

Effects of caudate microstimulation on spontaneous and purposive saccades

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Submitted 18 January 2013; accepted in final form 24 April 2013

Watanabe M, Munoz DP. Effects of caudate microstimulation on spontaneous and purposive saccades. *J Neurophysiol* 110: 334–343, 2013. First published May 1, 2013; doi:10.1152/jn.00046.2013.—Electrical stimulation has been delivered to the basal ganglia (BG) to treat intractable symptoms of a variety of clinical disorders. However, it is still unknown how such treatments improve behavioral symptoms. A difficulty of this problem is that artificial signals created by electrical stimulation interact with intrinsic signals before influencing behavior, thereby making it important to understand how such interactions between artificial and intrinsic signals occur. We addressed this issue by analyzing the effects of electrical stimulation under the following two behavioral conditions that induce different states of intrinsic signals: 1) subjects behave spontaneously without task demands; and 2) subjects perform a behavioral paradigm purposefully. We analyzed saccadic eye movements in monkeys while delivering microstimulation to the head and body of the caudate nucleus, a major input stage of the oculomotor BG. When monkeys generated spontaneous saccades, caudate microstimulation biased saccade vector endpoints toward the contralateral direction of stimulation sites. However, when caudate microstimulation was delivered during a purposive prosaccade (look toward a visual stimulus) or an antisaccade (look away from a stimulus) paradigm, it created overall ipsilateral biases by suppressing contralateral saccades more strongly than ipsilateral saccades. These results suggest that the impact of BG electrical stimulation changes dynamically depending on the state of intrinsic signals that vary under a variety of behavioral demands in everyday life.

basal ganglia; striatum; monkey; deep brain stimulation; suppression; reaction time; eye movement

ELECTRICAL STIMULATION HAS been used to treat intractable symptoms of a variety of neurological and psychiatric disorders (Perlmutter and Mink 2006). Such treatments target mainly nuclei in the basal ganglia [BG; e.g., deep brain stimulation in the subthalamic nucleus (STN) for Parkinson's disease (Benazzouz et al. 1996; Limousin et al. 1998) and in the ventral striatum for depression and obsessive compulsive disorder (Aouizerate et al. 2009; Bewernick et al. 2010)]. Although these treatments have been accepted widely, it is still unknown how they improve behavioral symptoms. One of the problems in addressing this issue is that behavior is controlled not only by artificial signals created by electrical stimulation, but also by intrinsic signals generated within inherent neural circuits. To understand the mechanisms underlying the therapeutic effects of electrical stimulation, it is important to uncover how artificial and intrinsic signals interact within neural circuits controlling behavior.

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The saccade control system is well-suited to challenge this issue because it has been studied extensively within and outside of the BG (Hikosaka et al. 2000; Munoz and Everling 2004; Scudder et al. 2002; Sparks 2002; Watanabe and Munoz 2011a). A previous study has shown that microstimulation in the head and body of the caudate nucleus, a major input stage of the oculomotor BG, biases saccades generated spontaneously toward the contralateral direction in cats (Kitama et al. 1991). Similar results have been reported in the tail of monkey caudate nucleus (Yamamoto et al. 2012). When there is no behavioral demand to perform a task, the cortical and BG oculomotor network is not recruited (Matsumura et al. 1992; Hikosaka et al. 1989; Hikosaka and Wurtz 1983; Bruce and Goldberg 1985; but see Schlag and Schlag-Rey 1987). Accordingly, interactions between artificial signals created by electrical stimulation and intrinsic signals generated within the oculomotor network are presumably limited under these circumstances.

In contrast, we have shown previously that microstimulation delivered to the head and body of the caudate nucleus suppresses saccade initiation in monkeys when they generate purposive saccades during a behavioral paradigm that requires directing a saccade toward a peripheral visual stimulus (prosaccade) or to the opposite direction of the stimulus (antisaccade; Watanabe and Munoz 2010b, 2011b). The recruitment of the cortical and BG oculomotor network is critical to perform this paradigm (Amador et al. 2004; Everling and Desouza 2005; Everling and Munoz 2000; Gottlieb and Goldberg 1999; Kunimatsu and Tanaka 2010; Watanabe and Munoz 2009; Yoshida and Tanaka 2009). Behavioral outcomes are determined presumably by interactions between intrinsic signals within the oculomotor network and artificial signals created by caudate microstimulation.

Differences in interactions between artificial and intrinsic signals might account for saccade facilitation and suppression induced by caudate microstimulation. However, it could be explained by other factors such as differences in stimulation sites (head/body and tail) or a difference in species (monkeys and cats). To resolve this issue, we examined microstimulation effects in the head and body of the caudate nucleus on both spontaneous and purposive saccades in the same monkeys.

METHODS

General. All experimental procedures were conducted in accordance with the Canadian Council on Animal Care policy on the use and care of laboratory animals and approved by the Queen's University Animal Care Committee. Surgical and electrophysiological procedures were described previously (Marino et al. 2008). Briefly, two male monkeys (*Macaca mulatta*), weighing 10 and 13.5 kg, were implanted with scleral search coils, a head-restraining device, and a

recording chamber. Horizontal and vertical eye positions were sampled at 1 kHz using the search-coil technique (Fuchs and Robinson 1966; Judge et al. 1980; Robinson 1963). Eye position data were processed offline by a digital filter (2nd-order Butterworth low-pass filter with the cutoff frequency of 200 Hz). The onset and end of saccades (amplitudes $>1^\circ$ and peak velocities $>100^\circ/\text{s}$) were identified by radial eye velocity criteria (threshold: $30^\circ/\text{s}$). The recording chambers were placed on the left hemisphere in both monkeys to cover the head and body of the caudate nucleus (circular, 19-mm inner diameter, tilted by 34° laterally and 13° anteriorly in *monkey O* and 36° laterally in *monkey E*). Using the grid system (Crist et al. 1988), we mapped the caudate nucleus as widely as possible in the area accessible by each chamber.

Behavioral paradigms. Monkeys performed the following two paradigms: 1) free-viewing paradigm for spontaneous saccades; and 2) pro- and antisaccade paradigm for purposive saccades. The sequence of the paradigms was counterbalanced across stimulation sites. In the free-viewing paradigm, monkeys viewed a blank screen freely. On each trial, we set a computer-controlled window ($\pm 20^\circ$) on the center of the screen and waited for up to 30 s for the eyes to enter the window. We analyzed first saccades initiated at least 300 ms after eyes entered the window. Trials were ended after eyes went out of the window or 800 ms after eyes entered the window. Each trial was followed by an intertrial interval (600-ms minimum) during which the screen remained blank. On half of the trials, microstimulation (see below) was delivered from 300 ms after eyes entered the window and lasted until the end of the trial (microstimulation duration: averages \pm standard deviation = 478 ± 65 ms in *monkey E* and 470 ± 79 ms in *monkey O*). Control and microstimulation trials were randomly interleaved in each block of trials. These task parameters resulted in ~ 4 -s intervals between consecutive microstimulation trains (i.e., from the end of the previous microstimulation train to the start of the current train; averages \pm standard deviation = 3.8 ± 7.7 s in *monkey E* and 4.3 ± 7.0 s in *monkey O*).

