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# Enhanced Modulation of Neuronal Activity during Antisaccades in the Primate Globus Pallidus

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## Abstract

The antisaccade task has been widely used to investigate the neural mechanisms underlying volitional movement control. In this task, subjects suppress reflexive saccades to the sudden appearance of peripheral visual stimuli (prosaccades) and generate a saccade in the opposite direction. Recent imaging studies suggest that the globus pallidus (GP) is involved in the generation of antisaccades. To understand the roles of the GP, we examined single neuron activity and the effects of local inactivation. Monkeys were trained to make either a pro- or antisaccade according to prior instruction provided by the color of the fixation point in each trial. Among 119 saccade-related neurons, 55% showed increased firing rates associated with saccades, while the remaining neurons showed decreased firing rates. For both populations of neurons, the activity modulation was enhanced during the preparation and execution of antisaccades, as compared to prosaccades. Inactivation of the recording sites in the external segment of the GP resulted in an increase in the number of error trials in the antisaccade tasks, suggesting that signals in the GP may play roles in suppressing inadequate prosaccades in the task. Signals in the GP might regulate eye movements through the nigro-collicular descending circuitry and through the basal ganglia-thalamocortical pathways.

## Introduction

In many situations, we select one from several possible responses to an external event, depending on internal behavioral goals or imposed task rules. For example, when we drive a car, we usually stop at a yellow light, but sometimes speed up on the same yellow light. The ability to alter the stimulus-response association depending on a given environment is essential for the volitional control of movements. To investigate the neural mechanisms underlying such flexible motor control, many previous studies have adopted the antisaccade paradigm (Hallett, 1978). Typically, subjects are instructed on each block or trial to make a saccade toward (prosaccade) or away from (antisaccade) the sudden appearance of peripheral visual stimuli. Compared to prosaccades, additional neural processes are needed to perform antisaccades correctly: the brain needs to suppress reflexive saccades to the stimulus and, instead, to generate a saccade command in the opposite direction. In humans, impairments in antisaccades have been reported in subjects with a variety of neurological or psychiatric disorders, including the attention deficit hyperactivity disorder, Parkinson's disease, Huntington's disease and schizophrenia, as well as those with focal lesions in the frontal and parietal cortices (for reviews, Everling and Fischer, 1998; Munoz and Everling, 2004). Consistent with the deficits in patients with cortical lesions and the basal ganglia diseases, imaging studies have revealed activation during antisaccades in many cortical areas as well as in the lentiform nucleus (the putamen and the globus pallidus) and the thalamus (O'Driscoll et al., 1995; Matsuda et al., 2004; Tu et al., 2006).

The purpose of the present study was to elucidate the roles of the globus pallidus (GP) in the generation of antisaccades. Although a previous clinical study suggested virtually no role when tested with a block of antisaccade trials (Vermersch et al., 1996), recent imaging studies have repeatedly detected significant activation in the GP during antisaccades (Matsuda et al., 2004; Tu et al., 2006). The external segment of the GP (GPe) contains neurons related to saccades (Kato and

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Hikosaka, 1995), and has traditionally been viewed as a part of the indirect pathway that relays signals in the striatum to the output nodes of the basal ganglia via the subthalamic nucleus (Alexander and Crutcher, 1990). Recent anatomical data show that neurons in the GPe also have direct access to all other nuclei in the basal ganglia (for reviews, Parent and Hazrati, 1995b; Chan et al., 2005), suggesting that the GPe plays a pivotal role in regulating the signal flow within the basal ganglia. The internal segment of the GP (GPi) has been thought to control somatic rather than ocular movements, but effects of deep brain stimulation in the GPi on eye movements have been reported (Straube et al., 1998; Fawcett et al., 2005). We examined the activities of many saccade-related neurons in the GPe and some in the GPi, when monkeys performed pro- or antisaccades depending on a prior instruction that was provided during each trial. Similarly to neurons in the supplementary eye field (SEF, Schlag-Rey et al., 1997; Amador et al., 2004) and the substantia nigra pars reticulata (SNr, Gore et al., 2005), the firing modulation of GP neurons was greater during antisaccades than during prosaccades. The following inactivation experiments confirmed that signals in the GPe play roles in the generation of antisaccades. Some of these data have appeared in an abstract form (Yoshida and Tanaka, 2007).

## **Materials and Methods**

#### Animal preparation

Experiments were conducted on two Japanese monkeys (*Macaca fuscata*, one male, 14 kg, and one female, 6 kg). All experimental protocols were approved in advance by the Animal Care and Use Committee of the Hokkaido University School of Medicine, and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The experimental procedures were similar to those described previously (Tanaka, 2005). Following initial chair training, a pair of head holders was implanted in the skull using titanium screws and dental acrylic under general halothane and pentobarbital anesthesia, and using sterile procedures. Using separate surgical

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procedures, a coil of stainless steel wire was implanted under the conjunctiva to record eye movements. During subsequent training and experimental sessions, the monkey's head was secured to the primate chair, and horizontal and vertical eye position was recorded using the search coil technique. After training for eye movement tasks, a recording cylinder was installed over a small craniotomy under the same surgical conditions. Animals received analgesia with either suppository acetaminophen or intramuscular injection of pentazocine or ketoprofen after each surgery. Topical antibiotics were administered around the implant and in the cylinder as necessary. Water intake was controlled daily so that monkeys were motivated to perform behavioral tasks.

## Visual stimulus and behavioral paradigms

Experiments were controlled by a Windows-based real-time data acquisition system (TEMPO; Reflective Computing, St. Louis, MO) running on Pentium PCs. All events were updated every 5 ms, and visual stimuli were presented on a 24-inch cathode-ray tube monitor (GDM-FW900; Sony, Tokyo, Japan; refresh rate: 60 Hz) that was located 38 cm away from the eyes, and subtended  $64 \times 44^{\circ}$  of visual angle. A 0.5° square spot served as a visual stimulus. Targets of different colors (gray, white, red and green) were used for different means in the trials (see below). Experiments were carried out in a darkened booth. Voltages proportional to horizontal and vertical eye position were calibrated before each experiment by having monkeys fixate on a stationary target spot at known visual angles. Thereafter, visual stimuli were presented in individual trials, and monkeys were rewarded with drops of water or apple juice for maintaining eye position within a 'window' that surrounded the target position at specific time intervals during each trial. The size of the window was 2° for initial fixation, and 6° (antisaccade task), 4° (memory saccade task) or 3° (prosaccade task) for the saccade target. A trial was aborted and followed by a newly selected trial if monkeys failed to maintain eye position within a specified window. Trials of different types and in different directions were presented in a random order within a block. The direction of the target step was in any of the 8 directions including four cardinal directions and four 45° oblique directions.

The present study used 3 saccade tasks (Fig. 1). In both the antisaccade task and the prosaccade task, a gray fixation point  $(10.7 \text{ cd/m}^2)$  initially appeared at the center of the screen. After 800 ms, color of the fixation point was changed either to green or red in order to instruct monkeys of the trial type. In trials with a green, unfilled fixation spot (4.6  $cd/m^2$ ), monkeys were required to perform antisaccades in response to the appearance of a peripheral target; in trials with a red spot  $(8.1 \text{ cd/m}^2)$ . they were required to perform prosaccades. Following the instruction period of 800 ms, the fixation point disappeared and a white target spot (22.6  $\text{cd/m}^2$ ) appeared 16° peripherally. Monkeys were required to make a saccade to the location diametrically opposite to the target (antisaccade task), or to the location of the target (prosaccade task) within 400 ms. Thus, in these tasks, monkeys were required to switch their eye movement responses to the same peripheral target depending on the prior instruction during fixation. In the antisaccade task, the target remained on for 400 ms, and then was relocated to a position opposite the initial location. In the prosaccade task, the target remained stationary. In both tasks, the peripheral target disappeared after a second fixation interval of 900–1200 ms, and then, a liquid reward was given. Introduction of the second fixation interval allowed us to temporally dissociate the neuronal responses to eve movements from those to reward. In the memory-guided saccade task, a white target spot appeared 16° peripherally for 200 ms during central fixation of 800–1300 ms. Monkeys were required to remember the location of the target, and to maintain fixation for an additional 800 ms. The color of the fixation point was red throughout the fixation interval in the memory-guided saccade task. Once the fixation point disappeared, monkeys made a saccade to the previously presented target location within 400 ms. The peripheral target reappeared at the same location 400 ms after the fixation point offset, and was visible for an additional 1600 ms until the end of the trial. Prior to the present experiments, both monkeys were extensively trained on a variety of memory-guided saccade tasks for another project (Tanaka 2007).

