Neuron

Primate nigrostriatal dopamine system regulates saccadic response inhibition

--Manuscript Draft--

Manuscript Number:	NEURON-D-18-00474R3
Full Title:	Primate nigrostriatal dopamine system regulates saccadic response inhibition
Article Type:	Research Article
Keywords:	response inhibition; inhibitory control; dopamine neurons; substantia nigra pars compacta; caudate nucleus; basal ganglia; primates
Corresponding Author:	Masayuki Matsumoto University of Tsukuba Tsukuba, Ibaraki JAPAN
First Author:	Takaya Ogasawara
Order of Authors:	Takaya Ogasawara
	Masafumi Nejime
	Masahiko Takada
	Masayuki Matsumoto, Ph.D.
Abstract:	Animals need to inhibit inappropriate actions that would lead to unwanted outcomes. Although this ability, called response inhibition, is impaired in neurological/psychiatric disorders with dopaminergic dysfunctions, how dopamine regulates response inhibition remains unclear. Here we investigated neuronal signals of the nigrostriatal dopamine system in monkeys performing a saccadic countermanding task. Subsets of dopamine neurons in the substantia nigra and striatal neurons receiving the dopaminergic input were activated when the monkey was required to cancel a planned saccadic eye movement. These activations were stronger when canceling the eye movements was successful than failed, and were enhanced in demanding trials. The activated dopamine neurons were distributed mainly in the dorsolateral, but not in the ventromedial part of the nigra. Furthermore, pharmacological blockade of dopaminergic neurotransmission in the striatum dampened the performance of canceling saccadic eye movements. The present findings indicate that disruption of the nigrostriatal dopamine signaling causes impairments in response inhibition.
Suggested Reviewers:	Naoshige Uchida Professor, Harvard University uchida@mcb.harvard.edu Dr. Uchida is one of the pioneers who worked with the physiological property of dopamine neurons and revealed many new aspects of these neurons. We would like to recommend him as a possible reviewer who could impartially judge our study.
	Peter Redgrave Professor, University of Sheffield p.redgrave@sheffield.ac.uk Dr. Redgrave is one of the pioneers who worked with the physiological and anatomical properties of dopamine neurons and established “reward-unrelated” aspects of these neurons. We would like to recommend him as a possible reviewer who could impartially judge our study.
	Minoru Kimura Professor, Tamagawa University mkimura@lab.tamagawa.ac.jp Dr. Kimura is one of the pioneers who worked with the basal ganglia and established the role of the brain structures in motivation and reinforcement. We would like to recommend him as a possible reviewer who could impartially judge our study.
	Hagai Bergman Professor, Hebrew University hagaibe@ekmd.huji.ac.il Dr. Bergman is one of the pioneers who worked with the basal ganglia and established

	the roles of the brain structures in regulating animal behaviors. We feel that his review comments would be helpful to judge our study.
Opposed Reviewers:	Wolfram Schultz Professor, University of Cambridge ws234@cam.ac.uk Dr. Schultz is famous for his influential theory, the reward theory of dopamine. However, recent studies, including our study, have reported data that are at least partly inconsistent with his theory. We are afraid that he might provide biased review comments.
	Christopher Fiorillo Associate professor, Korea Advanced Institute of Science and Technology (KAIST) fiorillo@kaist.ac.kr Dr. Fiorillo has collaborated with Dr. Schultz and also proposed the reward theory of dopamine that is at least partly inconsistent with our present findings. We are afraid that he might provide biased review comments.

Oct 10, 2018

Dear Dr. Babayan,

Enclosed please find a revised manuscript entitled “Primate nigrostriatal dopamine system regulates saccadic response inhibition (NEURON-D-18-00474R2)” by Takaya Ogasawara, Masafumi Nejime, Masahiko Takada, and Masayuki Matsumoto. We would like to submit this manuscript for publication in *Neuron* as a **Research Article**. The manuscript contains one main text (MS-Word file) with seven figures (TIFF files) and supplemental information with eight figures (PDF).

Thank you very much for your suggestions to improve our manuscript. In order to pass the restriction of 72,000 characters, as you suggested, we removed some citations, a minor analysis, and minor discussions that are not directly related to the present study. The submitted main manuscript is completely the same as the manuscript that I sent you by email in advance. As you suggested, we also revised our manuscript so that it can fulfill Neuron’s stylistic guidelines. Please let us know if further changes are necessary.

With best regards,

Masayuki Matsumoto, PhD
Division of Biomedical Science,
Faculty of Medicine,
University of Tsukuba
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan
TEL/FAX: +81-29-853-6012
E-MAIL: mmatsumoto@md.tsukuba.ac.jp

Masahiko Takada, DDS, PhD
Systems Neuroscience Section,
Department of Neuroscience,
Primate Research Institute,
Kyoto University

41-2 Inuyama, Aichi 484-8506, Japan

TEL: +81-568-63-0572

FAX: +81-568-63-0576

E-MAIL: takada.masahiko.7x@kyoto-u.ac.jp

Response to editor's suggestions

Currently, your manuscript is 75,432 characters, including spaces. It is important that the revised paper meets our length restrictions of 65,000 characters including spaces. This character count includes the title, author list and affiliations, summary, introduction, results, discussion, author contributions, acknowledgements, references, and main figure legends but excludes STAR Methods and supplemental item legends. Given the individuality of each piece and the amount of information this study requires, we can allow up to 72,000 characters, but no further. Please edit your manuscript so that it fits within 65,000 to 72,000 characters. If you have any questions on how best to do this, please contact me (bbabayan@cell.com).

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Thank you for the suggestion. In order to pass the restriction of 72,000 characters, we removed some citations, a minor analysis, and minor discussions that are not directly related to the present study. The submitted main manuscript is completely the same as the manuscript that I sent you by email in advance.

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We removed one copy of the Key Resources Table, the separate Word file.

Supplemental figures and tables, as well as their corresponding titles (including "Related to" information) and legends, should be provided in a PDF instead of a Word file. Please note that a descriptive title is required for supplemental tables, but a legend is optional. Please see our Supplemental Information Guidelines for more details.

Response to the above suggestion:

We converted the supplementary information file into PDF.

With respect to your response to one of reviewer #2's comments asking about

distributions of RT plots, if you wish to, you can add one supplemental figure with this data. We can exceptionally allow for one additional supplementary figure.

Response to the above suggestion:

Thank you for allowing us to present the additional supplemental figure. We presented the RT data as Figure S7 in the revised manuscript.

Primate nigrostriatal dopamine system regulates saccadic response inhibition

Takaya Ogasawara[1,2], Masafumi Nejime[3], Masahiko Takada[1*], Masayuki Matsumoto[2-5*]

[1] *Systems Neuroscience Section, Department of Neuroscience, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan*

[2] *Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan*

[3] *Division of Biomedical Science, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan*

[4] *Transborder Medical Research Center, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan*

[5] *Lead contact*

** Correspondence:* mmatsumoto@md.tsukuba.ac.jp (M.M.) and takada.masahiko.7x@kyoto-u.ac.jp (M.T.)

SUMMARY

Animals need to inhibit inappropriate actions that would lead to unwanted outcomes. Although this ability, called response inhibition, is impaired in neurological/psychiatric disorders with dopaminergic dysfunctions, how dopamine regulates response inhibition remains unclear. Here we investigated neuronal signals of the nigrostriatal dopamine system in monkeys performing a saccadic countermanding task. Subsets of dopamine neurons in the substantia nigra and striatal neurons receiving the dopaminergic input were activated when the monkey was required to cancel a planned saccadic eye movement. These activations were stronger when canceling the eye movements was successful than failed, and were enhanced in demanding trials. The activated dopamine neurons were distributed mainly in the dorsolateral, but not in the ventromedial part of the nigra. Furthermore, pharmacological blockade of dopaminergic neurotransmission in the striatum dampened the performance of canceling saccadic eye movements. The present findings indicate that disruption of the nigrostriatal dopamine signaling causes impairments in response inhibition.

KEYWORDS

response inhibition, inhibitory control, dopamine neurons, substantia nigra pars compacta, caudate nucleus, basal ganglia, primates

INTRODUCTION

Animals need to inhibit inappropriate actions that would lead to unwanted outcomes. This ability, called response inhibition or inhibitory control, is an essential factor of executive function, and its neural substrate has been explored in humans and experimental animals using the stop-signal task (Logan and Cowan, 1984). In this task, subjects are required to cancel a planned or ongoing motor action when they occasionally encounter a cue referred to as "stop signal". Numbers of previous studies have demonstrated that neural circuitry involving the frontal cortex and the basal ganglia participates in the brain mechanism that regulates response inhibition. In particular, a series of electrophysiological works in monkeys by Schall and colleagues has shown that specific regions of the lateral prefrontal and medial frontal cortex play crucial but different roles in saccadic response inhibition (Hanes et al., 1998; Ito et al., 2003; Stuphorn et al., 2010; Stuphorn and Schall, 2006; Stuphorn et al., 2000) (See also Xu et al. (2017)). Although whether the basal ganglia contribute to response inhibition remains to be determined in monkeys, it has electrophysiologically been shown in rodents that neurons in some nuclei of the basal ganglia transmit signals associated with canceling motor actions (Mallet et al., 2016; Schmidt et al., 2013). Several studies in humans also suggest the involvement of the cortico-basal ganglia circuitry in response inhibition (Aron et al., 2003; Aron and Poldrack, 2006; Chen et al., 2009; Duann et al., 2009; Li et al., 2006). Especially, the major neural pathways, i.e., the indirect and hyperdirect pathways, constituting the cortico-basal ganglia circuitry have recently attracted much attention as essential substrates for response inhibition (Jahfari et al., 2011; Li et al., 2008; Schmidt et al., 2013; Zandbelt and Vink, 2010). Whereas the direct pathway is thought to promote motor behavior, the indirect and

hyperdirect pathways are considered to serve as a brake to stop it through their inhibitory actions on cortico-basal ganglia signaling (DeLong, 1990; Nambu et al., 2002).

