISNs in the caudate nucleus have increased presaccade activity for ipsilateral antisaccades and decreased presaccade activity for contralateral antisaccades (Fig. 5, caudate, red arrows), but show no differences between ipsilateral and contralateral prosaccades (Fig. 5, caudate, blue bar). This discharge pattern would lead to an increase in activity of neurons in ipsilateral SNr and a decrease in the contralateral SNr; this in turn would lead to decreased activity in the ipsilateral SC (Fig. 5, right SC, red arrow) and increased activity in the contralateral SC on antisaccade trials (Fig. 5, left SC, red arrow). A similar model of BG function to that discussed here has been proposed by Watanabe and Munoz (2008). This model would predict that a lesion specific to the indirect BG pathway would lead to increased errors in the antisaccade task—indeed, clinical evidence suggests this is the case. Although human lesions localized to the striatum do not appear to influence antisaccade error rates (Condy et al. 2004), these lesions likely influenced neurons in both the direct and indirect BG pathways. In contrast, patients with Huntington’s disease, which is marked by the degeneration of neurons in the CN that project to the indirect pathway (Crossman et al. 1988; Mitchell et al. 1989), do exhibit increased error rates in the antisaccade task (Blekher et al. 2004, 2006; Lasker and Zee 1997; Lasker et al. 1987; Peltsh et al. 2008).

Although we do not know whether CSNs and ISNs receive inputs from separate cortical areas, similarities between our results and previous single-neuron recording studies in several saccade-related cortical areas suggest this is a possibility. Neurons in the frontal eye fields have been shown to have higher activity for contralateral prosaccades than for ipsilateral prosaccades and these differences are smaller for antisaccades (Everling and Munoz 2000). Our CSNs show a similar pattern of activity, with significant differences between contralateral and ipsilateral prosaccades, but no significant difference between contralateral and ipsilateral antisaccades. It is therefore possible that neurons in the frontal eye fields may project to
CSNs in the CN. In addition, previous work has shown that neurons in the prefrontal cortex (Johnston and Everling 2006) and supplementary eye fields (Schlag-Rey et al. 1997) have increased activity for antisaccades compared with that for prosaccades. This is similar to the pattern of activity we found for ISNs, with significant differences in saccade-related activity between ipsilateral and contralateral antisaccades and increased ipsilateral activity for antisaccades compared with that for prosaccades. It is therefore possible that neurons in the prefrontal cortex and supplementary eye field project to ISNs, although further work is necessary to test these hypotheses. Certainly the connections discussed earlier are not the only ones involved. Of specific note is the hyperdirect BG pathway, which projects from the cortex to the subthalamic nucleus (STN) through the output nuclei of the basal ganglia and then to the thalamus. Evidence shows lesions to the STN result in involuntary movement (Crossman et al. 1984) and the STN is involved in switching from automatic to voluntarily controlled eye movements (Isoda and Hikosaka 2008) and may therefore play a general role in saccade inhibition. In this way the hyperdirect pathway may place a role similar to the function of the indirect path discussed herein. How these pathways interact to control movement remains to be determined.

In conclusion, our study indicates a role of the caudate nucleus in antisaccade task performance and suggests a potential mechanism by which the discharge pattern of neurons in the caudate may be modulating activity in the SC.

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