## Procedures for recording and microinjection

A tungsten microelectrode (FHC, Bowdoin, ME) was lowered through a 23-gauge guide tube using a hydraulic micromanipulator (MO-97S; Narishige, Tokyo, Japan). At the beginning of each experimental session, the location of electrode penetration was adjusted using an X-Y stage attached to the top of the cylinder. Signals through the electrodes were amplified, filtered, and monitored using oscilloscopes and an audiomonitor. For each experiment, we were able to locate the dorsal surface of the GP rather easily by recording the characteristic tonically firing neurons with relatively short action potential duration. In some penetrations, we could determine the border of the internal and external segments of the GP by comparing the pattern of baseline firing that was much more irregular in the external segment than in the internal segment, and by the existence of larger, tonic 'border neurons' between the segments (Delong, 1971). Once task-related neuronal activity was encountered, spikes of single neuron were isolated using a real-time spike sorter with template-matching algorithms (MSD; Alpha Omega Engineering, Nazareth, Israel). The occurrence of action potentials was time-stamped, and saved in files with the data of eye movements, location and timing of visual stimuli during the experiments.

When we searched for task-related neurons, monkeys performed a block of 12 randomized trials consisting of 8 memory-guided saccade trials in different directions (45° apart) and horizontal antisaccade trials and smooth pursuit trials. The pursuit trials were included because recent studies show that the basal ganglia are also involved in the control of pursuit. Once a task-related neuron was isolated, we determined the optimal eye movement direction by analyzing the data in the memory-guided saccade trials during experiments. We then configured the block of saccade trials to include tasks only along the preferred axis for the neuron under study. For neurons that showed no clear directional preference, neuronal activity was examined during horizontal eye movements.

After the recording experiments were terminated, the effects of local inactivation were examined in separate experiments. A 30-gauge injection needle connected to a microsyringe was inserted through the guide tube, and a small amount of the GABA<sub>A</sub> agonist (muscimol hydrobromide; 5.0  $\mu$ g/ $\mu$ l in saline, volume 1.0  $\mu$ l) was pressure-injected around the recording sites at a rate of ~0.2  $\mu$ l/30 s. The effect of inactivation was assessed by comparing eye movements before and 15–60 min after injection.

#### Data acquisition and analysis

Horizontal and vertical eve position signals were directly obtained from the eve coil electronics (MEL-25; Enzanshi Kogyo, Chiba, Japan). Data were digitized and sampled at 1 kHz, and were stored in files during the experiments for further off-line analysis that was performed using Matlab (Mathworks, Natick, MA). For each neuron, data were aligned on either the initiation of saccades or the onset and offset of visual fixation point. Saccades were detected using automated algorithms. After applying a 29-point finite impulse response filter to eve position data, horizontal and vertical eye velocities were obtained by digital differentiation. Saccade onset was defined as the time when the net eye speed exceeded  $40^{\circ}$ /s, and offset was defined as the time when it crossed  $20^{\circ}$ /s on the way back to zero. We determined the velocity criteria for detecting onset and offset of each saccade by comparing the raw traces of eye position and eye velocity so that the filtering procedure did not shift the timing of saccade measurements considerably. In some antisaccade trials, monkeys initially generated a saccade toward the target, but redirected their eyes to the location opposite to the target within 400 ms ("turn-around saccade"). Since we detected error trials by analyzing eye position before and 400 ms after fixation point offset during experiments, monkeys were rewarded in such trials. We detected turn-around saccades off-line and considered them as errors. When we count the number of error trials (Table 2 and Fig. 9), we also included trials in which the endpoint of primary saccade following the fixation point offset was deviated >  $8^{\circ}$  from the goal of the saccade in a given trial, or eye position 400 ms after the fixation offset was within  $6^{\circ}$  from the location diametrically opposite to the endpoint of the required saccade. Other error trials in which monkeys made early Page 9 of 56

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fixation break before the fixation offset, made non-targeting saccade (neither to the target nor to the location opposite to the target), or made no saccade within 400 ms following the fixation point offset were removed from the analysis. For inactivation experiments, we compared the occurrence of early saccades to the cue (within 4°) during the delay period before versus after inactivation, whereas those error trials were not included in the other quantitative measures shown in Fig. 9. During experiments, the error trials were repeated in the recording sessions, but were not in the inactivation experiments unless monkeys maintained fixation until the time of the fixation point offset. Traces of horizontal and vertical eye positions were reviewed with rasters and spike density profiles that were constructed from neuronal data. To obtain spike densities, means of the millisecond-by-millisecond occurrence of action potentials across multiple trials were convolved using a Gaussian filter. The  $\sigma$  of the Gaussian was 15 ms to reveal the time courses of individual neuronal activity and to construct the population activity. Other quantitative measures were performed on the basis of spike counts for specific time windows. Analytical measures are reported in the relevant text in the Results.

Directionality of saccade-related transient activity was quantified by computing the modulation index (MI), which was defined by,

## MI = |Pref - Opp| / (Pref + Opp)

where *Pref* and *Opp* indicate the firing rate measured during the 150 ms starting from 100 ms before saccade initiation for trials in the optimal saccade direction and those in the opposite direction, respectively (Fig. 4). The indices were also computed for the 150 ms postsaccadic interval starting from 50 ms after saccade initiation (Supplementary Fig. S2). The MI was close to 0 for neurons showing no directional firing modulation, whereas the value was greater for neurons showing a strong directional modulation. For each neuron, the MIs were computed for both the antisaccade trials and the prosaccade trials. We also examined the time course of directional signals in the population of GP neurons. For each saccade paradigm, the firing rates for trials in opposite directions were compared for every 50-ms window starting from 600 ms before the initiation of saccades to 600 ms after (in

25-ms steps). In this and most of the other analyses, the difference in firing rate was assessed using Wilcoxon rank-sum test. We also used *t*-test and ANOVA to evaluate statistical significance. Each statistical test that was applied is stated in the relevant text.

## *Histological procedures*

The sites of recorded neurons were reconstructed from histological sections for one monkey (monkey *E*, Fig. 10). The remaining monkey is still in use for another project. At the end of the experiments on the first monkey, several electrolytic lesions were made by passing a direct current (10–20  $\mu$ A) through the recording electrodes for 30–40 s. The monkey was deeply anesthetized with a lethal dose of pentobarbital (> 50 mg/kg), and was perfused transcardially with 0.1 M phosphate buffer followed by 10% formalin. Then, the brain was removed, blocked, and fixed with the same solution overnight. Once the brain was equilibrated with 0.1 M phosphate buffer containing 30% sucrose, histological sections were cut from each hemisphere using a freezing microtome. Sections were stained with cresyl violet.

## Results

#### Neuronal and behavioral databases

Data were collected from three GPs of two monkeys. The data were classified into two groups which were obtained from either the GPe or the GPi. For one monkey (monkey *E*, two hemispheres), the locations of recorded neurons were reconstructed from the histological sections. For the other monkey (monkey *D*, one hemisphere), the border of the segments were determined based on the stereotaxic coordinates of electrode penetrations, the relative depth from the dorsal surface of the GP, and the firing pattern of the recorded and nearby neurons (Methods). Among 203 task-related neurons, 159 (78%, n = 132 from GPe) neurons modulated their activity associated with saccades, while the remaining neurons responded to reward delivery, smooth pursuit, and/or visual stimuli. The firing

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modulation during pursuit or reward delivery was detected visually and was not analyzed further in this study. Among 120 neurons that were tested for the three saccade paradigms formally, 119 neurons showed a significant firing modulation around the time of saccades (the 300 ms period starting from 100 ms before saccade) in one or more tasks as compared to the baseline activity 300 ms before the cue (memory saccade task) or instruction (other two tasks) onset (Wilcoxon rank-sum test, P < 0.05). This study focused on the activities of these 119 saccade-related neurons. Table 1 summarizes the number of these neurons and shows that most of the data presented in this study were collected from the GPe (85%). Slightly more than half of these neurons (n = 66, 55%) elevated their firing rates before and during saccades ('increase-type' neurons), whereas the other neurons reduced the firing rates associated with saccades (n = 53, 45%, 'decrease-type' neurons). As shown in Table 1, some of these neurons also had properties that were related to the other task events.