Clinical observations also suggest a possible neural basis for response inhibition. Impairments in response inhibition often accompany neurological/psychiatric disorders with dysfunctions of the dopamine system, for example, Parkinson's disease (Gauggel et al., 2004; Obeso et al., 2011). Although Parkinson's disease is characterized by motor symptoms, patients with this disease develop deficits across many aspects of executive function, including working memory, attention, and response inhibition (Nieoullon, 2002). In those patients, deep brain stimulation of the subthalamic nucleus, a component of the basal ganglia, improves not only motor functions (Deep-Brain Stimulation for Parkinson's Disease Study et al., 2001), but also the performance of response inhibition (van den Wildenberg et al., 2006) and enhances the electroencephalographic frontal activity related to response inhibition (Swann et al., 2011). These studies suggest the contribution of the dopamine system to the fronto-basal ganglia link that mediates response inhibition. Since dopamine released in the striatum where the indirect pathway originates is an important modulator of this pathway (Gerfen and Surmeier, 2011; Kreitzer and Malenka, 2008), the dopamine's contribution is consistent with the idea that the indirect pathway is involved in response inhibition (Jahfari et al., 2011; Li et al., 2008). However, although dopamine neurons are well known for their strong responses to rewards and for their crucial roles in motivation and reinforcement (Cohen et al., 2012; Kawagoe et al., 2004; Montague et al., 1996; Morris et al., 2004; Satoh et al., 2003; Schultz, 1998; Wise, 2004), how dopamine regulates response inhibition remains unclear.

To address this issue, we here examined the roles in response inhibition of dopamine signals transmitted to the striatum. Using a saccadic version of the stop-signal task in macaque monkeys, we found that topographically distributed dopamine neurons in the substantia nigra and striatal neurons located in the caudate nucleus were activated when the monkey was required to cancel a planned saccadic eye movement. Pharmacological blockade of dopaminergic neurotransmission in the caudate nucleus impaired the performance of canceling saccadic eye movements. Our findings suggest the causal contribution of the nigrostriatal dopamine signaling to response inhibition.

RESULTS

Saccadic countermanding task and behavioral performance

We trained two monkeys (monkeys M and E) to perform a saccadic countermanding task (**Figure 1A**) that has been used in human patients and nonhuman primates to evaluate the capability of response inhibition and elaborate its neural substrate (Hanes and Carpenter, 1999; Hanes and Schall, 1995; Thakkar et al., 2011). Each trial started with the presentation of a fixation point. While the monkey was fixating the point, the point disappeared and a saccadic target was simultaneously presented on the right or left side of the point. In 70% of the trials (no-stop signal trials), the monkey was required to make a saccadic eye movement to the target. In the remaining 30% (stop signal trials), the fixation point reappeared as a stop signal with a delay after the onset of the saccadic target. The monkey was required to cancel a planned saccadic eye movement. The delay between saccadic target onset and stop signal onset is referred to as "stop-signal delay" and ranged from 184 to 334 ms in 50-ms step for monkey M and from 84 to 234 ms in 50-ms step for monkey E.

In stop signal trials, the monkeys successfully canceled a saccadic eye movement to the target if the stop-signal delay was short. As the stop-signal delay became longer, the monkeys increasingly failed to cancel the eye movement (**Figure 1B**; see also **Figure S1A** for the variation of performance across sessions). The probability of trials in which the monkey failed to cancel a saccadic eye movement (non-canceled trials) as a function of the stop-signal delay was significantly fit by a logistic function (monkey M, $R^2 = 0.78$, $P < 1.0 \times 10^{-5}$, n = 141 sessions; monkey E, $R^2 = 0.77$, $P < 1.0 \times 10^{-5}$, n = 100 sessions, F test). The behavioral data indicated that canceling saccadic eye movements became more demanding as the stop-signal delay increased.

A critical behavioral index in quantifying the performance of canceling planned saccadic eye movements is the duration that is required to cancel the eye movements. This duration, termed stop-signal reaction time (SSRT), cannot directly be measured as raw data, but can be estimated by a mathematical model that assumes a race between the GO and the STOP processes (**Figure 1C**) (Logan and Cowan, 1984). The GO process is invoked by the presentation of the saccadic target, and a saccadic eye movement is generated when the GO process finishes. The STOP process is invoked by the presentation of the stop signal, and the eye movement is canceled if the STOP process finishes before the GO process. The SSRT is the duration that the STOP process needs to finish and can be estimated from the distribution of the durations of the GO process (i.e., the reaction times of saccadic eye movements in no-stop signal trials) (see STAR METHODS for the estimation method; see **Figure S1B** for saccadic reaction times in no-stop signal trials). We estimated the SSRTs based on the behavioral data obtained from single-unit recording in each session (mean ± SD = 89.8 ± 18.3 ms in monkey M and 112.6 ± 16.9 ms in monkey E) (**Figures 1D** and **S1C**).

Response of dopamine neurons to the stop signal

While the monkeys were performing the countermanding task, we first recorded single-unit activity from 76 dopamine neurons (40 in monkey M and 36 in monkey E) in the ventral midbrain, including the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). Recent electrophysiological studies have found that dopamine neurons not only respond to rewarding events, but also represent signals related to novel, salient, and even aversive experiences (Brischoux et al., 2009; Bromberg-Martin et al., 2010; Horvitz, 2000; Joshua et al., 2008; Matsumoto et al.,

2016; Matsumoto, 2015; Redgrave and Gurney, 2006). Here we examined how dopamine neurons responded to the stop signal that invoked the process for canceling saccadic eye movements. We identified putative dopamine neurons based on the well-known electrophysiological criteria: (1) a low background firing rate at around five spikes/s, (2) a broad spike waveform in clear contrast to neighboring neurons with a high background firing rate in the substantia nigra pars reticulata (**Figure S2**), and (3) a phasic increase in discharge caused by an unexpectedly delivered reward.

An example dopamine neuron was activated when the stop signal was presented (**Figure 2A**). This activation was stronger in trials in which the monkey successfully canceled a saccadic eye movement (canceled trials) compared with those in which the animal failed to cancel it (non-canceled trials). The activation occurred irrespective of the direction of planned saccadic eye movements that were canceled by the stop signal (i.e., ipsilateral or contralateral to the recording hemisphere). To clarify whether dopamine neurons are involved in canceling planned saccadic eye movements, we compared their activity in trials in which saccadic eye movements were canceled (canceled trials) vs. those in which the eye movements were properly executed (latency-matched no-stop signal trials) (see STAR METHODS for details). **Figure 2B** shows the receiver operating characteristic (ROC) value of all recorded dopamine neurons that was determined by comparing their activities in the two trial types. A subset of the dopamine neurons exhibited a phasic activation aligned at stop signal onset in canceled trials, and the same pattern of activation was observed in both the ipsilateral and the contralateral conditions.

Of the 76 dopamine neurons, 28 neurons showed a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials in at

least one of the ipsilateral and contralateral conditions (ipsilateral condition, 22 neurons; contralateral condition, 16 neurons; both conditions, 10 neurons; $P < 0.05$, Wilcoxon rank-sum test). The proportion of the neurons with a significant increase was not significantly different in the ipsilateral vs. contralateral conditions ($P = 0.32$, chi square test). **Figure 3A** shows the averaged activity of these 28 neurons in a combination of the ipsilateral and contralateral conditions (see STAR METHODS for details of population analyses; see **Figure S3** for data in individual monkeys). Given that the excitatory dopamine signal regulates the performance of canceling saccadic eye movements, the dopamine neuron activation evoked by the stop signal is expected to start earlier than the SSRT, i.e., the duration that the brain needs for canceling saccadic eye movements. **Figure 3B** shows dopamine neuron activity aligned at the SSRT. We found that the onset of the dopamine neuron activation preceded the SSRT by at least 12 ms as a population though only a few neurons exhibited an activation preceded the SSRT at the single neuron level (see **Figure S4A** for the onset of each dopamine neuron).

We next found that the dopamine neuron activation evoked by the stop signal was enhanced as the stop-signal delay increased (**Figure 3C**), which was proven by a significantly positive correlation coefficient between the dopamine neuron activation and the stop-signal delay (r, mean ± SD = 0.16 ± 0.19, $P = 3.2 \times 10^{-4}$, n = 28, Wilcoxon signed-rank test) (**Figure 3D**). Furthermore, we found that the dopamine neuron activation evoked by the stop signal was modulated depending on whether canceling a saccadic eye movement was successful or failed (**Figure 3E**). Although the dopamine neuron activation was modulated by the stop-signal delay as well (**Figure 3D**), we here removed this effect by analyzing neuronal activity only in trials with a certain

stop-signal delay (the third shortest stop-signal delay, 284 and 184 ms in monkeys M and E, respectively). In this stop-signal delay, canceling saccadic eye movements was equally successful and failed (**Figure 1B**), and we were able to collect enough data to compare neuronal activities in canceled vs. non-canceled trials. On average, the activation evoked by the stop signal was significantly stronger in canceled trials than in non-canceled trials (canceled trials, mean ± SD = 12.7 ± 7.4 spikes/s; non-canceled trials, mean ± SD = 7.6 ± 6.4 spikes/s; $P = 2.1 \times 10^{-4}$, n = 28, Wilcoxon signed-rank test). Even at the individual neuron level, 6 of the 28 neurons exhibited a significantly stronger activation in canceled trials than in non-canceled trials in spite of the limited number of these trials ($P < 0.05$, Wilcoxon rank-sum test) (see **Figure 2C** for the modulation of each neuron). These results indicated that the magnitude of dopamine neuron activation evoked by the stop signal was correlated with the performance of canceling planned saccadic eye movements.