We also trained the monkeys to perform a randomly interleaved prosaccade (look toward a stimulus) and antisaccade paradigm (Bell et al. 2000). Each trial was preceded by a 600-ms intertrial interval during which the screen was illuminated with a diffuse light. After the removal of the background light, a fixation point appeared, and the monkeys were required to direct eyes toward the fixation point within 30 s. After they maintained fixation for 900–1200 ms, a red stimulus was presented 15° either left or right from the fixation point, and the monkeys generated a saccade either toward the stimulus (prosaccade) or to the opposite direction of the stimulus (antisaccade) within 600 ms based on fixation point color (red: pro, green: anti). Another 150- to 350-ms fixation was required on the peripheral red stimulus on prosaccade trials or on a peripheral green stimulus presented at the mirror position of the peripheral red stimulus after saccade onset on antisaccade trials. The monkeys received a liquid reward after each correct performance. A 200-ms gap was introduced before stimulus appearance during which the fixation point disappeared and the monkeys maintained fixation on the blank screen. Microstimulation (see below) was delivered from 200 ms before stimulus appearance until saccade initiation on half of the trials. The pro-/anti-instructions, left/right stimulus locations, and microstimulation/control trials were randomly interleaved in each block of trials.

Spontaneous saccade analysis. To quantify the effect of microstimulation on horizontal saccade endpoints, we calculated the following index (DeAngelis and Uka 2003):

$$\text{endpoint index} = \frac{M - C}{|M - C| + 2\text{RMS}_{\text{error}}} \quad (1)$$

$$\text{RMS}_{\text{error}} = \sqrt{\text{SSE}/(N - 2)} \quad (2)$$

where M and C stand for microstimulation and control trials, respectively. Both M and C indicate the average of horizontal saccade vector

endpoints (i.e., endpoint minus initial point). Root-mean-square error ($\text{RMS}_{\text{error}}$) was calculated using Eq. 2. SSE is the squared sum error around the averages on control and microstimulation trials. N indicates the total number of trials. The absolute value of this index is close to 1 if the difference between the averages of horizontal saccade vector endpoints on microstimulation and control trials ($M - C$) is much larger than the variance in horizontal saccade vector endpoints ($\text{RMS}_{\text{error}}$), whereas it is close to 0 when the difference between the averages of horizontal saccade vector endpoints is negligible compared with the variance in horizontal saccade vector endpoints. Positive and negative indices indicate that microstimulation biased saccade vector endpoints toward contralateral (contra) and ipsilateral (ipsi) direction, respectively. We used the same procedure for vertical saccade vector endpoints. Positive and negative indices indicate upward and downward biases by microstimulation, respectively.

Purposive saccade analysis. To quantify the influence of microstimulation on pro- and antisaccade reaction times, we first calculated a reaction-time index defined by the same formula as the endpoint index (Eq. 1) with the replacement of saccade vector endpoints to reaction times (Watanabe and Munoz 2010b, 2011b). Positive and negative indices indicate that microstimulation prolonged and shortened reaction times, respectively.

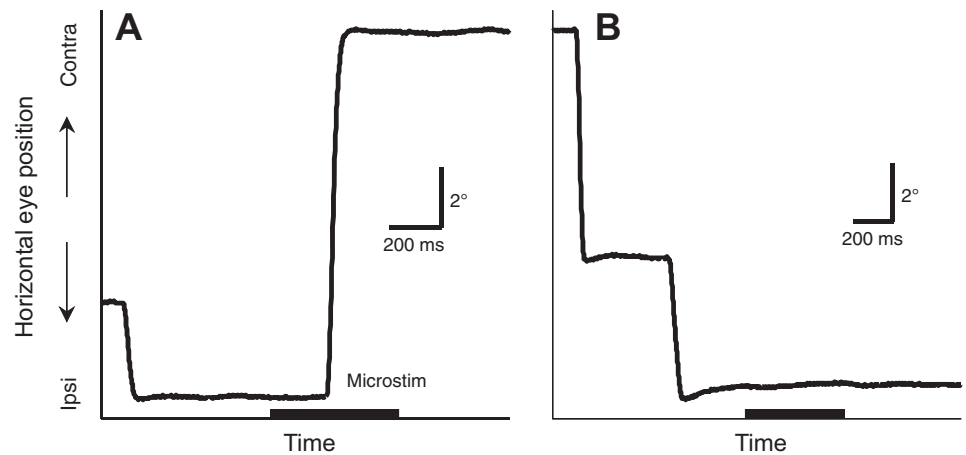
We have shown previously that the effects of microstimulation on reaction times were highly correlated between pro- and antisaccade trials [*monkey E*: Pearson's $r = 0.63$ (contra) and 0.77 (ipsi), both $P < 0.0001$; *monkey O*: $r = 0.61$ (contra) and 0.67 (ipsi), both $P < 0.0001$; see also Fig. 5 in Watanabe and Munoz 2010b]. We therefore characterized direction biases induced by caudate microstimulation at each stimulation site using the following multiple-linear-regression analyses and treat task instruction (pro/anti) as a control variable:

$$\text{normalized reaction times} = a_0 + a_1 \times \text{saccade direction} + a_2 \times \text{task instruction} \quad (3)$$

where α is the regression coefficient, saccade direction indicates contralateral (+1) or ipsilateral (−1) direction, and task instruction indicates a pro- (+1) or an antisaccade (−1) instruction. Normalized reaction time was calculated in each condition (pro-contra, pro-ipsi, anti-contra, and anti-ipsi) by normalizing reaction times on trials with microstimulation by the average and standard deviation of reaction times on corresponding control trials. Adding an interaction term between saccade direction and task instruction did not change the results. We report the t values of regression coefficients (i.e., regression coefficient divided by standard error; the same conclusion was confirmed by the analyses of regression coefficients). Trials with reaction times < 70 ms or > 600 ms during a pro- and antisaccade paradigm were excluded from data analysis.

Electrical microstimulation. Constant-current charge-balanced biphasic pulses (anode-first, 500- μs pulse width, 50 μA , 100 Hz) were delivered to the caudate nucleus via a monopolar tungsten microelectrode (impedance: 0.1–1 M Ω ; Frederick Haer, Bowdoin, ME) using a stimulator (Grass S88; Grass Technologies, West Warwick, RI) attached to a pair of constant-current stimulus isolation units (Grass PSIU6). Current was measured by the voltage drop across a 1-k Ω resistor in series with the return lead of the stimulator. We chose the polarity of anode-first because it induced stronger suppression effects on contralateral pro- and antisaccades than the opposite polarity (i.e., cathode-first) in *monkey E* evaluated by reaction-time indices [pro: averages \pm standard deviation = 0.27 ± 0.20 (anode), 0.17 ± 0.17 (cathode), $t_{(23)} = 2.96$, $P < 0.01$ (paired t -test); anti: 0.29 ± 0.15 (anode), 0.20 ± 0.18 (cathode), $t_{(20)} = 2.15$, $P < 0.05$; Tehovnik et al. 2003]. Stimulation sites were reconstructed and confirmed by MRI (3 T; Siemens) in one monkey (*monkey O*) whose implant was compatible with MRI.

Fig. 1. Horizontal eye traces on single trials with microstimulation (Microstim) at an example stimulation site in *monkey O*. *A*: single trial with a contralateral (Contra) saccade generated during microstimulation delivery. Ipsi, ipsilateral. *B*: another trial without saccade generation during microstimulation delivery. Black bars on the horizontal axes indicate the temporal periods of microstimulation delivery.