Table 2 summarizes the behavioral data obtained during recording sessions for 119 saccade-related single neurons (90 and 29 neurons for monkeys *E* and *D*, respectively). Overall, both monkeys performed the task correctly in > 94% of trials, and made more errors in the antisaccade tasks than in the other tasks ( $\chi^2$  test, *P* < 0.001). Latencies in correct trials were similar between antisaccades and prosaccades, and were shortest for memory-guided saccades (latency distributions are shown in Supplementary Fig. S1). The multiple comparisons revealed that the latencies were significantly different between any pair of the 3 tasks (Scheffé, *P* < 0.001). The shortest reaction times for memory-guided saccades might be attributed to the fact that the directional information was provided during fixation in this task, whereas the direction of upcoming saccade was uncertain before the fixation point offset in the other tasks. The relatively small difference in latencies between antisaccades found in both monkeys could be due to the prior extensive training on the memory-guided prosaccade tasks (Methods), because the training on these tasks is known to alter saccade latencies in the block of mixed anti/prosaccade trials and to reduce the difference in latencies between the tasks (Amador et al., 1998). When the distributions of saccade latencies were examined

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(Supplementary Fig. S1), the proportions of early saccades within the 'express' range (< 90 ms) were different between the correct prosaccades and the erroneous prosaccades in the antisaccade trials (0.8% vs. 10.5% for monkey *D*, 0.03% vs. 1.4% for monkey *E*), suggesting that the monkeys somehow inhibited the occurrence of reflexive saccades to the target even in the prosaccade trials. When we compared saccade accuracy by measuring the distance between target and eye position at the end of the response period (400 ms after the fixation point offset), the values were significantly different across the paradigms (one-way ANOVA,  $P < 10^{-10}$ ).

## Activity during saccades in different paradigms and directions

Many neurons in the GP exhibited a greater firing modulation during antisaccades than during prosaccades or memory-guided saccades. Figures 2A and B plot the data from two representative neurons aligned on saccade initiation. The neuron in Fig. 2A increased the firing rate before, during and after saccades in both directions, while the neuron in Fig. 2B reduced the firing rate. For both neurons, the size of the firing modulation during antisaccades was greatest across the paradigms, irrespective of saccade directions. To assess the firing modulation in the population of neurons, we computed the population activities for trials in the preferred directions. Figure 2C plots the data for neurons that increased (left panel, n = 66) or decreased (right, n = 53) their activity during saccades, and shows that the firing modulation was enhanced during antisaccades in the population.

To quantify the saccade-related activities in the three paradigms, the firing rates of individual neurons were measured in a 150-ms window starting from 100 ms before saccades in the preferred direction (black bars in Fig. 2C). Figure 3A compares the firing rates of individual increase-type neurons for antisaccades with that for prosaccades. A one-way ANOVA and post hoc comparison revealed that the firing rates of 24 of 66 increase-type neurons (38%) were statistically different between antisaccades and prosaccades (Scheffé, P < 0.05, filled symbols in Fig. 3A). For the

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population of the increase-type neurons, multiple comparisons showed that the activity during antisaccades was statistically different from that during saccades in the other tasks (Fisher's PLSD, P < 0.05), while the activities during prosaccades and memory-guided saccades were comparable (P >0.05). Figure 3B plots the data from individual decrease-type neurons. Among 53 decrease-type neurons, 17 (32%) showed activity that was statistically different between the pro- and antisaccades (Scheffé, P < 0.05, filled symbols in Fig. 3B). In the population of the decrease-type neurons, the firing rate during antisaccades was significantly smaller than those during prosaccades and memory-guided saccades (Fisher's PLSD, P < 0.05). To examine further the enhancement of neuronal activity during antisaccades, the firing modulation before and during saccades (-100 to +50)ms of saccade initiation) relative to the baseline activity (300 ms before the instruction) was measured for individual neurons. The triangular plot in Fig. 3C shows the relative magnitude of the firing modulation across the tasks for individual neurons. To generate this figure, the size of the activity modulation in each task was normalized according to the sum of the activity modulation in the three tasks. The x-coordinate of each data point is the difference in normalized activity between the prosaccade task and the memory saccade task (memory saccade minus prosaccade), and the y-coordinate is the normalized response in the antisaccade task times  $\sqrt{3}$  or 1.732 (Koida and Komatsu, 2007). Overall, 66% (n = 79) of neurons exhibited the greatest firing modulation during antisaccades across the tasks, while only 21% (n = 25) and 13% (n = 15) neurons preferred prosaccades and memory-guided saccades, respectively. Thus, activity modulation in the GP was enhanced during antisaccades compared to the modulation seen in the other tasks.

As shown in Figs. 2A and B, many neurons exhibited a similar amount of firing modulation for saccades in opposite directions. This is consistent with the previous study showing that the tuning of directional preference of GP neurons, if any, is generally broad (Kato and Hikosaka, 1995). Indeed, when we tested the directional tuning formally for each of 41 GP neurons (20 increase-type, 21 decrease-type neurons) by fitting a Gaussian curve to the means of firing rate during memory-guided saccades in 8 different directions, the preferred directions could be reliably determined ( $r^2 > 0.7$  for the fitted Gaussian) for 22 neurons only (54%). The remaining neurons (46%) showed a similar amount of firing modulation for saccades in all directions, and the magnitudes of the modulation did not correlate with saccade directions (Mardia's circular-linear correlation coefficient, D < 0.75, P >0.05). Overall, the preferred directions of these neurons (n = 22) were distributed contralaterally (Rayleigh test, P < 0.05, mean vector length r = 0.41), whereas the distribution of preferred directions of either type of neurons alone showed no significant directional bias (Rayleigh test, r = 0.47, P =0.052 for the increase-type neurons, and r = 0.37, P > 0.10 for the decrease-type neurons). The full width at half maximum of the firing modulation (computed as  $2\sigma\sqrt{2}\ln 2$  or  $2.35\sigma$ , where  $\sigma$  was derived from the fitted Gaussian) averaged 55.2° ± 40.0° (SD) for the increase-type neurons (n = 13) and 75.4 ± 70.1 for the decrease-type neurons (n = 9).

To assess to what extent neurons in the GP carry directional signals in different tasks, we computed the modulation indices (MIs) from the data for saccades in opposite directions in both the prosaccade task and the antisaccade task. The indices were computed based on neuronal activity during the 150 ms period starting from 100 ms before saccades (Materials and Methods). Figures 4A and B plot the MIs for individual neurons that either increased (open circles) or decreased (filled triangles) the firing rates during saccades. For neurons in the GPe (Fig. 4A), only a minority of neurons showed a significant directional modulation in both the tasks (Wilcoxon rank-sum test, P < 0.05, black bars in the histograms). Interestingly, however, the size of the directional preferences was different between the types of neurons. The MIs during prosaccades averaged  $0.09 \pm 0.10$  and  $0.15 \pm 0.15$  for the increase-type neurons and the decrease-type neurons, respectively, and these values were statistically different (unpaired *t*-test, P < 0.05). During antisaccades, the MIs averaged  $0.07 \pm 0.07$  and  $0.17 \pm 0.17$  for the increase-type neurons and the decrease-type neurons, respectively, and these values were again were also different (P < 0.001). The proportions of neurons showing a directional modulation in either or both tasks (MI > 0.2, corresponding to 1.5-fold firing modulation) were also

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different between the increase-type neurons (15%, n = 8/54) and the decreased-type neurons (38%, n = 18/47, P < 0.05). For neurons in the GPi (Fig. 4B), the MIs for the increase-type neurons were significantly greater than that for the decrease-type neurons during prosaccades (unpaired *t*-test, P < 0.05), but not during antisaccades (P = 0.07). As in Fig. 4B, all 4 out of 18 GPi neurons that showed a significant directional modulation (Wilcoxon rank-sum test, P < 0.05, black bars in the histograms) were the increase-type neurons. Thus, neurons in the GP generally showed no clear directional modulation before and during saccades, while some decrease-type neurons in the GPe and increase-type neurons in the GPi carried directional information. A similar tendency was found when the MIs were computed for the post-saccadic period (50-200 ms after saccade initiation, Supplementary Fig. S2), though the proportion of directional neurons was much larger during this time interval (see below).