As revealed by the difference in neuronal activity between canceled and non-canceled trials, the dopamine neuron activation evoked by the stop signal was correlated with the performance of canceling saccadic eye movements. Even in non-canceled trials, however, dopamine neurons were weakly but significantly activated by the stop signal, compared with latency-matched no-stop signal trials (non-canceled trials, mean ± SD = 7.6 ± 6.4 spikes/s; latency-matched no-stop signal trials, mean ± SD = 4.8 ± 3.7 spikes/s; P = 0.017, n = 28, Wilcoxon signed-rank test) (**Figure S4D**). Thus, dopamine neurons were activated by the stop signal even if the monkey failed to cancel a saccadic eye movement, suggesting that the activation did not simply reflect the performance itself. To further test how the dopamine neuron activation related to the performance of canceling saccadic eye movements, we next examined the correlation

between the magnitude of the activation and the probability of non-canceled trials (i.e., the probability of failed trials) that varied across recording sessions even for a given stop signal delay (see **Figure S1A** for the variation of performance across recording sessions). We observed a significant correlation between them ($r = 0.33$, $P = 7.4 \times 10^{-4}$, $n = 100$) (**Figure S4G**), suggesting that dopamine neurons were more strongly activated by the stop signal during recording sessions in which the monkey more often failed to cancel a saccadic eye movement.

Previous studies have demonstrated that dopamine neurons represent distinct signals in monkeys in a topographic fashion that is dependent on their locations in the SNc and VTA (Matsumoto and Hikosaka, 2009; Matsumoto and Takada, 2013). According to these studies, dopamine neurons in the ventromedial part of the SNc and the VTA represent a value-related signal called reward prediction error, whereas those in the dorsolateral part of the SNc represent a signal related to the salience of external events. In the present study, we also found that the dopamine neurons activated by the stop signal were not uniformly scattered over the SNc and VTA. These neurons were observed mainly in the dorsolateral part of the SNc (**Figure 4A**). This spatial localization was statistically verified by a significant negative correlation between the neuronal response to the stop signal and the depth of the recording site ($r = -0.45$, $P = 3.8 \times 10^{-5}$, $n = 76$) (**Figure 4B**). Moreover, we split all the recorded dopamine neurons into two groups according to their recording depth. **Figure 4C** indicates the averaged activities of the shallower and deeper groups. On average, the activation evoked by the stop signal was significantly stronger in the shallower group than in the deeper group (shallower, mean ± SD = 7.4 ± 4.8 spikes/s, $n = 38$; deeper, mean ± SD = 4.6 ± 3.7 spikes/s, $n = 38$; $P = 6.9 \times 10^{-4}$, Wilcoxon rank-sum test) (**Figure 4D**). These results

suggest that only a topographically distributed group of dopamine neurons participates in the neural process that cancels planned saccadic eye movements.

Response of caudate neurons to the stop signal

We so far found that dopamine neurons in the dorsolateral part of the SNc were predominantly activated by the stop signal that invoked the process for canceling planned saccadic eye movements, and that their activity was correlated with the performance of canceling the eye movements. To understand the role of this excitatory dopamine signal in the basal ganglia circuitry, we next investigated what signals are represented in the caudate nucleus that receives the dopaminergic input from the dorsolateral part of the SNc (Haber et al., 2000). We recorded single-unit activity from 165 neurons in the caudate nucleus (101 in monkey M and 64 in monkey E, see **Figure S5** for histology). The recording sites overlapped the region receiving projections from the frontal eye field and the supplementary eye field that have been shown to participate in saccadic response inhibition (Parthasarathy et al., 1992).

As seen in the dopamine neurons, we observed that a number of neurons in the caudate nucleus were so strongly activated when the stop signal was presented and the monkey successfully canceled a planned saccadic eye movement (canceled trials), as compared to latency-matched no-stop signal trials in which the stop signal was not presented and the animal properly executed the eye movement (see **Figure 5A** for an example neuron and **Figure 5C** for all recorded neurons). Unlike the dopamine neurons, the activity of some other caudate neurons was rather suppressed in canceled trials in comparison with latency-matched no-stop signal trials (see **Figure 5B** for an example neuron). These responses of caudate neurons were often modulated by the direction of

planned saccadic eye movements that were canceled by the stop signal.

Of the 165 caudate neurons, 59 neurons exhibited a significant increase in their activity in canceled trials, compared with latency-matched no-stop signal trials, in at least one of the ipsilateral and contralateral conditions (ipsilateral condition, 39 neurons; contralateral condition, 32 neurons; both conditions, 12 neurons; $P < 0.05$, Wilcoxon rank-sum test). Hereafter we classified these caudate neurons as the increase type. Conversely, 74 caudate neurons showed a significant decrease in their activity in canceled trials in at least one of the conditions (ipsilateral condition, 31 neurons; contralateral condition, 53 neurons; both conditions, 10 neurons; $P < 0.05$, Wilcoxon rank-sum test). These neurons were more strongly activated when the monkey executed a saccadic eye movement than when the animal canceled the eye movement. Hereafter we classified these caudate neurons as the decrease type. The proportion of the increase-type neurons was not significantly different in the ipsilateral vs. contralateral conditions ($P = 0.39$, chi square test), whereas that of the decrease-type neurons was significantly larger in the contralateral condition ($P = 0.009$, chi square test). Thus, although caudate neurons were more preferentially activated when the monkey executed a saccadic eye movement toward the contralateral than the ipsilateral direction (decrease type), consistent with previous findings (Hikosaka et al., 1989; Takikawa et al., 2002), they were activated when the monkey canceled the eye movement regardless of the direction (increase type).

Figure 6A shows the averaged activities of the increase-type (upper) and decrease-type (lower) neurons in a combination of the ipsilateral and contralateral conditions (see STAR METHODS for details of population analyses; see **Figure S3** for data in individual monkeys). The modulation of the increase-type neurons evoked by

the stop signal started earlier than the SSRT by at least 7 ms as a population (upper in **Figure 6B**) though only a few neurons exhibited a modulation started earlier than the SSRT at the single neuron level (see **Figure S4B** for the modulation onset of each increase-type neuron). The modulation of the decrease-type neurons started later than the SSRT even as a population (lower in **Figure 6B**; see **Figure S4C** for the modulation onset of each decrease-type neuron). The modulation of the increase-type neurons was enhanced as the stop-signal delay increased (correlation coefficient between the modulation and the stop-signal delay, mean ± SD = 0.12 ± 0.22, $P = 1.2 \times 10^{-4}$, n = 59, Wilcoxon signed-rank test) (upper in **Figure 6C** and **D**), whereas the modulation of the decrease-type neurons was not affected by the stop-signal delay (correlation coefficient between the modulation and the stop-signal delay, mean ± SD = 0.025 ± 0.27, P = 0.38, n = 74, Wilcoxon signed-rank test) (lower in **Figure 6C** and **D**). Notably, the increase-type neurons exhibited a significantly stronger activation in canceled trials than in non-canceled trials (canceled trials, mean ± SD = 8.7 ± 5.9 spikes/s; non-canceled trials, mean ± SD = 6.1 ± 7.3 spikes/s; $P = 7.5 \times 10^{-3}$, n = 59, Wilcoxon signed-rank test) (upper in **Figure 6E**; see upper in **Figure 5D** for the modulation of each increase-type neuron), while the decrease-type neurons displayed a significantly stronger activation in non-canceled trials than in canceled trials (canceled trials, mean ± SD = 4.5 ± 5.7 spikes/s; non-canceled trials, mean ± SD = 6.3 ± 7.4 spikes/s; $P = 6.8 \times 10^{-3}$, n = 74, Wilcoxon signed-rank test) (lower in **Figure 6E**; see lower in **Figure 5D** for the modulation of each decrease-type neuron). This indicates that the activities of both neuron types were correlated with the performance of canceling planned saccadic eye movements.