RESULTS

Spontaneous saccades during free-viewing paradigm. We delivered microstimulation at 162 sites in the head and body of the caudate nucleus (45 and 117 in *monkeys E* and *O*, respectively) while monkeys viewed a blank screen freely. Figure 1 shows two representative horizontal eye position traces from single trials with microstimulation at an example stimulation site in *monkey O*. During microstimulation delivery (indicated by the black bar on the *x*-axis), a saccade was sometimes generated toward the contralateral direction (e.g., Fig. 1*A*). Because the latency of the saccade from the initiation of microstimulation delivery was long (~ 350 ms), it seems likely that this saccade was not evoked directly by caudate microstimulation but rather biased its endpoint toward the contralateral direction. Indeed, on another example trial (Fig. 1*B*), a saccade was not generated by the same microstimulation.

When saccades were generated during microstimulation delivery, their vector endpoints were biased consistently toward the contralateral direction. Figure 2 reveals this for a representative stimulation site (same site as in Fig. 1). Saccade vector endpoints were clustered in the contralateral hemifield on microstimulation trials (Fig. 2*B*) compared with control trials [Fig. 2*A*; 2-sample *t*-test for horizontal endpoints: $t_{(163)} = 8.0$, $P < 0.0001$; saccade vector initial points were aligned at the origin]. In contrast, vertical endpoints were not systematically affected by this microstimulation [$t_{(163)} = 0.92$, $P > 0.3$].

Fig. 2. Effects of microstimulation on the saccade vector endpoints of spontaneous saccades at the same example stimulation site shown in Fig. 1. *A*: control trials. *B*: microstimulation trials. Each data point indicates the endpoint of each saccade for which the initial point is aligned at the origin. Gray triangles indicate an area within $\pm 45^\circ$ from the contralateral horizontal meridian, used for the latency analysis shown in Figs. 4 and 5. deg, Degrees.

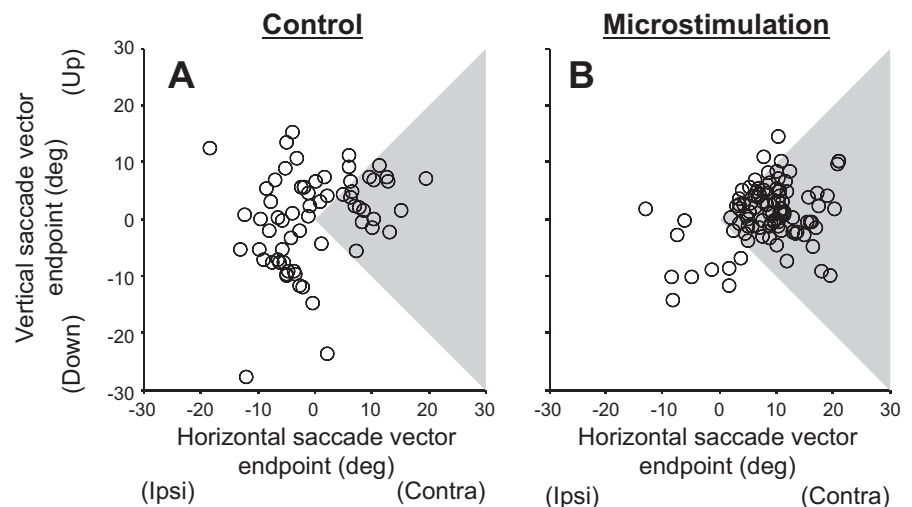


Figure 3 summarizes the effects of microstimulation on saccade vector endpoints across stimulation sites. We found that horizontal endpoint indices were biased toward positive values [1-sample *t*-test in *monkey E*: $t_{(44)} = 5.67$, $P < 0.0001$; *monkey O*: $t_{(116)} = 8.63$, $P < 0.0001$], indicating that microstimulation biased saccade vector endpoints toward the contralateral direction. We did not find a consistent bias along the vertical axis between the two monkeys [slight upward bias in *monkey E*: $t_{(44)} = 2.83$, $P < 0.01$; no bias in *monkey O*: $t_{(116)} = -1.75$, $P > 0.05$].

The latencies of saccades were not time-locked to the initiation of microstimulation delivery at the same example stimulation site shown in Figs. 1 and 2 (Fig. 4). For this analysis, we classified saccades based on their directions [$\pm 45^\circ$ from the contralateral horizontal meridian (gray triangles in Fig. 2 and Fig. 4*A*, *inset*) and the other directions (white regions in Fig. 2 and gray region in Fig. 4*B*, *inset*)]. Saccades toward the contralateral direction had latencies that were not clustered at a specific time but rather distributed evenly (black bars in Fig. 4*A*). A similar distribution was also observed in the latencies of saccades toward the other directions on control trials (white bars in Fig. 4*B*; latencies on control trials were calculated from 300 ms after eyes entered a computer-controlled window, at which stimulation was initiated on microstimulation trials). The gradual increases in cumulative distributions (Fig. 4, *C* and *D*) reflect the uniform distributions of latencies on both micro-

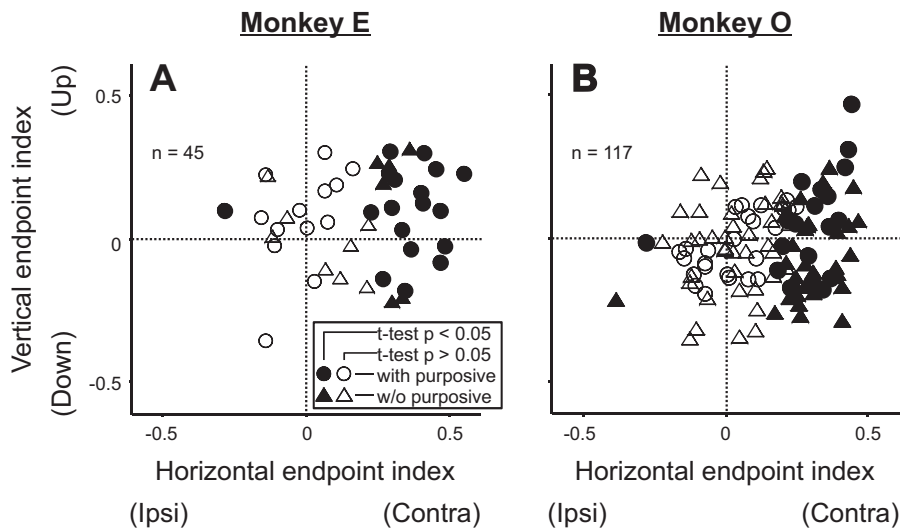


Fig. 3. Summary of microstimulation effects on the saccade vector endpoints of spontaneous saccades. *A*: monkey *E* ($n = 63$). *B*: monkey *O* ($n = 117$). Filled markers indicate stimulation sites where microstimulation biased horizontal saccade vector endpoints significantly ($P < 0.05$, *t*-test). Circles and triangles indicate stimulation sites where purposive saccades were examined or not. w/o, Without.

stimulation and control trials. Note that the summations of cumulative frequencies for saccades toward all directions did not reach 100% because there were trials on which saccades were not generated within the period of this analysis (500 ms after microstimulation onset).