To examine the time courses of the directional signals carried by GP neurons, we next compared the firing rates between trials in opposite directions for every 50-ms period starting from 600 ms before saccade initiation to 600 ms after (in 25 ms steps). Figures 4C and D plot the proportions of neurons exhibiting directional modulation in each of the three tasks (Wilcoxon rank-sum test, P < 0.05) as a function of time, relative to the initiation of saccades. Because no directional information was provided during the instruction period in the antisaccade tasks and in the prosaccade tasks, only a few neurons showed directional modulation > 150 ms before saccades. In contrast, approximately 10% of GPe neurons showed directional modulation during the delay period in the memory-guided saccade task (Fig. 4C). For all three tasks, the number of neurons showing directional modulation increased just before saccades, and about 23% of GP neurons carried directional signals at the time of saccade initiation. The directional signals in the population peaked around 200 ms after saccade initiation, and the proportion of directional neurons was up to 40% of all saccade-related neurons. Thus, more than half (56%) of the saccade-related neurons in the GP did not carry directional information in any of the three saccade paradigms. About half of the remaining

neurons exhibited directional modulation only postsaccadically, suggesting that the majority of neurons in the GP may not provide immediate drive signals for saccades, whereas only a minority of the decrease-type neurons in the GPe and the increase-type neurons in the GPi that showed directional modulation before saccades might play this role.

## **Context-dependent activity modulation during fixation**

On each anti- or prosaccade trial, monkeys were required to alter their eye movement response to the same peripheral target depending on the color of the fixation point. We next examined whether neurons in the GP exhibited activity modulation related to the task instructions. Figure 5 plots the data from two representative neurons aligned on the fixation point offset. Because the saccade target appeared at the time of the fixation point offset, data for trials in opposite directions were combined to examine the firing modulation during the instruction period. The neuron plotted in Fig. 5A showed an increased firing rate during the instruction period, while that in Fig. 5B showed a decreased firing rate. For both neurons, the firing modulation was greater before antisaccades than before prosaccades, while the baseline activity (300 ms before the instruction) was not statistically different between the tasks (Wilcoxon rank-sum test, P > 0.05). For the population of neurons, the baseline activity was smaller for the increase-type neurons than the decrease-type neurons (34.2 ± 19.5 versus. 46.6 ± 23.8 spikes/s, unpaired *t*-test, P < 0.01; Supplementary Fig. S3).

To quantify the modulation of the instruction-period activity for individual neurons, we measured the firing rates during the 200 ms before the fixation point offset. Figure 6A plots the data for neurons that increased the firing rate before and during saccades. Among 66 increase-type neurons, 29 (44%) displayed instruction period activity that was significantly different between the paradigms (unpaired *t*-test, P < 0.05; filled symbols in Fig. 6A). In the population of the increase-type neurons, the instruction period activity in the antisaccade task was statistically different from that in the prosaccade task (paired *t*-test, P < 0.001). The time course of the population activities plotted in

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Fig. 6B demonstrates that the task-dependent firing modulation emerged ~500 ms following the instruction, and became more evident towards the end of the fixed instruction interval (800 ms). A similar tendency was found among the decrease-type neurons. Among 53 neurons, 26 (49%) showed task-dependent firing modulation (Fig. 6C, filled symbols). In the population of the decrease-type neurons, the firing rate for antisaccacdes was statistically different from that for prosaccades (paired *t*-test, P < 0.001), and the traces of the population activities diverged during the latter half of the instruction period (Fig. 6D). When the same analyses were performed for neurons in the GPe and GPi separately, the task-dependent firing modulation during fixation was statistically significant for neurons in the GPe (paired *t*-test, P < 0.001, Supplementary Fig. S4), but for neither type of neurons in the GPi (P > 0.05). We also found that the levels of individual neuronal activity before the target onset were not correlated with the latencies of antisaccades in the preferred direction, while only a minority of neurons showed a significant correlation (P < 0.05, increase-type neurons, n = 4/66, 6%, decrease-type neurons, n = 5/53, 9%). Correlation coefficients for the increase-type neurons averaged  $-0.05 \pm 0.27$  (SD), and those for the decrease-types neurons averaged  $-0.01 \pm 0.32$ .

Because the instruction for saccade type was given by changing the color of the fixation point, the task-dependent firing modulation during fixation could possibly be due to the color selectivity of GP neurons, rather than due to a difference in task requirements. To address this issue, we examined the activity of 14 neurons (6 increase-type, 8 decrease-type neurons) during a block of fixation trials. In this task, the color of the fixation point changed as in the antisaccade task (that is., from gray to green), but the saccade target never appeared and the fixation point remained on until the end of the trial. Monkeys were required to maintain fixation throughout the trial to obtain a reward. If the color of the fixation point was actually responsible for the greater firing modulation in the antisaccade trials, similar changes in neuronal activity would be observed in the fixation tasks. We configured a separate block of fixation trials to include 30 fixation tasks followed by repeated sequences of one horizontal antisaccade trial and 9 fixation trials (Fig. 7A). In almost all antisaccade

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trials in the fixation block, monkeys made an erroneous prosaccade to the peripheral target, most likely because they did not prepare for antisaccades in advance during the instruction period. We compared neuronal activity during fixation across three different trials: 1) fixation trials, 2) error antisaccade trials and 3) correct antisaccade trials. Data for the latter two classes were collected from both the fixation block and the preceding randomized saccade block, and then were combined for analysis. Figure 7B compares the magnitudes of activity modulation between the fixation trials and the correct antisaccade trials for the 14 neurons. The size of the activity modulation was measured as the difference in the firing rate between 600–800 ms following (instruction period) and 300 ms before (baseline) the color change of the fixation point. All but two neurons (open triangles) showed greater activity modulation in the correct antisaccade trials than in the fixation trials (multiple comparisons with Scheffé, P < 0.05). For the population of neurons, Fig. 7C plots the means (± SDs) of firing modulation during fixation across the three different trials. A one-way ANOVA and post hoc multiple comparisons revealed that the instruction period activity prior to correct antisaccades was significantly greater than those before erroneous prosaccades in the antisaccade trials and those in the fixation trials (Scheffé, P < 0.05). The time courses of the population activities for both the increase-type neurons (Fig. 7D) and the decrease-type neurons (Fig. 7E) show that the instruction period activity found in the correct antisaccade trials (green traces) almost disappeared in error trials (red traces) and in the fixation trials (blue traces). Thus, neuronal activity during the instruction period was related to the preparation for antisaccades, rather than to the color of the fixation point *per* se.

We next examined the context-dependent firing modulation during fixation on a trial-by-trial basis. Figure 8A plots the means of firing modulation (n = 14) during fixation as a function of the trial number in the fixation block. As before, the level of neuronal activity was computed as the difference in firing rate during the 300 ms before and 600–800 ms after the color change of the fixation point. Although the magnitude of firing modulation was initially comparable to

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that measured in the antisaccade trials in the preceding saccade block (Fig. 8A, open square), the firing modulation decreased gradually during the repetition of 30 fixation trials (black circles). In addition, we also found that neuronal activity in the fixation trials was modulated immediately after the error trials. Figure 8B plots the sizes of neuronal activity recorded from the 14 neurons during 9 fixation trials prior to and following an error trial in the antisaccade task. The activity was greatest immediately following the antisaccade trial, and decreased progressively during the repetition of the fixation trials. The magnitude of firing modulation in the fixation trial just after erroneous antisaccade was significantly different from that in the fixation trial immediately before the antisaccade trial (paired *t*-test, P < 0.05, n = 14). These results again confirmed that neuronal activity during the instruction period was context-dependent, suggesting that the activity was likely related to the preparation for antisaccades.