As seen in dopamine neurons, the increase-type neurons exhibited a weak but

significant increase in their activity even in non-canceled trials, compared with latency-matched no-stop signal trials (non-canceled trials, mean ± SD = 6.1 ± 7.3 spikes/s; latency-matched no-stop signal trials, mean ± SD = 3.8 ± 5.1 spikes/s; $P = 1.1 \times 10^{-3}$, n = 59, Wilcoxon signed-rank test) (**Figure S4E**). Thus, the increase-type neurons were activated by the stop signal even if the monkey failed to cancel a saccadic eye movement. On the other hand, the activity of the decrease-type neurons did not significantly change in non-canceled trials vs. latency-matched no-stop signal trials (non-canceled trials, mean ± SD = 6.3 ± 7.4 spikes/s; latency-matched no-stop signal trials, mean ± SD = 7.5 ± 7.4 spikes/s; P = 0.051, n = 74, Wilcoxon signed-rank test) (**Figure S4F**). These results suggest that the activity of the decrease-type neurons reflected whether the monkey would execute or cancel a saccadic eye movement, whereas the activity of the increase-type neurons did not simply represent the performance itself. Furthermore, we found that the magnitude of the modulation of the increase-type neurons evoked by the stop signal was significantly correlated with the probability of non-canceled trials that varied across recording sessions (r = 0.20, P = 0.0089, n = 176) (**Figure S4H**), suggesting that these neurons were more strongly activated by the stop signal during recording sessions in which the monkey more often failed to cancel a saccadic eye movement. The modulation magnitude of the decreased-type neurons, on the other hand, was not significantly correlated with the probability of non-canceled trials (r = -0.043, P = 0.55, n = 198) (**Figure S4I**).

Taken together, in particular, the increase-type neurons in the caudate nucleus shared similar electrophysiological properties with dopamine neurons.

Pharmacological blockade of dopaminergic neurotransmission in the caudate nucleus

We found that dopamine neurons mainly in the dorsolateral part of the SNc were activated by the stop signal. A subset of caudate neurons, which would receive the dopamine signal, also exhibited an activation evoked by the stop signal especially when the monkey successfully canceled a planned saccadic eye movement. To test whether dopaminergic neurotransmission in the caudate nucleus has a causal role in canceling saccadic eye movements, we next injected the dopamine antagonist, SCH23390 (4 injections in monkey M and 8 injections in monkey E) or haloperidol (6 injections in monkey M and 8 injections in monkey E) that mainly prevents D1 or D2 receptor signaling, respectively, into the caudate nucleus of one hemisphere in the two monkeys (see **Figure S5** for the injection sites).

In an experiment in which the D2 antagonist was injected into a representative site, the monkey so often failed to cancel a saccadic eye movement after the injection, as compared to the pre-injection control (upper in **Figure 7A**; see also upper in **Figure S6A** for data in all injection experiments). In general, the caudate nucleus is thought to regulate saccadic eye movements contralateral to a given hemisphere. Notably, however, the effect of the D2 antagonist was observed when the monkey was required to cancel a saccadic eye movement ipsilateral, but not contralateral, to the injection hemisphere. The injection of the D1 antagonist into another representative site also impaired the performance of canceling saccadic eye movements only in the ipsilateral condition (lower in **Figure 7A**; see also lower in **Figure S6A** for data in all injection experiments). To statistically analyze the effects of the D1 and D2 antagonists on the performance of canceling saccadic eye movements, we compared the stop-signal delay at which the monkey failed in 50% of trials in the pre- vs. post-injection conditions (ΔZ in **Figure 7A**). In the representative experiments, the impaired performance in the ipsilateral

condition resulted in a significant decrease in the stop-signal delay incurring 50% failed trials (i.e., non-canceled trials) (D1 antagonist, ΔZ = -56.7 ms, $P < 0.002$; D2 antagonist, ΔZ = -59.2 ms, $P = 0.002$; bootstrap test with 1,000 repetitions).

On average, both the D1 and the D2 antagonist injections significantly decreased the stop-signal delay in the ipsilateral condition (D1 antagonist, ΔZ, mean ± SD = -68.1 ± 57.1 ms, $P = 2.4 \times 10^{-3}$, n = 12; D2 antagonist, ΔZ, mean ± SD = -59.6 ± 34.4 ms, $P = 1.2 \times 10^{-4}$, n = 14; Wilcoxon signed-rank test), but had no significant effect in the contralateral condition (D1 antagonist, ΔZ, mean ± SD = 14.5 ± 54.8 ms, P = 0.68, n = 12; D2 antagonist, ΔZ, mean ± SD = 5.9 ± 20.9 ms, P = 0.46, n = 14; Wilcoxon signed-rank test) (top in **Figure 7B**). When the same amount of saline was injected into similar loci of the caudate nucleus as a control, the stop-signal delay incurring 50% failed trials remained unchanged (**Figure S6C** and **D**). These results indicated that disruption of dopamine D1 and D2 signals in the caudate nucleus impaired the performance of canceling saccadic eye movements.

The performance of response inhibition largely depends on the SSRT, i.e., the duration that the brain needs for canceling motor actions. We next analyzed the effects of the D1 and D2 antagonists on the SSRT. We estimated the SSRT based on the race model (**Figure 1C**) and compared the SSRT in the pre- vs. post-injection conditions for each injection experiment. On average, the D2 antagonist injection significantly increased the SSRT in the ipsilateral condition (ΔSSRT, mean ± SD = 20.5 ± 18.2 ms, $P = 2.3 \times 10^{-3}$, n = 14; Wilcoxon signed-rank test), but had no significant effect in the contralateral condition (ΔSSRT, mean ± SD = -7.6 ± 15.8 ms, P = 0.12, n = 14; Wilcoxon signed-rank test) (middle in **Figure 7B**). On the other hand, the D1 antagonist injection exerted no significant effect in either the ipsilateral (ΔSSRT, mean ± SD = 6.4 ± 23.1 ms,

P = 0.34, n = 12; Wilcoxon signed-rank test) or the contralateral condition (ΔSSRT, mean ± SD = -2.5 ± 21.6 ms, P = 0.85, n = 12; Wilcoxon signed-rank test).

The unilateral increase in the SSRT after the D2 antagonist injection is well consistent with the ipsilateral deficit in the performance of canceling saccadic eye movements. It remains unclear, however, how the D1 antagonist injection impaired the performance without affecting the SSRT. Previous studies have reported that the performance of response inhibition also depends on the reaction time of motor actions (Emeric et al., 2007; Stuphorn and Schall, 2006). The more quickly animals attempt to execute a motor action, the more difficult canceling the action becomes. We then analyzed the effects of the D1 and D2 antagonists on the reaction time of saccadic eye movements. On average, both antagonists significantly decreased the reaction time in the ipsilateral condition (D1 antagonist, ΔRT, mean ± SD = -45.8 ± 36.6 ms, P = 1.5×10^{-3}, n = 12; D2 antagonist, ΔRT, mean ± SD = -36.6 ± 26.1 ms, P = 1.2×10^{-4}, n = 14; Wilcoxon signed-rank test), but exerted no significant effect in the contralateral condition (D1 antagonist, ΔRT, mean ± SD = 10.7 ± 34.2 ms, P = 0.30, n = 12; D2 antagonist, ΔRT, mean ± SD = 2.5 ± 15.2 ms, P = 0.63, n = 14; Wilcoxon signed-rank test) (bottom in **Figure 7B**) (see **Figure S7** for the distributions of saccadic reaction times in the pre- and post-injection conditions). Thus, while the D2 antagonist seemed to impair the performance of canceling planned saccadic eye movements by altering both the SSRT and the saccadic reaction time, the D1 antagonist was likely to impair the performance only by affecting the saccadic reaction time.

To test whether the effects of the D1 and D2 antagonists were contingent on the performance of canceling saccadic eye movements, we finally examined the effects of these antagonists on saccadic eye movements in a simple visually-guided saccade task

in which the stop signal was not presented (**Figure 7C**). As seen in the saccadic countermanding task, the D1 antagonist injection significantly decreased the saccadic reaction time in the ipsilateral condition (ΔRT, mean ± SD = -20.2 ± 13.2 ms, P = 0.031, n = 6; Wilcoxon signed-rank test). Notably, however, the D2 antagonist injection had no significant effect on the reaction time in the ipsilateral condition (ΔRT, mean ± SD = -2.8 ± 8.3 ms, P = 0.46, n = 8; Wilcoxon signed-rank test). Thus, the effect of the D2 antagonist on ipsilateral saccadic eye movements was observed only in the context in which the monkey was required to cancel the eye movement. Both antagonists, on the other hand, significantly increased the reaction time in the contralateral condition (D1 antagonist, ΔRT, mean ± SD = 46.8 ± 32.8 ms, P = 0.031, n = 6; D2 antagonist, ΔRT, mean ± SD = 15.8 ± 9.5 ms, P = 0.016, n = 8; Wilcoxon signed-rank test), which was not observed in the saccadic countermanding task.

DISCUSSION

In the present study, we have revealed that a topographically distributed group of dopamine neurons in the SNc and striatal neurons in the caudate nucleus receiving the dopaminergic input were activated when the monkey was required to cancel a planned saccadic eye movement. These excitatory signals were correlated with the performance of canceling saccadic eye movements. By injecting the D1 and D2 antagonists, we have further elucidated a causal role of dopaminergic neurotransmission to the caudate nucleus in the performance of canceling the eye movements.