To evaluate the timing of spontaneous saccades from microstimulation onset across stimulation sites, we collapsed data from stimulation sites where microstimulation biased spontaneous sac-

cades toward the contralateral direction (black markers with positive values of horizontal endpoint indices in Fig. 3; 23 and 49 sites in monkeys *E* and *O*, respectively). Figure 5, *A* and *B*, shows the cumulative distributions of the latencies of saccades toward the contralateral direction with their angles limited within $\pm 45^\circ$ from the contralateral horizontal meridian. The latencies of remaining saccades are shown in Fig. 5, *C* and *D*. The frequency of contralateral saccades was increased by caudate

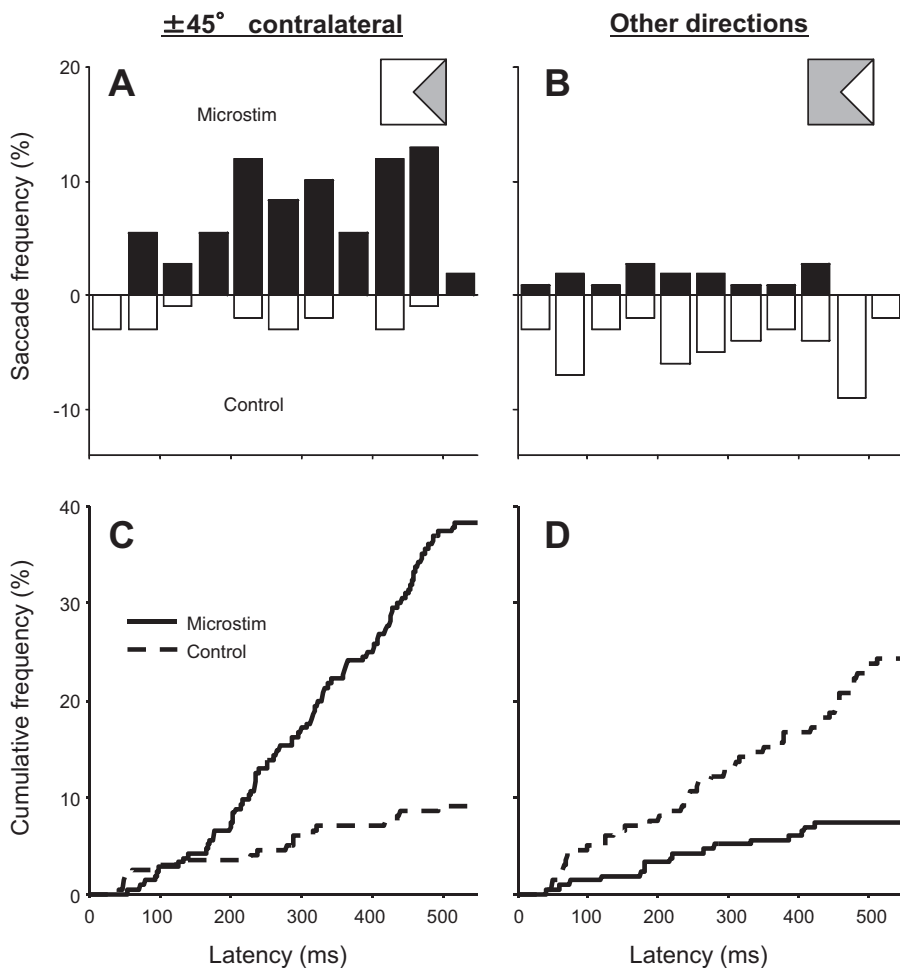


Fig. 4. Latencies of saccades from microstimulation onset at the same example stimulation site shown in Figs. 1 and 2. *A* and *B*: latency distribution of saccades directed within $\pm 45^\circ$ from the contralateral horizontal meridian (*A*; gray triangles in the inset) and toward other directions (*B*; gray areas in the inset), respectively. *C* and *D*: cumulative frequencies of latencies shown in *A* and *B*, respectively.

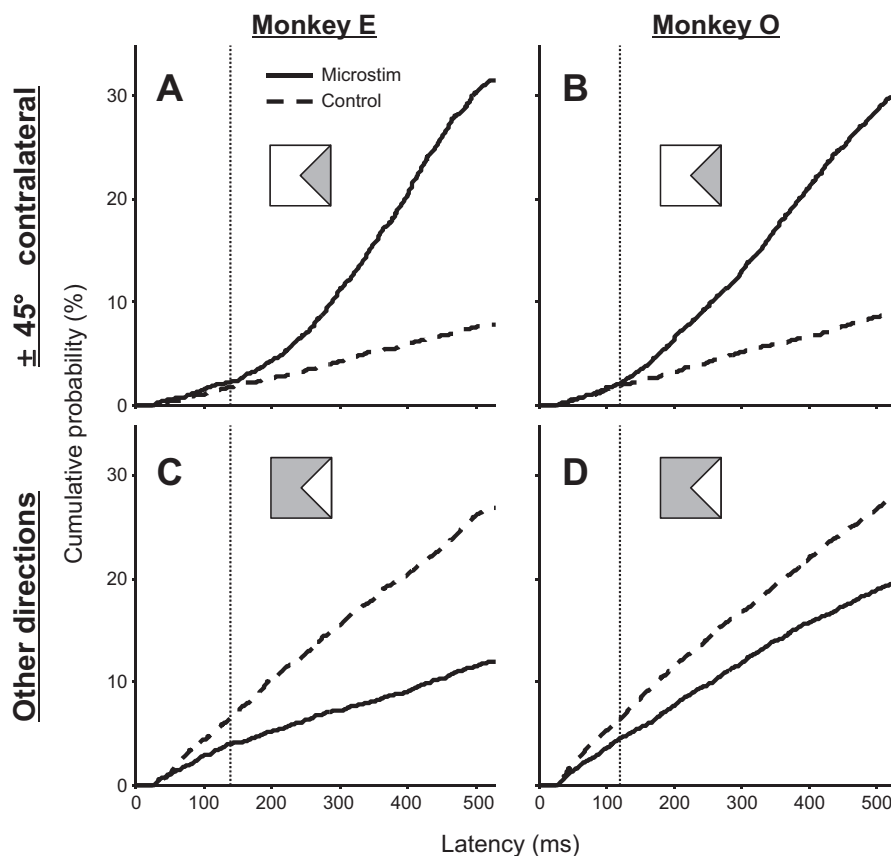


Fig. 5. Summary of microstimulation effects on spontaneous saccade latencies. *A* and *B*: saccades directed within $\pm 45^\circ$ from the contralateral horizontal meridian (gray triangles in the inset) in monkeys *E* and *O*, respectively. *C* and *D*: saccades toward other directions (gray areas in the inset) in monkeys *E* and *O*. Vertical dotted lines in each panel indicate the time when the distributions of microstimulation trials deviated from those of control trials (140 and 120 ms for monkeys *E* and *O*, respectively). This analysis was limited to stimulation sites where microstimulation biased spontaneous saccades toward the contralateral direction (black markers with positive values of horizontal endpoint indices in Fig. 3; $n = 35$ and 51 sites in monkeys *E* and *O*, respectively).

microstimulation (Fig. 5, *A* and *B*), whereas the frequency of saccades to the other directions was decreased at the same time (Fig. 5, *C* and *D*). The cumulative distributions for microstimulation trials started deviating from those for control trials at ~ 130 ms after the initiation of microstimulation (monkey *E*: 140 ms; monkey *O*: 120 ms, determined by χ^2 tests applied by 10 ms with Bonferroni correction).