## **Effects of local inactivation**

To explore the causal roles of neuronal signals in the GPe in the generation of antisaccades, we inactivated the recording sites reversibly by injecting a small amount of the GABA<sub>A</sub> agonist, muscimol (1 µl in volume, concentration 5 µg/µl dissolved in saline). Sites of inactivation were selected from those containing saccade-related neurons found in the separate experiments. We performed 4 muscimol injection experiments and 3 saline controls in the left hemispheres of two monkeys. For each monkey, the two inactivation sites were in the same coordinate but at different depth along the penetrations (1.0 mm apart). Figure 9A illustrates the data obtained from a single inactivation experiment (site *D1*). Before inactivation, the monkey made erroneous prosaccades to the target in about 15% of the antisaccade trials in both directions (red dashed traces). Following muscimol injection into the left GPe, the error rate increased significantly in the rightward antisaccade trials (contraversive saccades,  $\chi^2$  test, *P* < 0.01), while the error rate remained unchanged in the leftward antisaccade trials (*P* = 0.75). The effects of inactivation did not appear to be biasing

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the occurrence of leftward saccades, because the monkey performed saccades correctly in all prosaccade trials in both directions during inactivation, and because the errors of making a reflexive saccade to the peripheral cue in the memory-guided saccades tasks did not increase significantly in both directions ( $\chi^2$  test, P > 0.05). For this and the other experiments, Figure 9B plots the changes in error rate following muscimol (solid lines) or saline (dashed lines) injections for both anti- and prosaccades. Data connected with thick lines were for the experiment shown in Figure 9A (site *D1*). In all 4 muscimol experiments, the rate of erroneous prosaccades increased dramatically in the antisaccade trials in one direction ( $\chi^2$  test, P < 0.01), whereas the error rates remained unchanged following saline injection. In one inactivation experiment (site *E1*), we also found clear deficits in the performance of prosaccades; the monkey made many erroneous *antisaccades* in the ipsiversive prosaccade tasks, in addition to the deficits in the ipsiversive antisaccade trials (Supplementary Fig. S5), as if they failed to assign correct responses for a given instruction. The other saccade parameters for all experiments are summarized in Supplementary Tables 1 (latency) and 2 (accuracy). Although inactivation of the GPe altered saccade latency or accuracy slightly and often significantly, we did not find out any consistent effect.

## Localization of task-related neurons

Figure 10 illustrates the sites of recording and inactivation in monkey *E*, reconstructed from histological sections. Most saccade-related neurons were found in the anterior part of the GPe, within 2 mm from the level of the anterior commissure. Consistent with the previous study (Kato and Hikosaka, 1995), many of the saccade-related neurons were located in the dorsal portion of the GPe. As summarized in Table 1, 15% of saccade-related neurons were recorded from the GPi. Other task-related neurons (pursuit or reward) were distributed rather sparsely within the GP.

## Discussion

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The present study, for the first time, provides direct evidence for the involvement of the GP in the generation of antisaccades. Neurons in the GPe exhibited significant firing modulation during both the preparation and execution of antisaccades. Since the firing modulation during fixation decreased or even disappeared when the instruction stimulus normally calling for antisaccades was presented in a block of fixation trials, we concluded that the activity during the instruction period was related to the preparation of antisaccades, rather than to the visual feature of the fixation target. We also found that the error rate in the antisaccade task increased modestly following inactivation of the recording sites in the GPe, suggesting that signals in the GPe play roles in the generation of antisaccades.

## Enhanced modulation of transient activity during antisaccades

The most conspicuous feature of neuronal activity in the GP was the strong enhancement of firing modulation during antisaccades. For 32% of individual neurons (33% of increase-type neurons and 30% of decrease-type neurons), the modulation of the firing rate immediately before and during antisaccades was statistically greater than the modulation during prosaccades or memory-guided saccades. Furthermore, for both types of GP neurons, the enhancement of activity modulation during antisaccades was also statistically significant in the population of neurons. Previous studies have shown that the firing modulation in the population of neurons during antisaccades is smaller than or comparable to that during prosaccades in many cortical areas, including the lateral prefrontal cortex (PFC, Evering and DeSouza, 2005), the frontal eye field (Everling and Munoz, 2000), the lateral intraparietal area (Gottlieb and Goldberg, 1999; Zhang and Barash, 2000) and the anterior cingulate cortex (ACC, Johnston et al., 2007), whereas a subset of individual neurons in these areas display the enhanced activity during antisaccades (e.g., Johnston et al., 2007). Similarly, the magnitude of the presaccadic burst of activity in the SC is also smaller during antisaccades than during prosaccades, whereas the saccade-related firing modulation (suppression) of the fixation neurons in the rostral SC appears to be augmented during antisaccades, possibly because the firing rates of these neurons are

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elevated during the preparation for antisaccades (Everling et al., 1999). In contrast, most neurons in the SEF (Schlag-Rey et al., 1997; Amador et al., 2004) and SNr (Gore et al., 2005), as well as the SC-projecting neurons in the lateral PFC (Johnston and Everling, 2006) exhibit greater firing modulation during antisaccades than during prosaccades, showing a significant enhancement during antisaccades in the population, just like the neurons in the GP reported in the present study.

The enhancement of firing modulation in the SEF, PFC, GP and SNr suggests a strong functional linkage between these regions during antisaccades. Anatomically, the SEF sends outputs to the basal ganglia and receives signals from the basal ganglia via the thalamus (Shook et al., 1991; Tian and Lynch, 1997). Similarly, neurons in the lateral PFC receive the basal ganglia outputs through the thalamocortical pathways (Middleton and Strick, 2002). Within the basal ganglia, the GPe regulates signals in both the GPi and SNr directly or through the subthalamic nucleus (Parent and Hazrati, 1995a,b; Chan et al., 2005), which in turn send inhibitory projections to the SC and the thalamus.

What are the roles of the saccade-related, transient signals in these structures? Figure 11 illustrates a hypothetical diagram showing how the signals in the GP could regulate antisaccades. Although it is plausible that each of the intrinsic pathways in the basal ganglia mediates multiple signals, each pathway in the diagram is assigned to play a single role during antisaccades for simplicity. Because only a minority of neurons in the GP exhibited directional firing modulation prior to saccades (Fig. 4), most GP neurons are unlikely to provide the immediate drive or movement signals for antisaccades. Instead, one possible function of the pathways through the GP might be the active suppression of unnecessary prosaccades. The signals in the GP could suppress reflexive saccades through either or both of two possible pathways. The directional neurons in the indirect pathway might suppress saccades in particular direction by sending inhibitory signals to the SC and to the thalamus unilaterally (cf, Peltsch et al., 2008). Alternatively, the signals in the hyper-direct pathway might suppress saccades omnidirectionally, giving the time to produce the command of

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correct antisaccade (Fig. 11; cf, Isoda and Hikosaka, 2007). These hypotheses are consistent with our findings that inactivation of the GPe increased the occurrence of erroneous saccades toward the target in the antisaccade task. Similarly to the previous inactivation experiments in the lateral PFC (Condy et al., 2007), we also found directional antisaccade deficits following inactivation of the GPe (Fig. 9); however, unlike the PFC data, the impaired direction was different from site to site, while the effects were consistent during each post-lesional testing that lasted up to 60 min. The laterality of the antisaccade deficits observed in this study might result from the different inactivation effects on the crossed or uncrossed innervations from the output nodes of the basal ganglia to the thalamus (Hazrati and Parent, 1991) or to the SC (Jiang et al., 2003; Beckstead et al., 1981), whereas it remains unknown whether neurons regulating the crossed or uncrossed projections cluster separately within the GPe. In addition, the idea that signals from the frontal cortex through the basal ganglia to the SC and the thalamus suppress the initiation of saccades is also consistent with the previous findings that inactivation of the SNr increases the occurrence of reflexive saccades in the memory-guided saccade task (Hikosaka and Wurtz, 1985), that the lesions in the medial frontal cortex shorten the latency of antisaccades (Boxer et al., 2006), and that stimulation in the medial frontal cortex prolongs saccade latencies (Isoda and Hikosaka, 2007). The signals at the time of antisaccades might play roles in suppressing unnecessary reflexive saccades through the projections from the basal ganglia to the SC as well as to the thalamus.