Notably, the dopamine neurons activated by the stop signal were distributed mainly in the dorsolateral part of the SNc. Although dopamine neurons are well known to encode a value-related signal called reward prediction error, recent studies in monkeys have shown that dopamine neurons in the dorsolateral part of the SNc transmit a signal related to the salience, rather than the value, of external events (Matsumoto and Hikosaka, 2009; Matsumoto and Takada, 2013). Dopamine neurons in this region receive inputs from the superior colliculus (Redgrave and Gurney, 2006) in which neurons also represent the salience of external stimuli (McPeek and Keller, 2002; Shen and Pare, 2014; White et al., 2017) and respond to the stop signal in the same saccadic countermanding task in monkeys (Paré and Hanes, 2003). Since the distribution of dopamine neurons activated by the stop signal overlaps that of dopamine neurons signaling the salience, the dopamine neuron activation evoked by the stop signal may reflect the salience of the stop signal that is critically salient to achieve the saccadic countermanding task. Consistent with this idea, dopamine neurons were more strongly activated by the stop signal as the stop-signal delay increased. The behavioral data indicated that canceling saccadic eye movements became more demanding as the

stop-signal delay increased, and it can be considered that the stop signal turns more salient if it requires a more demanding action. In addition, the dopamine neurons activated by the stop signal tended to be activated by another salient stimulus in the countermanding task, i.e., the fixation point that signaled the start of trials (**Figure S4J** and **K**). By contrast, the dopamine neuron activation does not appear to reflect the value-related aspect of the stop signal. That is, although the monkey more often failed to cancel a saccadic eye movement (i.e., although the reward probability decreased) as the stop-signal delay increased, the dopamine neuron activation was enhanced by longer stop-signal delays. Taken together, our findings suggest that the nigrostriatal dopamine system may regulate saccadic response inhibition probably by signaling the salience of the stop signal.

How does the dopamine neuron activation evoked by the stop signal, which we observed in our single-unit recordings, regulate the performance of canceling saccadic eye movements? Given that the dopamine neuron activation participates in the cancel process, the activation is expected to start earlier than the SSRT. We found that although the latency of the dopamine neuron activation preceded the SSRT at least by 12 ms as a population, only a few neurons exhibited a latency preceding the SSRT at the single neuron level. The latency in the caudate nucleus, which receives the dopamine signal, preceded the SSRT only by 7 ms. Moreover, it should be noted here that the effect of released dopamine on postsynaptic activity is mediated by G-protein-coupled dopamine receptors that are generally regarded as receptors that signal with slow speed (Beaulieu and Gainetdinov, 2011). In addition, the conduction velocity of dopaminergic fibers is slower than that of non-dopaminergic fibers (e.g., 0.6 m/sec for mesolimbic dopaminergic fibers and 2.4 m/sec for mesolimbic non-dopaminergic fibers in rats; see

Thierry et al. (1980)). Therefore, it does not become immediately clear whether or not the latency preceding the SSRT by 12 ms as a population is short enough for the dopamine neuron activation to regulate the performance of canceling saccadic eye movements.

A possible role of the dopamine neuron activation in canceling saccadic eye movements is "proactive inhibition" that suppresses the eye movements in advance even before the presentation of the stop signal. Human imaging studies have shown that the basal ganglia are involved in this process (Aron, 2011; Majid et al., 2013). Although the role of the basal ganglia in proactive inhibition has not yet been elucidated at the single neuron level, electrophysiological studies in monkeys have shown that neurons in the supplementary eye field (SEF) regulate the performance of saccadic response inhibition in the proactive manner. Neurons in the SEF respond to the stop signal in the saccadic countermanding task as well, but most of their responses start later than the SSRT (Stuphorn et al., 2010). Nevertheless, electrical stimulation of the SEF improves the performance of canceling saccadic eye movements (Stuphorn and Schall, 2006). Accordingly, the SEF has been thought to contribute to canceling saccadic eye movements by "preparing" the cancel before the presentation of the stop signal (Stuphorn et al., 2010). Such a proactive inhibition process is guided by some information, for example, prior knowledge about task and environment. Dopamine is an ideal neurotransmitter that is involved in this process. Dopamine released in the striatum is known to modulate a synaptic efficacy of the corticostriatal pathway that connects the SEF and caudate nucleus (Gerfen and Surmeier, 2011; Kreitzer and Malenka, 2008). The synaptic effect seems instrumental in proactive inhibition that requires long-lasting changes in a circuit state to keep the circuit ready to cancel motor

actions. Thus, the dopamine neuron activation evoked by the stop signal would change the corticostriatal synaptic efficacy and, consequently, might inhibit subsequent saccadic eye movements by preparing for canceling the eye movements. Consistent with the idea that the nigrostriatal dopamine system regulates saccadic response inhibition in the proactive manner, we found that the magnitude of the dopamine neuron activation evoked by the stop signal was correlated with the reaction time of saccadic eye movement in the next no-stop signal trial (**Figure S8D** and **E**). In other words, as dopamine neurons were more strongly activated by the stop signal, the eye movement in the next trial more largely delayed. Furthermore, as seen in the SEF (Stuphorn et al., 2010), the activity of caudate neurons represented whether the monkey would successfully cancel or erroneously execute a saccadic eye movement even before the presentation of saccadic target (**Figure S8B** and **C**). These results suggest that dopamine neurons and the caudate nucleus may proactively regulate saccade promotion by biasing the balance between the cancel and the execution of saccadic eye movement.

As discussed above, it is a critical issue whether and how the nigrostriatal system cooperates with the SEF to regulate the performance of saccadic response inhibition. The SEF and caudate nucleus constitute an oculomotor functional unit known as the cortico-basal ganglia oculomotor loop circuit (DeLong and Wichmann, 2015). Consistent with the anatomical linkage, the neurophysiological property of caudate neurons observed in the present study resembles that of SEF neurons. For instance, as observed in the SEF (Stuphorn et al., 2000), a subset of caudate neurons was more strongly activated when the monkey canceled a saccadic eye movement (canceled trials) than when the animal executed the eye movement (no-stop signal trials), whereas another subset of caudate neurons was more strongly activated when the monkey executed a

saccadic eye movement. In addition, neuronal modulations in both the SEF and the caudate nucleus were stronger when the monkey correctly canceled a saccadic eye movement than failed. Such a correlation with the performance, as well as the diversity of neuronal modulations and the proactive influence on the modulations, is a common feature across these structures. However, they do not share all the electrophysiological features. Whereas the activation of caudate neurons evoked by the stop signal was modulated by the stop-signal delay, that of SEF neurons was not (Stuphorn et al., 2000). These results suggest that the caudate nucleus does not simply receive signals from the SEF.

Although the role of the basal ganglia in response inhibition has not yet been elucidated in monkeys, human imaging studies have proposed that the indirect pathway of the basal ganglia plays a crucial role in response inhibition (Jahfari et al., 2011; Li et al., 2008; Zandbelt and Vink, 2010). How do our findings in monkeys fit this proposal? In the present study, we injected the D1 and D2 antagonists that prevent dopaminergic neurotransmission to the direct and indirect pathways, respectively, into the caudate nucleus. While the D1 antagonist impaired the performance of canceling saccadic eye movements by decreasing the reaction time of the eye movements, the D2 antagonist impaired it not only by decreasing the reaction time, but also by increasing the SSRT. Since the SSRT is the duration that is necessary for the brain to cancel saccadic eye movements (i.e., the duration that is necessary for the STOP process in the race model), the impact on this behavioral index implies that the dopamine signal to the indirect pathway participates in the neural process that cancels the eye movements (i.e., STOP process). On the other hand, the saccadic reaction time is the duration that is necessary for the brain to generate saccadic eye movements (i.e., the duration that is

necessary for the GO process in the race model). Thus, the effects of the D1 and D2 antagonists on the saccadic reaction time indicate that dopaminergic neurotransmission is involved in the promotion of saccadic eye movements (i.e., GO process) not only through the direct pathway, but also through the indirect pathway. However, it is most likely that these pathways affect the saccade promotion in different ways. In this respect, the present work has clearly demonstrated that the D1 antagonist decreased the reaction time regardless of the task context, while the D2 antagonist did so only in the context in which the monkey was required to cancel a saccadic eye movement. The context-dependent effect of the D2 antagonist is consistent with the idea that dopaminergic neurotransmission to the indirect pathway contributes to the proactive inhibition process that has been described in the above paragraph, because the proactive inhibition process is executed only in the context in which animals are required to cancel motor actions. Our findings suggest that although the dopaminergic input to the direct pathway simply affects the promotion of saccadic eye movements, the input to the indirect pathway serves as a brake to cancel the eye movements even before the presentation of the stop signal.

Our electrophysiological and pharmacological findings lead to an idea that the dopamine neuron activation evoked by the stop signal regulates the performance of canceling saccadic eye movements through the indirect pathway of the basal ganglia. However, this idea does not perfectly fit the conventional theory of the indirect pathway. According to the conventional theory, excitatory dopamine signals suppress the activity of striatal neurons with D2 receptors in the indirect pathway that inhibits motor actions. Consequently, the excitatory dopamine signals “disinhibit” motor actions through the indirect pathway (Gerfen and Surmeier, 2011). Contrary to this theory, our findings

suggest that the excitatory dopamine signal evoked by the stop signal "inhibits" saccadic eye movements. A hint that may bridge the gap between the conventional theory and our findings is provided by our pharmacological observations that blockade of dopamine D2 signaling in the caudate nucleus impaired the performance of canceling saccadic eye movements ipsilateral, but not contralateral, to the injected hemisphere. In general, the direct and indirect pathways involving the caudate nucleus are thought to regulate contralateral saccadic eye movements. However, it has also been documented that the caudate nucleus influences ipsilateral saccadic eye movements as well, but in an opposite manner. Electrical stimulation of the caudate nucleus in monkeys facilitates contralateral saccadic eye movements, whereas it suppresses ipsilateral ones (Nakamura and Hikosaka, 2006). This suggests that the caudate nucleus regulates contralateral and ipsilateral saccadic eye movements in opposite ways. Such reversed effects on contralateral and ipsilateral eye movements might be mediated by uncrossed and crossed projections from the substantia nigra pars reticulata, which receives inputs from the caudate nucleus, to the superior colliculi of both hemispheres (Beckstead et al., 1981; Jiang et al., 2003). Taken together, the excitatory dopamine signal evoked by the stop signal would suppress caudate neurons in the indirect pathway. The suppression of the indirect pathway exerts a disinhibitory effect on contralateral saccadic eye movements, while the effect on ipsilateral ones turns to be inhibitory. Consequently, the excitatory dopamine signal transmitted to the indirect pathway could contribute to canceling ipsilateral saccadic eye movements.