The above results indicate clearly that microstimulation in the head and body of the caudate nucleus created contralateral biases for spontaneous saccades in monkeys, consistent with the previous report in cats (Kitama et al. 1991) and in the tail of the caudate nucleus in monkeys (Yamamoto et al. 2012).

Purposive saccades during pro- and antisaccade paradigm. We delivered microstimulation at 228 sites in the head and body of the caudate nucleus in the same monkeys (119 and 109 sites in monkeys *E* and *O*, respectively) while they generated purposive saccades during the pro- and antisaccade paradigm. Figure 6 shows results from the same stimulation site shown in Figs. 1 and 2. Microstimulation prolonged the reaction times of prosaccades when they were directed toward the contralateral side [Fig. 6*A*; 2-sample t -test: $t_{(43)} = 4.53$, $P < 0.0001$]. In contrast, the same microstimulation did not influence ipsilateral prosaccades [Fig. 6*B*; $t_{(45)} = 1.83$, $P > 0.05$]. Therefore, microstimulation created an overall ipsilateral bias on prosaccade trials. At this particular stimulation site, microstimulation did not influence the reaction times of antisaccades [contra: $t_{(32)} = 0.88$, $P > 0.3$; ipsi: $t_{(35)} = 1.76$, $P > 0.05$].

We found overall ipsilateral biases in purposive saccades across stimulation sites (Fig. 7). We first quantified the effects of caudate microstimulation on pro- and antisaccade reaction times using the same methods that we used previously (reac-

tion-time index; Eq. 1 with the replacement of saccade vector endpoints to reaction times; positive indices indicate prolonged reaction times by microstimulation). We found stronger suppression effects on contralateral than ipsilateral saccades [repeated-measures 2-way analysis of variance with the main factors of saccade direction (contra/ipsi) and task instruction (pro/anti); saccade direction: $F_{(1,118)} = 13.1$, $P < 0.0005$ (monkey *E*), $F_{(1,108)} = 52.9$, $P < 0.0001$ (monkey *O*)]. This analysis also confirmed our previous observations that suppression effects were stronger on prosaccades than antisaccades [task instruction: $F_{(1,118)} = 4.5$, $P < 0.05$ (monkey *E*), $F_{(1,118)} = 29.1$, $P < 0.0001$ (monkey *O*); Watanabe and Munoz 2010b, 2011b].

To quantify the overall ipsilateral biases induced by caudate microstimulation at individual stimulation sites, we used multiple linear regressions (Eq. 3). The distributions of t values for saccade direction (regression coefficient divided by standard error) were biased toward positive values in both monkeys [Fig. 5; monkey *E*: $t_{(118)} = 4.6$, $P < 0.0001$; monkey *O*: $t_{(108)} = 7.3$, $P < 0.0001$]. This confirms that microstimulation suppressed contralateral saccades more strongly than ipsilateral saccades and created overall response biases toward the ipsilateral direction for purposive saccades.

Because the effects of caudate microstimulation on direction errors are correlated with those on reaction times (Watanabe and Munoz 2010b), ipsilateral biases observed in reaction times could also be reflected in direction errors. We confirmed this prediction in monkey *O* by the following analysis. We compared changes in direction error rates induced by microstimulation ($\Delta = \% \text{direction errors on microstimulation trials minus \% \text{direction errors on control trials}$) on pro- and antisac-

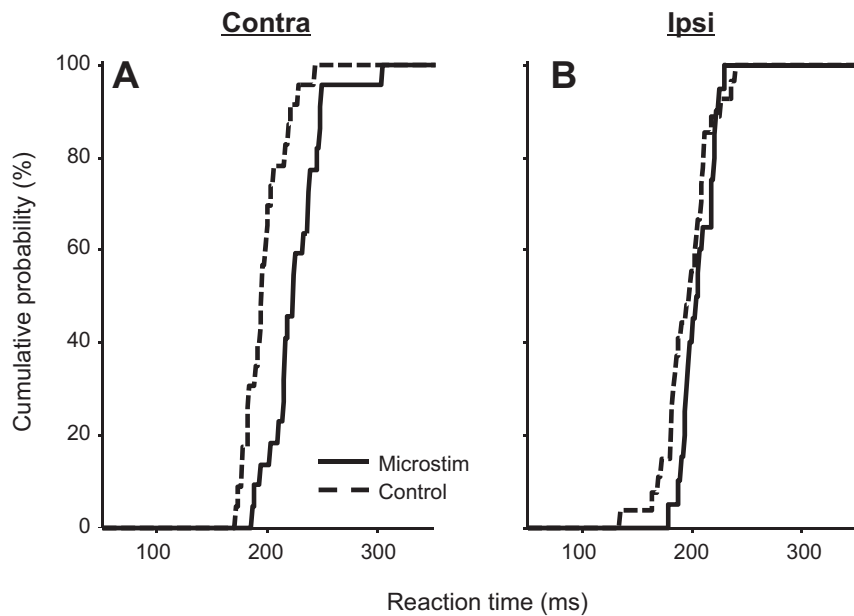


Fig. 6. Effects of microstimulation on the reaction times of purposive prosaccades at the same stimulation site shown in Figs. 1, 2, and 4. *A*: contralateral saccade trials. Microstimulation delayed saccade initiation. *B*: ipsilateral saccade trials. Microstimulation did not influence saccade initiation.

cade trials with the same stimulus location (e.g., contralateral prosaccade trials vs. ipsilateral antisaccade trials). We designed this comparison because antisaccade behavior is determined by competition between saccade commands programmed automatically toward the stimulus and those programmed volitionally to the opposite direction of the stimulus (e.g., Watanabe and Munoz 2009). On trials with an ipsilateral stimulus, microstimulation increased direction errors on antisaccade trials [$\Delta_{\text{anti}} = 9.1 \pm 24.7$ (averages \pm standard deviation)] more strongly than those on prosaccade trials [$\Delta_{\text{pro}} = 0.5 \pm 9.3$; $t_{(108)} = 3.21$, $P < 0.005$ (paired t -test)], indicating overall ipsilateral biases. In contrast, on trials with a contralateral stimulus, microstimulation increased direction errors on prosaccade trials more strongly ($\Delta_{\text{pro}} = 5.3 \pm 12.2$) than those on antisaccade trials [$\Delta_{\text{anti}} = -1.9 \pm 10.2$; $t_{(108)} = 4.1$, $P < 0.0001$], again indicating overall ipsilateral biases. Such biases in direction error rates were not confirmed in *monkey E* [contra stimulus: $\Delta_{\text{pro}} = 5.2 \pm 14.0$, $\Delta_{\text{anti}} = 8.5 \pm 20.9$, $t_{(118)} = 1.4$, $P > 0.1$; ipsi stimulus: $\Delta_{\text{pro}} = 3.6 \pm 11.0$, $\Delta_{\text{anti}} = 3.5 \pm 12.9$, $t_{(118)} = 0.1$, $P > 0.9$].

Relationship between spontaneous and purposive saccades. Microstimulation induced contralateral biases in spontaneous saccades (Figs. 3 and 5), whereas it induced ipsilateral biases in purposive saccades (Figs. 7 and 8). The opposite direction

biases induced by microstimulation might be explained by differences in stimulation sites within the head/body of the caudate nucleus. To control for this, we examined the effects of microstimulation on both spontaneous and purposive saccades at 75 sites (31 and 44 in *monkey E* and *O*, respectively).