The other possible role of the transient signals in the GPi might be to boost the commands of antisaccades in the thalamus and the cortex (Fig. 11). It has been accepted widely that 'disinhibition' of the tonic inhibitory outputs from the SNr to the SC is essential for conveying the signals in the basal ganglia to the brainstem, and for giving access to the SC from the cortex (for review, Hikosaka et al., 2000). Similarly, the decrease-type neurons in the GPi might transmit non-directional signals to the thalamus by the disinhibition mechanism, enabling the enhancement of the antisaccade signals within the reciprocal positive feedback circuitry between the thalamus and the cortex. Unfortunately,

however, we could neither test nor prove the hypothesis in the present study. The hypothesis could be tested by analyzing the effects of inactivation in the GPi or the thalamus during antisaccades in the future studies.

## Context-dependent sustained activity during fixation

In addition to the transient activity during saccades, many neurons in the GP also exhibited task-related firing modulation even before the target onset. Similarly to the activity during saccades, the firing modulation during fixation was also enhanced for antisaccades compared with prosaccades. For each neuron, the direction of the firing modulation during saccade preparation was identical to that during saccade execution; the increase-type neurons elevated the firing rates during the preparation for antisaccades, while the decrease-type neurons reduced the firing rates during the preparation for antisaccades (Fig. 6). This pattern of firing modulation was similar to those reported previously in the SEF (Schlag et al., 1997), the SNr (Gore et al., 2005) and the lateral PFC (Johnston and Everling, 2006), but seems to be different from that reported in the rostral SC; the activities of the collicular fixation neurons are strongly suppressed around the time of antisaccades, but the firing rate is elevated during the preparation for antisaccades, as compared to prosaccades (Everling et al., 1999). Although it is possible that the preparatory signals in the basal ganglia regulate the firing of fixation neurons in the rostral SC, the difference in the pattern of firing modulation between the GPe and the SC suggests that the signals in the basal ganglia may not be sent exclusively to the rostral SC to suppress reflexive prosacacdes — some of the signals in the basal ganglia may regulate eye movements through the thalamocortical pathways, as discussed in the previous section. The existence of the task-related neurons in the GPi (15% of tested neurons) also supports this possibility. The elevation of regional blood flow in the thalamus during antisaccades (O'Driscoll et al., 1995; Matsuda et al., 2004; Tu et al., 2006) could be due to the signals originating from the GPi and the SNr. Another line of evidence also suggests that signals through the thalamocortical pathways likely play

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roles in the initiation of voluntary saccades (Petit et al., 1993; Vermersch et al., 1996; Tanaka, 2006, 2007).

Neuronal activity related to the task instruction has been reported in several cortical areas during antisaccades (PFC, Everling and DeSouza, 2005; ACC, Johnson and Everling, 2007; SEF, Schlag-Rey et al., 1997; Amador et al., 2004), and those activities have been thought to be related to the stimulus-response association in the task (for review, Munoz and Everling, 2004). Similarly, neurons in the GP that discriminate the tasks during the instruction period may also play this role, by sending signals through the thalamus to the cortex (Fig. 11). A recent study showed that error rates in the antisaccade trials were significantly greater for subjects with Parkinson's disease than normal controls when the antisaccade trials were mixed with the prosaccade trials randomly within a block, suggesting that the basal ganglia may play roles in online switching of eye movement responses according to a given instruction (Rivaud-Péchoux et al., 2007, but see Chan et al., 2005). Furthermore, this proposal appears to be consistent with the current view of the neural mechanisms underlying the conditional learning that implicates the basal ganglia-thalamocortical circuitry in the stimulus-response associations (Toni and Passingham, 1999; Brasted and Wise, 2004; Pasupathy and Miller, 2005; Boettiger and D'Esposito, 2005). As suggested in the somatic motor system, the cortico-basal ganglia 'cognitive' and 'oculomotor' loops might also play roles in the evolution of the rule-dependent signals for the generation of voluntary eye movements, including antisaccades. To examine the possible roles played by the thalamocortical pathways in antisaccades, further recording and inactivation experiments in the thalamus are needed.

## Notes

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## **Figure legends**

**Figure 1.** Sequence of events in the three saccade tasks. In both the antisaccade task and the prosaccade task, the color of the fixation point was initially gray, and it became green (antisaccade task) or red (prosaccade task) for 800 ms. A white target spot appeared 16° eccentrically at the time of the fixation point offset. Monkeys were required to make a saccade toward (prosaccade) or away from (antisaccade) the target within 400 ms. In the memory-guided saccade task, the color of the fixation point was red throughout the trial. A peripheral target was flashed briefly (200 ms) during central fixation, and monkeys were required to maintain fixation and remember the location of the target. After a delay of 800 ms, the fixation point disappeared and monkeys made a saccade to the location of the previously flashed target within 400 ms. All of these trials were randomly interleaved in a block.

**Figure 2.** Comparison of the responses in the 3 saccade paradigms for representative neurons. *A*, An increase-type neuron. *B*, A decrease-type neuron. In all panels, data are aligned on saccade initiation (vertical line). Trials are sorted by saccade latency, and the rasters are shown for contraversive saccades only, while the spike density traces are shown for both directions. The purple diamond on each raster line indicates the time of the fixation target offset. *C*, Traces of mean spike density for the population of neurons. The dashed traces indicate the mean +/- SE for pro- and antisaccades. The population activity was computed for the preferred direction only. The firing rates of individual neurons measured at the time of black rectangle are shown in Fig. 3. *Anti*, antisaccade; *Pro*, prosaccade; *Mem*, memory-guided saccade.

**Figure 3.** *A*, *B*, Comparison of the firing rates during antisaccades versus prosaccades for individual neurons. Filled symbols indicate the data showing a significant difference (Scheffé, P < 0.05). *C*, Relative magnitudes of the firing modulation. The size of the activity modulation (saccade activity minus baseline) in each task was normalized according to the sum of the activity modulation in the three tasks. Overall, 66% (n = 79/119) of neurons exhibited the greatest firing modulation during

antisaccades across the tasks.

**Figure 4.** Quantification of the directionality during saccades. *A*, *B*, Comparison of the modulation indices (MIs) computed for the 150 ms period starting from 100 ms before saccade initiation between the antisaccade task and the prosaccade task. Neurons with strong directional preference show greater MI values. Open circles indicate the increase-type neurons and filled triangles indicate the decrease-type neurons. Two outliers (both are decrease-type neurons) have been omitted from the plot in A. Filled bars in the histograms indicate neurons showing a significant directional modulation (Wilcoxon rank-sum test, P < 0.05). Note that the directional neurons in the GPe tended to be the decrease-type neurons (A, filled triangles), while all directional neurons in the GPi were the increase-type neurons (B, open circles). *C*, *D*, Time series analysis of the directionality for neurons in the GPe (C) and GPi (D). The proportion of directional neurons is plotted as a function of the time relative to saccade initiation. The firing rates in a 50-ms window centered at the value on the abscissa were compared for saccades in opposite directions (Wilcoxon rank-sum test, P < 0.05). Note that approximately 10% of neurons showed directional activity during the delay period in the memory-guided saccade tasks.

**Figure 5.** Activity modulation during the instruction period. *A*, An increase-type neuron. *B*, A decrease-type neuron. The color of the fixation point was different for the two saccade tasks during the periods between the two vertical lines (Instruction period). The saccade target appeared at the time of the fixation point offset (time 0). To examine the activity during the instruction, data for saccades in both directions were combined. The blue diamond on each raster line indicates the time of saccades.

Figure 6. Quantitative comparison of the instruction period activity. A, C, Activities of individual increase-type neurons (A) and decrease-type neurons (C) are compared between the antisaccade trial and the prosaccade trial. Circle indicates the GPe neurons, while triangles indicate the GPi neurons.