It remains unclear, on the other hand, why blockade of dopamine D2 signaling (and D1 signaling as well) influenced the performance of canceling only ipsilateral saccadic eye movements. According to the above consideration, the pharmacological blockade

should improve the performance of canceling contralateral saccadic eye movements. Consistent with our findings, a previous study in monkeys also reported that D1 and D2 antagonist injections into the caudate nucleus affected ipsilateral saccadic eye movements, but not contralateral ones, in a saccade task named the self-timed memory-guided saccade task (Kunimatsu and Tanaka, 2016). In this task, the monkey was required to wait for a predetermined time interval (e.g., 1100 ± 300 ms after cue offset) before making a saccadic eye movement to a remembered cue location. Thus, the monkey needed to "inhibit" the eye movement during the interval. Together with the previous study, our findings suggest that the caudate nucleus may predominantly regulate ipsilateral saccadic eye movements in the context in which animals need to inhibit the eye movements. Further investigations are called for to determine the precise mechanism underlying how the nigrostriatal dopamine system regulates ipsilateral saccadic eye movements.

In summary, we have defined a neural correlate of saccadic response inhibition in the nigrostriatal dopamine system as well as the causal relationship between this system and the performance of saccadic response inhibition. The indirect pathway, rather than the direct pathway, of the basal ganglia might mediate the role of the nigrostriatal dopamine system in saccadic response inhibition. Our data indicate that disruption of the nigrostriatal dopamine signaling causes impairments in response inhibition, which is observed in neurological/psychiatric disorders such as Parkinson's disease.

ACKNOWLEDGEMENTS

We thank Drs. I. E. Monosov, H. Yamada and T. Kawai for valuable discussion, K. Nagaya for technical assistance, and K. Bunzui for animal care. This research was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (16H06567 to M.M., 26710001 to M.M.) and by Takeda Science Foundation (to M.M.).

AUTHOR CONTRIBUTIONS

T.O., M.T. and M.M. designed the experiments. T.O. performed all experiments and analyzed the data. M.N. performed part of the pharmacological experiments. T.O., M.T. and M.M. discussed the results and wrote the manuscript. M.M. organized the entire project.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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FIGURE LEGENDS

Figure 1. Saccadic countermanding task and behavioral performance

(A) Saccadic countermanding task. (B) Probability of non-canceled trials in which the monkey failed to cancel a saccadic eye movement as a function of the stop-signal delay (SSD). Filled and open circles indicate data in monkeys M and E, respectively. Solid and dotted curves represent the fitted logistic functions in monkeys M and E, respectively. Error bars indicate SD. (C) Schematic diagram of the race model. If the GO process reaches its threshold before the STOP process, a saccadic eye movement is generated (top; non-canceled trials). The duration of the GO process is the reaction time of the eye movement (saccadic RT). If the STOP process reaches its threshold before the GO process, the saccadic eye movement is canceled (middle; canceled trials). The duration of the STOP process is the stop-signal reaction time (SSRT). The SSRT is estimated from the distribution of the durations of the GO process (i.e., the reaction times of saccadic eye movements in no-stop signal trials) (bottom). (D) Distributions of the SSRTs in each recording session for each monkey. See also Figure S1.

Figure 2. Response of individual dopamine neurons to the stop signal

(A) Activity of an example dopamine neuron when a saccadic target was presented ipsilateral (left) or contralateral (right) to the recording hemisphere. Rasters and spike density functions (SDFs) are aligned at target and stop signal onsets. Canceled, non-canceled, and latency-matched no-stop signal trials are indicated in red, orange, and black, respectively. Magenta dots represent stop signal onset in each trial. Dotted line represents the SSRT. (B) Stop-signal evoked neuronal modulation of all recorded dopamine neurons (n = 76) aligned at stop signal onset in the ipsilateral (left) and

contralateral (right) conditions. The modulation of each neuron is presented as a row of pixels. The color of each pixel represents the receiver operating characteristic (ROC) value that was determined by comparing the discharge rate in canceled vs. latency-matched no-stop signal trials. The ROC value was calculated using a 50-ms test window sliding with a 10-ms step. Warmer colors (ROC > 0.5) indicate higher discharge rates in canceled trials, whereas cooler colors (ROC < 0.5) indicate higher discharge rates in latency-matched no-stop signal trials. Open circles indicate the SSRT of each recording session. (C) Neuronal modulation between canceled and non-canceled trials of the 28 neurons with a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials in at least one of the ipsilateral and contralateral conditions ($P < 0.05$, Wilcoxon rank-sum test). Warmer colors (ROC > 0.5) indicate higher discharge rates in canceled trials, whereas cooler colors (ROC < 0.5) indicate higher discharge rates in non-canceled trials. See also Figure S2.

Figure 3. Averaged activity of dopamine neurons

(A) Averaged activity of the 28 dopamine neurons with a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test). The data in the ipsilateral and contralateral conditions are combined. SDFs are aligned at target and stop signal onsets and shown for canceled trials (red curve) and latency-matched no-stop signal trials (black curve). (B) Averaged activity of the 28 dopamine neurons aligned at the SSRT. SDFs are shown for canceled trials (red curve) and latency-matched no-stop signal trials (black curve). Vertical dotted line represents the time when the difference in the averaged discharge rate between canceled and latency-matched no-stop signal trials becomes significant ($P <$

0.05, bootstrap test with 1,000 repetitions). (C) Effect of the stop-signal delay on the averaged activity of the 28 dopamine neurons. Their averaged neuronal modulation evoked by the stop signal (i.e., the difference in their averaged activity between canceled and latency-matched no-stop signal trials) is aligned at stop signal onset and shown for the shortest stop-signal delay (SSD1, light red), the second shortest stop-signal delay (SSD2, red), and the third shortest stop-signal delay (SSD3, dark red). Data obtained with the longest stop-signal delay (SSD4) was precluded from the analysis, because the monkey failed to cancel an eye movement in most of the trials and, consequently, we were unable to collect enough data for statistically valid analysis. (D) Distribution of the correlation coefficients of the 28 dopamine neurons between the stop-signal delay and the magnitude of neuronal modulation evoked by the stop signal. Filled bars indicate neurons with a significant correlation ($P < 0.05$). Arrowhead denotes the mean correlation coefficient. Double asterisk represents a significant deviation from zero ($P < 0.01$, Wilcoxon singed-rank test). (E) Averaged activity of the 28 dopamine neurons in canceled (red curve) and non-canceled (orange curve) trials with SSD3 in which canceling saccadic eye movements was equally successful and failed. SDFs are aligned at stop signal onset. See also Figures S3, S4 and S8.

Figure 4. Locations of dopamine neurons with a significant response to the stop signal

(A) Recording sites of 30 dopamine neurons in the right hemisphere of monkey M. Red circles indicate dopamine neurons showing a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test). The approximate anteroposterior distance (mm) from the interaural line is shown at the left-bottom corner of each panel. cp, cerebral peduncle; RN, red

nucleus; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; VTA, ventral tegmental area. (B) Relationship between the response of each dopamine neuron to the stop signal and the depth of the recording site. The recording depth was measured from a reference depth (the recording depth of the shallowest dopamine neuron in each monkey). Red circles indicate dopamine neurons with a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test), whereas open circles indicate dopamine neurons with no significance ($P > 0.05$, Wilcoxon rank-sum test). Gray line represents the regression line. (C) Averaged activities of the shallower (red solid curve, $n = 38$) and deeper (red dotted curve, $n = 38$) dopamine neurons. SDFs are aligned at stop signal onset. (D) Distributions of the response magnitude of the shallower (red solid line) and deeper (red dotted line) dopamine neurons. Filled bars indicate neurons with a significant response to the stop signal ($P < 0.05$, Wilcoxon signed-rank test). Arrowheads denote the mean response magnitudes. Double asterisk represents a significant difference between the distributions ($P < 0.01$, Wilcoxon rank-sum test).

Figure 5. Response of individual caudate neurons to the stop signal

(A, B) Activity of two example caudate neurons showing a significant increase (A) and decrease (B) in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test). (C) Stop-signal evoked neuronal modulation of all recorded caudate neurons ($n = 165$). (D) Neuronal modulations between canceled and non-canceled trials of the 59 increase-type neurons (upper) and the 74 decrease-type neurons (lower). All conventions are as in Figure 2. See also Figure S5.

Figure 6. Averaged activity of caudate neurons

(A) Averaged activities of the 59 and 74 caudate neurons with a significant increase (increase type; upper) or decrease (decrease type; lower), respectively, in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test). (B) Averaged activities of the 59 increase-type neurons (upper) and 74 decrease-type neurons (lower) aligned at the SSRT. (C) Effects of the stop-signal delay on the averaged activities of the 59 increase-type neurons (upper) and 74 decrease-type neurons (lower). (D) Distributions of the correlation coefficients of the 59 increase-type neurons (upper) and 74 decrease-type neurons (lower) between the stop-signal delay and the magnitude of the neuronal modulation evoked by the stop signal. (E) Averaged activities of the 59 increase-type neurons (upper) and 74 decrease-type neurons (lower) in canceled and non-canceled trials with SSD3. All conventions are as in Figure 3. See also Figures S3, S4, S5 and S8.