At these selected stimulation sites, we confirmed the contralateral biases of spontaneous horizontal saccade vector endpoints [circles in Fig. 3; *monkey E*: $t_{(30)} = 4.51$, $P < 0.0001$; *monkey O*: $t_{(43)} = 4.91$, $P < 0.0001$]. We also confirmed the ipsilateral biases of purposive saccades [black bars in Fig. 8; *monkey E*: $t_{(30)} = 3.2$, $P < 0.005$ (*monkey E*), $t_{(43)} = 2.1$, $P < 0.05$ (*monkey O*)]. There was no correlation between contralateral biases in spontaneous saccades and ipsilateral biases in purposive saccades [Pearson's $r = -0.27$, $P > 0.1$, $n = 31$ (*monkey E*); $r = -0.13$, $P > 0.4$, $n = 44$ (*monkey O*)].

These results suggest that the contralateral biases of spontaneous saccades and the ipsilateral biases of purposive saccades might be mediated by independent mechanisms.

Topography of stimulation sites. We examined whether direction biases in spontaneous and purposive saccades created by microstimulation depended on locations within the head and body of the caudate nucleus (Fig. 9). We focused this analysis on 75 stimulation sites where we examined both spontaneous and purposive saccades (*monkey E*: 31 sites; *monkey O*: 44

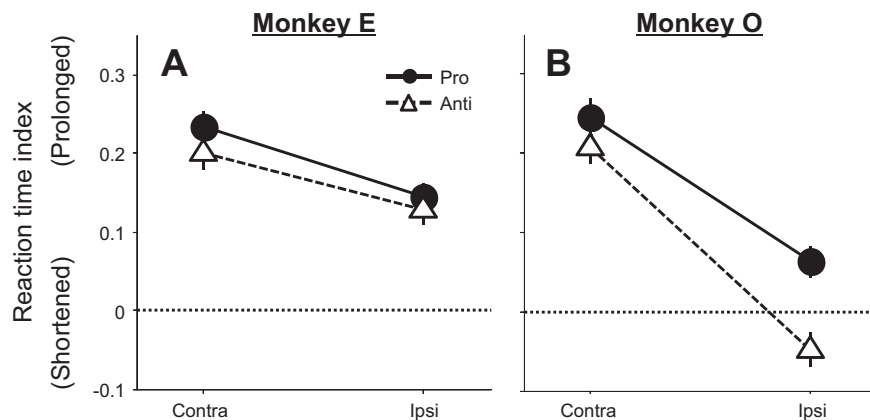
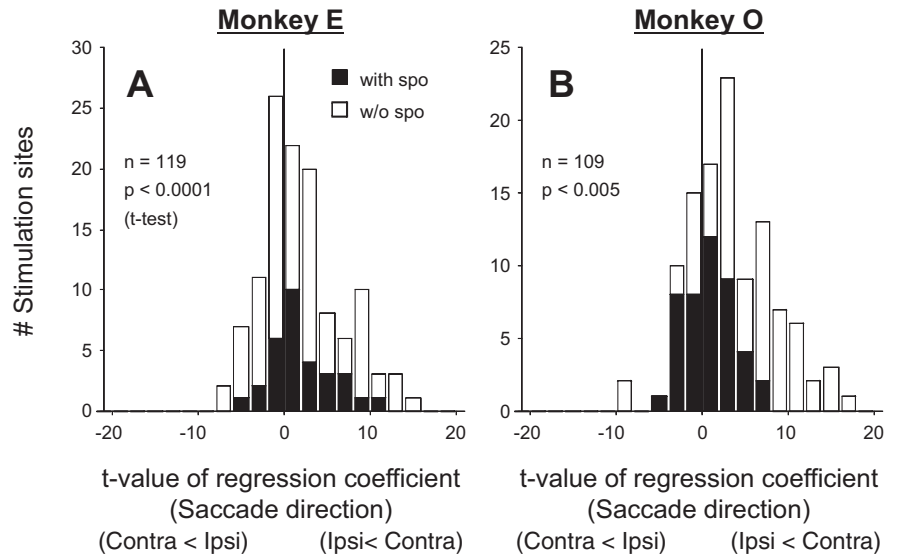


Fig. 7. Summary of microstimulation effects on purposive saccade reaction times. *A*: *monkey E* ($n = 119$). *B*: *monkey O* ($n = 109$). Reaction-time indices (Eq. 1 with the replacement of saccade vector endpoints to reaction times; positive indices indicate prolonged reaction times by microstimulation) were quantified at individual stimulation sites and averaged. Positive and negative values indicate that microstimulation prolonged and shortened reaction times, respectively. Error bars indicate standard errors. Pro, prosaccade; Anti, antisaccade.

Fig. 8. Direction biases in purposive saccades at individual stimulation sites. *A: monkey E* ($n = 119$). *B: monkey O* ($n = 109$). t Values of regression coefficients for saccade direction (Eq. 3) were quantified at individual stimulation sites. Positive values indicate that microstimulation induced stronger suppression effects on contralateral saccades than ipsilateral saccades. Black bars indicate stimulation sites where spontaneous saccades (spo) were examined. #, Number of.



sites). Stimulation sites spanned from -2 mm posterior to 5 mm anterior with respect to the anterior commissure along the rostral-caudal axis in both monkeys. In *monkey E*, sampling was rather limited in the dorsal-lateral region (approximately 3–7 mm from the midline along the medial-lateral axis and 5 mm from the dorsal surface along the dorsal-ventral axis). In *monkey O*, stimulation sites were distributed fairly along the

medial-lateral (1–6 mm from the midline) and the dorsal-ventral (7 mm from the dorsal surface) axes. For spontaneous saccades, horizontal endpoint indices were correlated with coordinates along the anterior-posterior axis [*monkey E*: Pearson's $r = 0.42$, $P < 0.05$ (Fig. 9A); *monkey O*: $r = 0.32$, $P < 0.05$ (Fig. 9D)] and the dorsal-ventral axis [*monkey E*: $r = 0.40$, $P < 0.05$ (Fig. 9C); *monkey O*: $r = 0.31$,