## **Cerebral Cortex**

The firing rate was measured for a 200-ms interval immediately before the fixation point offset (black bars in *B* and *D*). Filled symbols represent data showing a significant difference between the tasks (two-tailed *t*-test, P < 0.05). *B*, *D*, Population activities for the increase-type neurons (*B*) and the decrease-type neurons (*D*). Data are aligned on the fixation point offset. Dashed traces indicate plus or minus SEM. Supplementary Fig. S4 plots the population activities for each of the GPe and the GPi neurons separately.

**Figure 7.** Context-dependent modulation of the instruction period activity. *A*, Design of the fixation experiment. Following 30 fixation trials, an antisaccade trial was presented in every nine fixation trials. In the fixation task, the color of the fixation point changed from gray to green as in the antisaccade task, but a peripheral target never appeared throughout the trial (See Materials and Methods). *B*, Comparison of the magnitude of the instruction period activity between the successful antisaccade trials and the fixation trials for individual neurons. Data of successful antisaccade trials were obtained from a separate block, and are the same as those plotted in Fig. 6. Different shapes of symbols indicate different types of neurons. Filled symbols indicate data showing a statistically significant difference (Wilcoxon rank-sum test, P < 0.05). *C*, Comparison of the mean firing modulation during the instruction period for successful antisaccade trials ('Correct'), error antisaccade trials ('Error') and fixation trials ('Fixation'). Each data point indicates mean ( $\pm$  SD) response sizes across the 14 neurons. *D*, *E*, Time courses of the population activity for the increase-type neurons (*D*) and the decrease-type neurons (*E*). Data are aligned on the fixation point offset. Dashed traces indicate plus or minus SEM.

**Figure 8.** Immediate modulation of the instruction period activity in a block of fixation trials. *A*, Decay of neuronal activity during the repetition of 30 fixation trials. Black circles plot the means of firing modulation as a function of trial number in the block. Open square indicates the magnitude of the instruction-period activity measured from the antisaccade trials in the preceding block of saccade

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trials. *B*, Activity modulation following the antisaccade task. Means of activity modulations are plotted for the 9 fixation trials prior to and following the antisaccade task in the fixation block. Note that neuronal activity during the instruction period is minimal in the block of fixation trials, as shown in Fig. 7. The asterisk indicates significant difference in neuronal activity in comparison with that in the fixation trial immediately before the antisaccade trial (paired *t*-test, P < 0.05). For both panels, error bar indicates ± SEM.

**Figure 9.** Effects of local inactivation on the performance of antisaccades. *A*, Data obtained from antisaccade trials before and after muscimol injection. Eye position traces are aligned on the fixation point offset. Red dashed traces indicate error trials. The number on each panel indicates the proportion of error trials. *B*, Changes in error rate for all injection experiments, including 4 muscimol (solid lines) and 3 saline (dashed lines) experiments. Data connected by thick lines plot the data shown in Fig. 9A. Asterisks indicate significant inactivation effects ( $\chi^2$  test, *P* < 0.01). Data for the experiment showing the prosaccade deficits are shown in Supplementary Fig. S5.

**Figure 10.** Sites of the task-related neurons and local inactivation in monkey *E*. Different symbols are used for neurons showing different response properties. Levels of frontal sections are shown as millimeters from the anterior commissure. AC, anterior commissure; GPe and GPi, external and internal segments of the globus pallidus, respectively.

**Figure 11.** A hypothetical diagram showing how the signals in the GP could regulate antisaccades. Plus and minus signs indicate elevation and reduction of firing rates, respectively, for neurons in each structure when neurons in the cerebral cortex are activated. Note that Cx, STN and SC/Thal contain excitatory neurons, while other structures contain inhibitory neurons. About one-third of the decrease-type neurons in the GPe and the increased-type neurons in the GPi showed directional activity modulation (rectangles). Other types of neurons in the GP generally showed non-directional firing modulation before saccades. Although it is possible that each intrinsic pathway in the basal ganglia plays multiple roles, each pathway is assigned to a single function for simplicity. Cx, cerebral cortex; Cd, caudate nucleus; Put, putamen; SC, superior colliculus; SNr, substatia nigra pars reticulate; STN, subthalamic nucleus; Thal, thalamus.

| lask-related activity                     |          |          |
|---|----------|----------|
| Monkey D (29)                             |          |          |
| Increased saccade-related activity (13)   | GPe (13) | GPi (0)  |
| + visual                                  | 0        | 0        |
| + pursuit                                 | 5        | 0        |
| + reward                                  | 1        | 0        |
| Decreased saccade-related activity (16)   | GPe (14) | GPi (2)  |
| + visual                                  | 1        | 0        |
| + pursuit                                 | 5        | 1        |
| + reward                                  | 2        | 1        |
|   |          |          |
| Monkey E (90)                             |          |          |
| Increased saccade-related activity (53)   | GPe (41) | GPi (12) |
| + visual                                  | 2        | 1        |
| + pursuit                                 | 12       | 5        |
| + reward                                  | 10       | 5        |
| Decreased saccade-related activity (37)   | GPe (33) | GPi (4)  |
| + visual                                  | 1        | 0        |
| + pursuit                                 | 12       | 1        |
| + reward                                  | 13       | 1        |
| Number in parenthesis indicates subtotal. |          |          |
|   |          |          |

Table 1. Number of saccade-related neurons and those also showing other types of task-related activity

|                     | Anti         | Pro           | Mem           |
|---------------------|--------------|---------------|---------------|
| Monkey D            |              |               |               |
| N (correct/all)     | 1249/1418    | 1298/1331     | 1318/1322     |
| Error rate (%)      | 11.9         | 2.5           | 0.3           |
| Latency (ms)        | $183 \pm 35$ | $174 \pm 36$  | $143 \pm 34$  |
| Accuracy (deg)      | 2.2 ± 1.3    | $0.9 \pm 0.5$ | $1.5 \pm 0.8$ |
| Error latency (ms)  | $151 \pm 47$ | $233 \pm 47$  | $150 \pm 26$  |
|                     |              |               |               |
| Monkey E            |              |               |               |
| N (correct/all)     | 3349/3769    | 3704/3901     | 3677/3718     |
| Error rate (%)      | 11.1         | 5.1           | 1.1           |
| Latency (ms)        | 213 ± 38     | $207 \pm 36$  | $174 \pm 34$  |
| Accuracy (deg)      | 2.8 ± 1.6    | $1.1 \pm 0.7$ | $1.5 \pm 0.9$ |
| Error latency (ms)  | 201 ± 53     | 239 ± 39      | 158 ± 79      |
| Each entry shows me | ran ± SD.    | 70-6          |               |
|                     |              |               |               |

| Table 2. Comparison of | saccade parameters du | iring recording sessions. |
|------------------------|-----------------------|---------------------------|
|                        |                       |                           |



Figure 1, Yoshida & Tanaka

73x104mm (600 x 600 DPI)



114x207mm (600 x 600 DPI)





Figure 4, Yoshida & Tanaka

84x101mm (600 x 600 DPI)





Figure 5, Yoshida & Tanaka

105x104mm (600 x 600 DPI)



Figure 6, Yoshida & Tanaka

81x103mm (600 x 600 DPI)





Figure 7, Yoshida & Tanaka

85x119mm (600 x 600 DPI)







Figure 8, Yoshida & Tanaka

71x153mm (600 x 600 DPI)



Figure 9, Yoshida & Tanaka

83x136mm (600 x 600 DPI)





Figure 10, Yoshida & Tanaka

87x92mm (600 x 600 DPI)





Figure 11, Yoshida & Tanaka

82x61mm (600 x 600 DPI)





## **Supplementary Figure S1**

Distributions of saccade latencies for different tasks and monkeys. Blue bars indicate the data for correct trials, while red bars indicate those for error trials.

117x144mm (600 x 600 DPI)



## **Supplementary Figure S2**

Modulation indices computed for the post-saccadic activity (50-200 ms after saccade initiation). Red circles and blue triangles are the data for the increase-type neurons and those for the decrease-type neurons, respectively. For the data of GPe neurons, one outlier (decrease-type) has been omitted. Note that the range of both axes are different from that in Figs. 4A and B.