Figure 7. Effects of D1 and D2 antagonist injections into the caudate nucleus on monkey's behavior

(A) Effects of caudate nucleus injections of the D2 (upper) and D1 (lower) antagonists on the performance of canceling saccadic eye movements in representative injection experiments. The probability of non-canceled trials in which the monkey failed to cancel a saccadic eye movement is plotted as a function of the stop-signal delay. Data are shown for the pre-injection condition (black circles) and the post-injection condition (D2, green circles; D1, blue circles), and for the ipsilateral condition (left) and the contralateral condition (right). Black and colored curves indicate the fitted logistic

functions. ΔZ represents the difference in the stop-signal delay incurring 50% failed (non-canceled) trials in the pre- vs. post-injection conditions. Red asterisks denote a significant difference between the two conditions ($P < 0.05$, bootstrap analysis with 1,000 repetitions). (B) Changes in the stop-signal delay incurring 50% failed (non-canceled) trials (ΔZ; top), SSRT (ΔSSRT; middle), and the reaction time of saccadic eye movements (Δsaccadic RT; bottom) in the pre- vs. post-injection conditions. Data are shown for the ipsilateral condition (left) and the contralateral condition (right). Each circle represents data obtained by each injection experiment. Filled circles denote data showing a significant deviation from zero (ΔZ and ΔSSRT, $P < 0.05$, bootstrap analysis with 1,000 repetitions; Δsaccadic RT, $P < 0.05$, Wilcoxon rank-sum test). Horizontal red lines indicate the mean of each data. Especially, solid red lines indicate the mean values showing a significant deviation from zero ($P < 0.05$, Wilcoxon signed-rank test), whereas dotted ones denote the mean values with no significance ($P > 0.05$, Wilcoxon signed-rank test). (C) Changes in the reaction time of saccadic eye movements (Δsaccadic RT) in the pre- vs. post-injection conditions in the visually-guided saccade task. See also Figures S5, S6 and S7.

STAR METHODS

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, Peptides, and Recombinant Proteins		
Haloperidol	Sumitomo Dainippon Pharma	Serenace
SCH23390	Sigma	D054
Experimental Models: Organisms/Strains		
Macaca mulatta	Primate Research Institute, Kyoto University	N/A
Software and Algorithms		
MATLAB	MathWorks	https://www.mathworks.com/
TEMPO	Reflective Computing	http://reflectivecomputing.com/

Contact for Reagent and Resources Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Masayuki Matsumoto (mmatsumoto@md.tsukuba.ac.jp).

Experimental Model and Subject Details

Two adult rhesus monkeys (Macaca mulatta; monkey M, male, 8.6 kg; monkey E, male, 10.2 kg) were used in the present study. All procedures for animal care and experimentation complied with the guidelines for the Care and Use of Laboratory Animals by the University of Tsukuba, and were approved by the University of Tsukuba Animal Experiment Committee (permission number, 12-415).

Method Details

Behavioral task

Behavioral task events and data collection were controlled by TEMPO system (Reflective Computing). The monkeys sat in a primate chair facing a computer monitor in a sound-attenuated and electrically shield room. Eye movements were monitored using an infrared eye-tracking system (Eyelink, SR research) by sampling at 500 Hz.

The monkeys were trained to perform a saccadic countermanding task (**Figure 1A**). Each trials began with the presentation of a central fixation point on the monitor (0.5° diameter), and the monkey was required to fixate the point. After the monkey maintained the fixation for 800 ms, the fixation point disappeared, and simultaneously a saccadic target (0.5° diameter) was presented at the right or left side of the point (8° eccentricity in monkey M, 10° eccentricity in monkey E). In 70% of the trials, the monkey was required to make a saccadic eye movement to the target within 550 ms and to fixate the target for 500 ms (no-stop signal trials). In the remaining 30% of the trials, the fixation point reappeared as a "stop signal" with a delay (referred as the stop-signal delay) after the onset of the saccadic target (stop signal trials). The monkey was then required to cancel the planned eye movement and fixate the stop signal for 600 ms (canceled trials). The correct behavior was signaled by a tone (1 kHz), and simultaneously a liquid reward was delivered. If the monkey failed to cancel and generated the eye movement to the target in stop signal trials, the stop signal and the target remained for 600 ms, and then a beep tone (100 Hz) was given without a liquid reward (non-canceled trials). Four stop-signal delays (184 to 334 ms in monkey M and 84 to 234 ms in monkeys E) were used during single-unit recording and six stop-signal

delays (167 to 334 ms in monkey M, 67 to 234 ms in monkey E) were used in pharmacological experiments. All trials were presented with a random intertrial interval that was ranging from 2000 to 3000 ms.

The monkeys were also trained to perform a visually-guided saccade task. The task procedure was the same as the saccadic countermanding task except that the stop signal never appeared.

Electrophysiology

A plastic head holder and two recording chambers were fixed to the skull under general anesthesia and sterile surgical condition. One recording chamber was placed over the frontoparietal lobes, tilted laterally by 36°, and aimed at the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA). The other recording chamber was placed over the midline of the frontoparietal lobes, and aimed at the caudate nucleus. The head holder and the recording chambers were embedded in dental acrylic that covered the top of the skull and were firmly anchored to the skull by plastic screws. After implanting the head holder and recording chambers, the monkeys underwent a magnetic resonance image (MRI) scan to determine the position of the recording electrode.

Single-unit recordings were performed using tungsten electrodes with impedance of 1.2 to 2.5 MΩ (Frederick Haer) that were introduced into the brain through a stainless-steel guide tube by an oiled-driven micromanipulator (MO-97-S, Narishige). The recording sites were determined using a grid system, which allowed recordings at every 1 mm between penetrations. For finer mapping of neurons, we also used a complementary grid which allowed electrode penetrations between the holes of the

original grid.

Single-unit potentials were amplified and band-pass filtered (100Hz to 8 kHz) using a multichannel processor (MCP Plus 8, Alpha Omega) and isolated online using a voltage-time window discrimination system (ASD, Alpha omega). The time of occurrence of each action potential was stored with 1-ms resolution.

Drug injection

After all single-unit recording sessions, we injected haloperidol (5 µg/µl) and SCH23390 (10 µg/µl) that mainly prevents D2 and D1 receptor signaling, respectively, into the caudate nucleus of the two monkeys unilaterally. The injection sites were determined based on the region where we found task-related neurons in single-unit recording during the saccadic countermanding task (see **Figure S5** for injection sites). The drug solutions were pressure-injected, 0.2 µl every 30 s for 10 times (2 µl in total), using a 10-µl microsyringe with a 30 gage needle (Hamilton). These doses were chosen based on previous studies in monkeys (Sawaguchi and Goldman-Rakic, 1994; Watanabe and Kimura, 1998). We also injected the same amount of saline as a control.

For each injection experiment, the monkeys performed 600 or 700 trials of the saccadic countermanding task or 400 trials of the visually-guided saccade task before injection as a pre-injection control. We then injected haloperidol, SCH23390 or saline. Ten minutes after the injection, the monkeys started performing post-injection trials, 600 or 700 trials of the saccadic countermanding task or 400 trials of the visually-guided saccade task.

Histology

After all single-unit recording and injection experiments in monkey M, we selected representative locations of electrode penetration into the SNc and caudate nucleus, and made electrolytic microlesions (12 μA and 30 s). Then monkey M was deeply anaesthetized with pentobarbital sodium and perfused with 10% formaldehyde. The brain was blocked and equilibrated with 30% sucrose. Frozen sections were cut every 50 μm in the coronal plane, and then stained with cresyl violet.

Quantification and Statistical Analysis

To evaluate the effect of the stop-signal delay on the performance of canceling saccadic eye movements, the probability of trials in which the monkey failed to cancel an eye movement (non-canceled trials) was fit by the following logistic function

$$P = 1/\{1 + e^{-k(stop\ signal\ delay - x_0)}\}$$

where P indicates the probability of non-canceled trials, k indicates the slope and x_0 indicates the x-value of the midpoint of the logistic curve.

The stop-signal reaction time (SSRT), the duration that the STOP process needs to finish, was estimated based on a mathematical model that assumes a race between the GO and the STOP process (**Figure 1C**) (Logan and Cowan, 1984). For the estimation, the distribution of the durations of the GO process (i.e., the reaction times of saccadic eye movements in no-stop signal trials) was integrated from saccadic target onset until the integral equals the proportion of non-canceled trials (**Figure 1D**). The saccadic reaction time at the end of the integral is the longest reaction time at which the GO process can finish before the STOP process. Thus, the time between the onset of the stop signal and

this saccadic reaction time is the SSRT. We estimated the SSRTs at each stop-signal delay and averaged them for each behavioral data obtained in all single-unit recording sessions. The averaged value was assigned as the SSRT of each recording session.

To calculate spike density functions (SDFs), each spike was replaced by a combination of growth and decay exponential functions that resembles a postsynaptic potential given by the following equation

$$R(t) = \left\{1 - e^{(-t/\tau_g)}\right\} \cdot e^{(-t/\tau_d)}$$

where rate as a function of time, *R(t)*, varies according to τ_g (the time constant for the growth phase) and τ_d (the time constant for the decay phase). The same equation was used by Hanes et al. (1998). τ_g and τ_d were set to 1 and 20 ms, respectively, according to physiological data from excitatory synapses (Mason et al., 1991; Sayer et al., 1990).