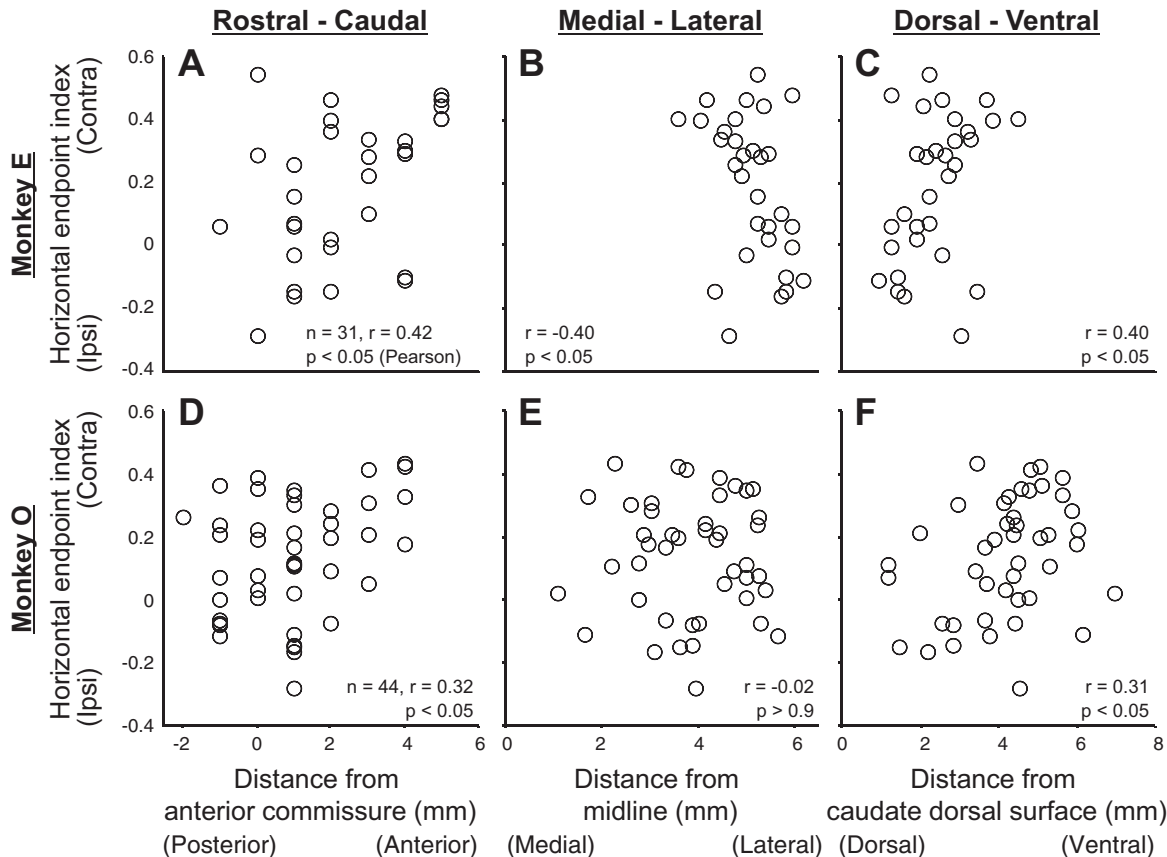


Fig. 9. A–F: correlation between horizontal endpoint indices for spontaneous saccades (Fig. 3) and the 3-dimensional coordinates of stimulation sites. The contralateral biases of spontaneous saccades were relatively stronger at anterior-ventral stimulation sites. In *monkey E*, the coordinates of stimulation sites along the dorsal-ventral and medial-lateral axes had 1-on-1 correspondence because we examined only single penetration at each rostral-caudal level.

$P < 0.05$ (Fig. 9F)]. This indicates stronger contralateral biases at anterior-ventral stimulation sites. In *monkey E*, the absolute values of correlation coefficients were identical along the dorsal-ventral and medial-lateral axes because coordinates along these axes had one-on-one correspondence. Such correspondence was resulted because we examined only single penetration at each rostral-caudal level in *monkey E*.

For purposive saccades, there was no correlation between direction biases (regression coefficient saccade direction in Eq. 3) and one of the three-dimensional coordinates of stimulation sites (*monkey E*: $|r| < 0.34$, $P > 0.05$; *monkey O*: $|r| < 0.17$, $P > 0.2$).

These results indicate that the strength of contralateral biases in spontaneous saccades depended on the coordinates of stimulation sites, whereas ipsilateral biases in purposive saccades did not have such dependence.

DISCUSSION

We have shown that microstimulation delivered to the head and body of monkey caudate nucleus created contralateral biases for spontaneous saccades during the free-viewing paradigm (Figs. 3 and 5). However, during the pro- and antisaccade paradigm, the same microstimulation created overall ipsilateral biases for purposive saccades (Figs. 7 and 8). Although the results demonstrate that the impact of caudate microstimulation depend on the states of intrinsic signals set by the behavioral paradigms, it is still unclear how such differences emerged. Before discussing this issue, we first describe the potential mechanisms of contralateral bias for spontaneous saccades and ipsilateral bias for purposive saccades in turn.

Contralateral biases for spontaneous saccades. Microstimulation delivered to the head and body of the caudate nucleus during the free-viewing paradigm biased spontaneous saccades toward the contralateral direction (Figs. 3 and 5), which is consistent with previous reports (Kitama et al. 1991; Yamamoto et al. 2012). The gradual increase of the frequency of contralateral saccades (Fig. 5) indicates that their latencies were not fixed with respect to microstimulation onset but rather had uniform latency distributions. This suggests that caudate microstimulation did not evoke contralateral saccades directly but instead biased spontaneous saccades toward the contralateral direction when they were generated during microstimulation delivery.

The contralateral biases of spontaneous saccades are presumably explained by unbalanced spatial activity on the saccade motor map of the superior colliculus (SC), a midbrain structure integrating saccade commands from multiple brain areas and sending its signals to the brain stem (Sparks 2002). If caudate microstimulation induced higher SC activity in the same hemisphere compared with the opposite hemisphere, signals generated spontaneously outside of the SC (e.g., supplementary eye field: Schlag and Schlag-Rey 1987) and/or the fluctuations of neural activity within the SC are likely to generate motor bursts by recurrent excitatory connections controlled by NMDA receptors within the SC (Saito and Isa 2003) and trigger spontaneous saccades toward the contralateral direction. Because neural noise is not time-locked to microstimulation, the timing of contralateral saccades should be random, which should result in the uniform distribution of their latencies (Fig. 5).

The enhanced SC neural activity in the same hemisphere with respect to microstimulation delivery might be realized by

the following mechanism. The caudate nucleus sends direct projections to the substantia nigra pars reticulata (SNr), the output stage of the oculomotor BG (Hikosaka et al. 2000; Watanabe and Munoz 2011a). This direct pathway suppresses the SNr activity and facilitates contralateral saccades by disinhibiting the SC in the same hemisphere (red pathway in Fig. 10A). The recruitment of the direct pathway by caudate microstimulation could therefore bias spontaneous saccades toward the contralateral direction.

The same microstimulation presumably also recruited caudate neurons activating SNr neurons indirectly via the external segment of globus pallidus (GPe) and STN (Hikosaka et al. 2000; Watanabe and Munoz 2011a). However, the recruitment of this indirect pathway (blue pathway in Fig. 10A) would suppress contralateral saccades. To account for the contralateral biases of spontaneous saccades, the influences of the indirect pathway should be weaker than those of the direct pathway. We therefore speculate that artificial signals created by caudate microstimulation might be attenuated along the polysynaptic indirect pathway before reaching to the SNr. This idea might be supported by the fact that STN neurons do not change activity in relation to spontaneous saccades (Matsumura et al. 1992).

Lateral inhibitory interactions within the caudate nucleus (Tepper et al. 2004) may also be activated by the same microstimulation. However, we speculate that their contributions were limited because the majority of caudate neurons do not have activity in relation to spontaneous saccades (Hikosaka et al. 1989).