127x109mm (600 x 600 DPI)



## **Supplementary Figure S3**

Comparison of the baseline activity 300 ms before the instruction between the decrease-type neurons (blue symbols) and the increase-type neurons (red symbols). Despite significant overlap, the baseline firing tended to be less for the increase-type neurons than the decrease-type neurons. Five out of 119 neurons showed differential activity between the tasks (cross, Wilcoxon rank-sum test, P < 0.05). Open squares indicate the data for the neurons shown in Fig. 5.

122x102mm (600 x 600 DPI)





# **Supplemantary Figure S4** Comparison of the population activities between neurons in the GPe and the GPi.

117x150mm (600 x 600 DPI)



## **Supplemantary Figure S5** Deficits in prosaccades following inactivation of GPe (*site E1*).

108x80mm (600 x 600 DPI)

| Supplementary Table 1. Saccade latencies before and after injection. |      |            |            |             |            |            |                 |
|--|------|------------|------------|-------------|------------|------------|-----------------|
|  |      |            |            | Target lo   | ocation    |            |                 |
|  |      |            | lpsi       |             |            | Contra     |                 |
| Monkey D   |      | Anti       | Pro        | Mem         | Anti       | Pro        | Mem             |
| 1 (MUS)  | Pre  | 190 ± 5    | 187 ± 6    | 120 ± 4     | 166 ± 6    | 156 ± 2    | 117 ± 3         |
|  | Post | 237 ± 7*** | 158 ± 2*** | 119 ± 4     | 167 ± 4    | 172 ± 2*** | 129 ± 5**       |
| 2 (MUS)  | Pre  | 263 ± 11   | 168 ± 3    | 142 ± 7     | 200 ± 6    | 192 ± 4    | 175 ± 7         |
|  | Post | 268 ± 9    | 170 ± 4    | 172 ± 4 *** | 237 ± 11** | 178 ± 2**  | 164 ± 6         |
| 3 (saline)   | Pre  | 211 ± 10   | 175 ± 10   | 131 ± 14    | 196 ± 14   | 167 ± 4    | 162 ± 7         |
|  | Post | 216 ± 6    | 165 ± 4    | 124 ± 7     | 188 ± 7    | 151 ± 2*** | $138 \pm 6^{*}$ |
|  |      |            |            |             |            |            |                 |
| Monkey E   |      | Anti       | Pro        | Mem         | Anti       | Pro        | Mem             |
| 1 (MUS)  | Pre  | 162 ± 3    | 179 ± 3    | 150 ± 5     | 194 ± 8    | 175 ± 4    | 158 ± 3         |
|  | Post | 177 ± 5*   | 221 ± 6*** | 170 ± 4**   | 243 ± 9*** | 193 ± 5*   | 177 ± 4***      |
| 2 (MUS)  | Pre  | 211 ± 6    | 207 ± 6    | 169 ± 6     | 221 ± 6    | 168 ± 3    | 158 ± 4         |
|  | Post | 232 ± 10*  | 202 ± 6    | 178 ± 9     | 237 ± 11   | 195 ± 6*** | 177 ± 8*        |
| 3 (saline)   | Pre  | 196 ± 5    | 191 ± 5    | 150 ± 7     | 224 ± 10   | 187 ± 7    | 174 ± 6         |
|  | Post | 208 ± 6    | 199 ± 5    | 167 ± 9     | 201 ± 6    | 190 ± 9    | 173 ± 7         |
| 4 (saline)   | Pre  | 205 ± 4    | 185 ± 3    | 162 ± 6     | 205 ± 6    | 162 ± 4    | 172 ± 5         |
|  | Post | 201 ± 7    | 185 ± 4    | 159 ± 9     | 205 ± 8    | 187 ± 9*   | 164 ± 6         |

| Supplementary Table 1. Saccade latencies b | before and after injection. |
|--|-----------------------------|
|--|-----------------------------|

Each entry shows mean  $\pm$  SD. MUS indicates muscimol injection. \*p < 0.05, \*\*p < 0.01 and \*\*\*\*p < 0.001, Wilcoxon rank-sum test.

> 123x109mm (600 x 600 DPI) 9mm (600 x 600 ניזע)

|            |      |                | Target location |                |             |               |             |
|------------|------|----------------|-----------------|----------------|-------------|---------------|-------------|
| lpsi       |      |                | Contra          |                |             |               |             |
| Monkey D   |      | Anti           | Pro             | Mem            | Anti        | Pro           | Mem         |
| 1 (MUS)    | Pre  | 1.5 ± 0.12     | 0.8 ± 0.04      | 1.7 ± 0.1      | 1.4 ± 0.13  | 0.8 ± 0.05    | 1.1 ± 0.09  |
|            | Post | 1.9 ± 0.19     | 1.0 ± 0.03***   | 1.1 ± 0.08 *** | 1.4 ± 0.07  | 0.8 ± 0.03    | 1.1 ± 0.08  |
| 2 (MUS)    | Pre  | $2.2 \pm 0.30$ | $0.6 \pm 0.04$  | 1.1 ± 0.09     | 1.5 ± 0.15  | 0.8 ± 0.10    | 1.6 ± 0.12  |
|            | Post | 1.5 ± 0.11     | 0.8 ± 0.04***   | 0.8 ± 0.05*    | 1.4 ± 0.19  | 0.6 ± 0.05    | 1.2 ± 0.08* |
| 3 (saline) | Pre  | $2.5 \pm 0.46$ | 1.3 ± 0.06      | 1.0 ± 0.09     | 2.6 ± 0.22  | 0.8 ± 0.04    | 2.2 ± 0.19  |
|            | Post | $2.2 \pm 0.21$ | 1.7 ± 0.05***   | 1.4 ± 0.09*    | 2.4 ± 0.15  | 1.2 ± 0.06*** | 2.5 ± 0.14  |
|            |      |                |                 |                |             |               |             |
| Monkey E   |      | Anti           | Pro             | Mem            | Anti        | Pro           | Mem         |
| 1 (MUS)    | Pre  | 1.9 ± 0.16     | $0.6 \pm 0.05$  | 1.1 ± 0.10     | 2.0 ± 0.19  | 1.4 ± 0.07    | 2.0 ± 0.14  |
|            | Post | 1.9 ± 0.15     | $0.8 \pm 0.06$  | 1.2 ± 0.11     | 2.6 ± 0.34  | 1.2 ± 0.08*   | 1.7 ± 0.18* |
| 2 (MUS)    | Pre  | $2.5 \pm 0.21$ | 0.7 ± 0.05      | 1.2 ± 0.09     | 2.4 ± 0.22  | 0.8 ± 0.05    | 1.1 ± 0.12  |
|            | Post | $3.4 \pm 0.42$ | 0.6 ± 0.06      | 1.6 ± 0.20     | 3.1 ± 0.44* | 0.8 ± 0.06    | 1.4 ± 0.19  |
| 3 (saline) | Pre  | $2.6 \pm 0.33$ | $0.9 \pm 0.05$  | 0.9 ± 0.09     | 1.8 ± 0.40  | 0.7 ± 0.08    | 1.4 ± 0.13  |
|            | Post | $2.2 \pm 0.22$ | $0.9 \pm 0.07$  | 1.1 ± 0.14     | 2.8 ± 0.48  | 1.1 ± 0.14    | 1.5 ± 0.15  |
| 4 (saline) | Pre  | $2.2 \pm 0.17$ | 0.7 ± 0.05      | 1.0 ± 0.08     | 1.7 ± 0.22  | 0.6 ± 0.05    | 1.1 ± 0.10  |
|            | Post | 1.7 ± 0.17     | 0.5 ± 0.07      | 1.0 ± 0.12     | 2.4 ± 0.42* | 0.6 ± 0.08    | 1.2 ± 0.18  |

| Supplementary | Table 2. | Saccade | accuracies | before | and | after inje | ection. |
|---------------|----------|---------|------------|--------|-----|------------|---------|
|---------------|----------|---------|------------|--------|-----|------------|---------|

Each entry shows mean  $\pm$  SD. MUS indicates muscimol injection. \*p < 0.05, \*\*p < 0.01 and \*\*\*p < 0.001, Wilcoxon rank-sum test.

123x108mm (600 x 600 DPI)