Ipsilateral biases for purposive saccades. Caudate microstimulation created ipsilateral biases for purposive saccades in

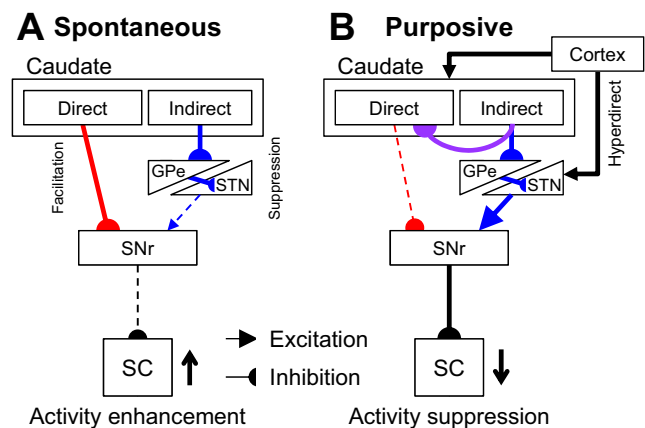


Fig. 10. Hypothetical mechanisms for saccade direction biases. **A:** contralateral biases for spontaneous saccades. Activation of the direct pathway (red) suppresses substantia nigra pars reticulata (SNr) neurons, which in turn enhances the activity of the superior colliculus (SC) and facilitates contralateral saccades. Artificial signals created by caudate microstimulation may be attenuated along the polysynaptic pathway because the subthalamic nucleus (STN) is not recruited during spontaneous saccades. **B:** ipsilateral biases for purposive saccades. Activation of lateral inhibitory interactions within the caudate nucleus (purple arrow) suppresses caudate neurons remote from the stimulation site. If these neurons give rise to the direct pathway (red), this mechanism enhances the activity of SNr neurons and suppresses the activity of SC neurons (lateral inhibition on caudate neurons giving rise to the indirect pathway is not shown for simplicity). Activation of the indirect pathway (blue) enhances the activity of SNr neurons and suppresses the activity of SC neurons. The gain of signals carried by the indirect pathway may be modulated by direct cortical input to the STN (hyperdirect pathway indicated by black arrow). GPe, external segment of globus pallidus.

both monkeys (Figs. 7 and 8). Similar ipsilateral biases have also been reported in a perceptual decision task (Ding and Gold 2012). The ipsilateral biases in purposive saccades were induced by stronger suppression of contralateral saccades than ipsilateral saccades (Fig. 7). Because of the active recruitment of the oculomotor network, including the caudate nucleus (e.g., Watanabe and Munoz 2010a), during the pro- and antisaccade paradigm, it is more challenging to infer mechanisms underlying the ipsilateral biases of purposive saccades. Nevertheless, we suggest the following two potential mechanisms that might account for our results.

First, in contrast with spontaneous saccades, lateral inhibitory interactions within the caudate nucleus (purple arrow in Fig. 10B) might have significant impact on purposive saccades and explain the overall ipsilateral bias by caudate microstimulation. This is because caudate neurons increasing activity before contralateral saccades are more prevalent than those preferring ipsilateral saccades (Hikosaka et al. 1989; Itoh et al. 2003; Watanabe and Munoz 2010a). If caudate neurons preferring contralateral saccades give rise to the direct pathway and facilitate the SC in the same hemisphere (red pathway in Fig. 10B), lateral inhibition within the caudate nucleus could attenuate signals facilitating contralateral saccades.

Second, caudate microstimulation could activate the indirect pathway and enhance inhibitory signals from the SNr (blue pathway in Fig. 10B). The effects of microstimulation on contralateral saccades were likely mediated by “uncrossed” projections from the SNr to the SC in the same hemisphere, whereas those on ipsilateral saccades were presumably mediated by “crossed” projections from the SNr to the SC in the opposite hemisphere (Cebrian et al. 2005; Jiang et al. 2003). Assuming that the indirect pathway controls both crossed and uncrossed projections, the overall ipsilateral bias in purposive saccades might be explained by stronger connection between the SNr and the SC in the same hemisphere by uncrossed projections than those in the opposite hemispheres by crossed projections (indicated by thinner blue crossed line in Fig. 8B; Cebrian et al. 2005; Jiang et al. 2003).

Because the above two mechanisms (red and blue pathways in Fig. 8B) are not mutually exclusive, they might be recruited together to generate the overall ipsilateral bias.

Opposite biases depending on behavioral demands. To account for the opposite biases for spontaneous and purposive saccades, the contributions of the direct and indirect pathways as well as lateral inhibitory interactions within caudate nucleus should change depending on which behavioral paradigm monkeys were required to perform. Because we adopted the same parameters of microstimulation and delivered electrical currents at the same stimulation sites during the free-viewing paradigm and the pro- and antisaccade paradigm, neural elements stimulated directly by microstimulation should be the same for spontaneous and purposive saccades. We speculate that the direct impact of microstimulation on the direct pathway was similar between spontaneous and purposive saccades because the connection between the caudate nucleus to the SNr is monosynaptic so that artificial activation of caudate neurons giving rise to this pathway should be transmitted to the SNr without much modification along this pathway.

In contrast, it seems likely that the impact of microstimulation on the polysynaptic indirect pathway could be modified significantly because artificial signals created by microstimu-

lation need to be transmitted through the GPe and the STN before reaching to the SNr. This hypothesis is supported by the fact that STN neurons that increase activity in relation to purposive saccades do not respond to spontaneous saccades (Matsumura et al. 1992). Furthermore, because the GPe and the STN are controlled not only by the caudate nucleus, but also by direct cortical input to the STN (known as the hyperdirect pathway; Fig. 10B; Nambu et al. 2002), it is possible that such cortical input determines the impact of microstimulation delivered to the head and body of the caudate nucleus on saccade behavior by modulating the gain of artificial signals created by microstimulation along the indirect pathway.

The impact of lateral inhibitory interactions within the caudate nucleus (purple arrow in Fig. 10B) on behavior might also change between spontaneous and purposive saccades because caudate signals facilitating purposive saccades might be suppressed by this mechanism, although such suppression should not have significant impact on spontaneous saccades because the majority of caudate neurons do not carry signals for spontaneous saccades (Hikosaka et al. 1989).

There are multiple potential mechanisms that were presumably recruited by caudate microstimulation to account for opposite direction biases between spontaneous and purposive saccades. It is therefore difficult to disentangle individual contributions of those mechanisms to behavioral outcomes by simply considering them separately. Computational simulations may help clarify this issue (e.g., Frank 2005; Gurney et al. 2001).

Conclusion. We have shown that microstimulation in the head and body of monkey caudate nucleus induced contralateral biases for spontaneous saccades, whereas it induced overall ipsilateral biases for purposive saccades. We speculate that the different direction biases are explained by anatomic constraints within the BG as well as interactions between artificial signals created by caudate microstimulation and intrinsic signals adopted for different behavioral paradigms. Further research, including computational simulations, will be required to disentangle the interactions between the artificial and intrinsic signals in the entire saccade control system. Nevertheless, our results suggest that the impact of electrical stimulation in the BG changes dynamically depending on intrinsic signals that vary under a wide variety of behavioral demands in everyday life.

ACKNOWLEDGMENTS

We thank A. Lablans for animal care, S. Hickman and M. Lewis for technical assistance, and J. Jantz for comments on this manuscript.

GRANTS

This work was supported by the Canadian Institutes of Health Research (Grant MOP-77734). D. P. Munoz was supported by the Canadian Research Chair Program.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

M.W. and D.P.M. conception and design of research; M.W. performed experiments; M.W. analyzed data; M.W. and D.P.M. interpreted results of experiments; M.W. prepared figures; M.W. drafted manuscript; M.W. and

D.P.M. edited and revised manuscript; M.W. and D.P.M. approved final version of manuscript.

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