To determine whether recorded neurons are involved in canceling planned saccadic eye movements, we compared their activity in trials in which a saccadic eye movement was canceled (canceled trials) vs. trials in which the eye movement was properly executed (no-stop signal trials). According to the race model, a planned saccadic eye movement is canceled in canceled trials if the STOP process finishes before the GO process. Therefore, to properly compare the activity in no-stop signal trials with that in canceled trials, we need to use only the no-stop signal trials in which the initiation of the eye movement was slow enough that the eye movement would have been canceled if the stop signal had been presented. These trials are referred as "latency-matched no-stop signal trials" (Hanes et al., 1998), and the latencies of saccadic eye movements in these trials are longer than the stop-signal delay plus the SSRT. The comparison

between canceled trials and latency-matched no-stop signal trials allowed us to detect a neuronal modulation related to the STOP process and counteract a neuronal modulation related to the GO process, because the GO-related neuronal modulation is presumed to be equivalent in the canceled and latency-matched no-stop signal trials.

We also compared the activity in trials in which the monkey failed to cancel a saccadic eye movement (non-canceled trials) vs. trials in which the eye movement was properly executed (no-stop signal trials). According to the race model, a planned saccadic eye movement is executed in non-canceled trials if the GO process finishes before the STOP process. Therefore, to properly compare the activity in no-stop signal trials with that in non-canceled trials, we used only the no-stop signal trials in which the initiation of the eye movement was fast enough that the eye movement would have been executed even if the stop signal had been presented. These trials are also referred as "latency-matched no-stop signal trials", and the latencies of saccadic eye movements in these trials are shorter than the stop-signal delay plus the SSRT.

For the above two comparisons (i.e., canceled vs. latency-matched no-stop signal trials and non-canceled vs. latency-matched no-stop signal trials), we calculated the neuronal activity aligned at stop signal onset not only in canceled and non-canceled trials but also in latency-matched no-stop signal trials in which the stop signal was not presented. To obtain the stop-signal aligned activity in the latency-matched no-stop signal trials, we applied a permutation procedure that has been used in previous studies (Mayse et al., 2015). For each neuron, we first randomly sampled trials from the latency-matched no-stop signal trials to form a new data-set that had the same number of trials as the canceled trials. We then assigned the same stop-signal delays used in the canceled trials to the randomly-sampled latency-matched no-stop signal trials. We

calculated the stop-signal aligned activity of the randomly-sampled latency-matched no-stop signal trials using the assigned stop-signal delays. This procedure allowed us to obtain the neuronal activity aligned at the timing when the stop signal would have been presented in the latency-matched no-stop signal trials.

To visualize the time course of neuronal modulation evoked by the stop signal for each neuron, we calculated receiver operating characteristic (ROC) value for discriminating the discharge rate in canceled vs. latency-matched no-stop signal trials using a 50-ms test window sliding with a 10-ms step. We also calculated ROC value for discriminating the discharge rate in canceled vs. non-canceled trials using the same procedure.

To analyze neuronal modulation elicited by the stop signal in dopamine neurons, we calculated the discharge rate of each dopamine neuron during 80 to 190 ms after stop signal onset and compared the discharge rate in canceled vs. latency-matched no-stop signal trials. In caudate neurons, we calculated the discharge rate during 100 to 300 ms after stop signal onset. These time windows were chosen such that they included a major part of the neuronal modulation in canceled vs. latency-matched no-stop signal trials.

We classified caudate neurons into “increase” and “decrease” types based on their response to the stop signal. We calculated the discharge rate of each caudate neuron using a 120-ms sliding window shifting from stop signal onset in 1-ms step. If a caudate neuron showed a significant increase or decrease in the discharge rate in canceled trials compared with latency-matched no-stop signal trials during the first and at least 19 of the 20 consecutive windows, it was classified as increase or decrease type, respectively ($P < 0.05$, Wilcoxon rank-sum test). To filter out caudate neurons showing a significant

but small modulation from the classification, we removed neurons of which the discharge rate was smaller than 2 spikes/s during the time windows in canceled and latency-matched no-stop signal trials.

To conduct population analyses, we combined the activity of each neuron in the ipsilateral and contralateral conditions if the neuron exhibited a significant modulation (i.e., increase or decrease) in the discharge rate in canceled trials vs. latency-matched no-stop signal trials in both conditions ($P < 0.05$, Wilcoxon rank-sum test). If the neuron exhibited a significant modulation only in either of the conditions, we used the activity in that condition.

To determine whether neuronal modulation elicited by the stop signal started earlier than the SSRT, we calculated the latency of the neuronal modulation aligned at the SSRT. We defined the latency as the time when the difference in spike density function between canceled and latency-matched no-stop signal trials exceeded by 2 SD of the difference during the 250-ms time window before target onset. We used the averaged spike density function across neurons to determine the latency at the population level (**Figures 3B** and **6B**), and used the spike density function of each neuron to determine the latency at the single neuron level (**Figure S4A - C**).

To examine the correlation between the stop-signal delay and the neuronal modulation evoked by the stop signal, we removed the data obtained using the longest stop-signal delay because the monkey failed to cancel an eye movement in most of the trials and consequently we were unable to collect enough data for statistically valid analysis.

To evaluate the effect of the D1 and D2 antagonists on the performance of canceling saccadic eye movements, we calculated the stop-signal delay at which the monkey failed

in 50% of trials by fitting the performance with the logistic function, and compared the stop-signal delay in the pre- vs. post-injection conditions for each injection site (ΔZ in **Figure 7A**). We also compared the SSRT (ΔSSRT) and the reaction time of saccadic eye movements (Δsaccade RT) in the two conditions. To test a statistic significance of the effects of drug injection on the stop-signal delay incurring 50% failed trials (i.e., non-canceled trials) and the SSRT for each injection site, we applied a bootstrap procedure. For each injection site, trials were randomly resampled with replacements to form a new bootstrap data-set which had the same number of trials as the original data-set. Using the new data-set, we compared the stop-signal delay incurring 50% failed trials and the SSRT in the pre- vs. post-injection conditions. Such random resampling and comparison were repeated 1,000 times. If the stop-signal delay and SSRT were larger in the pre-injection condition than in the post-injection condition or vice versa in more than 975 repetitions, the changes in these behavioral indices in the two conditions were regarded as significant.

Data and Software Availability

All data and computer codes are available upon request to the Lead Contact.

Figure 1

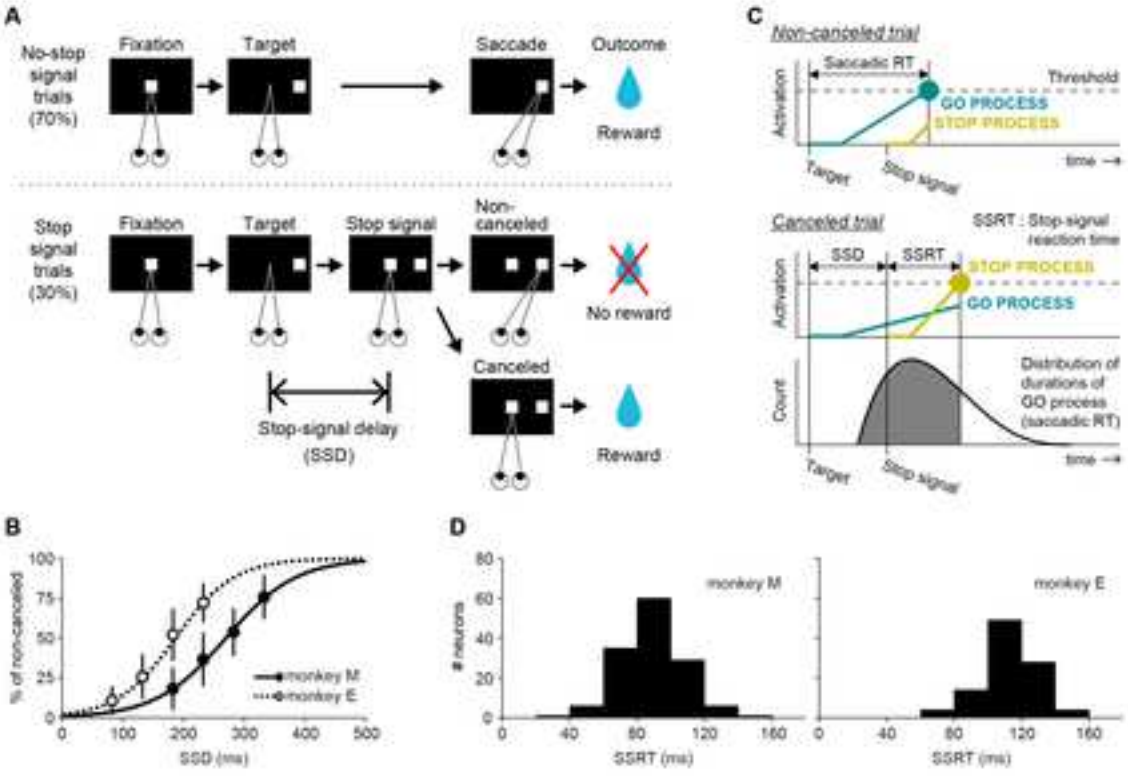

Figure 1 Ogasawara et al.

Figure 2

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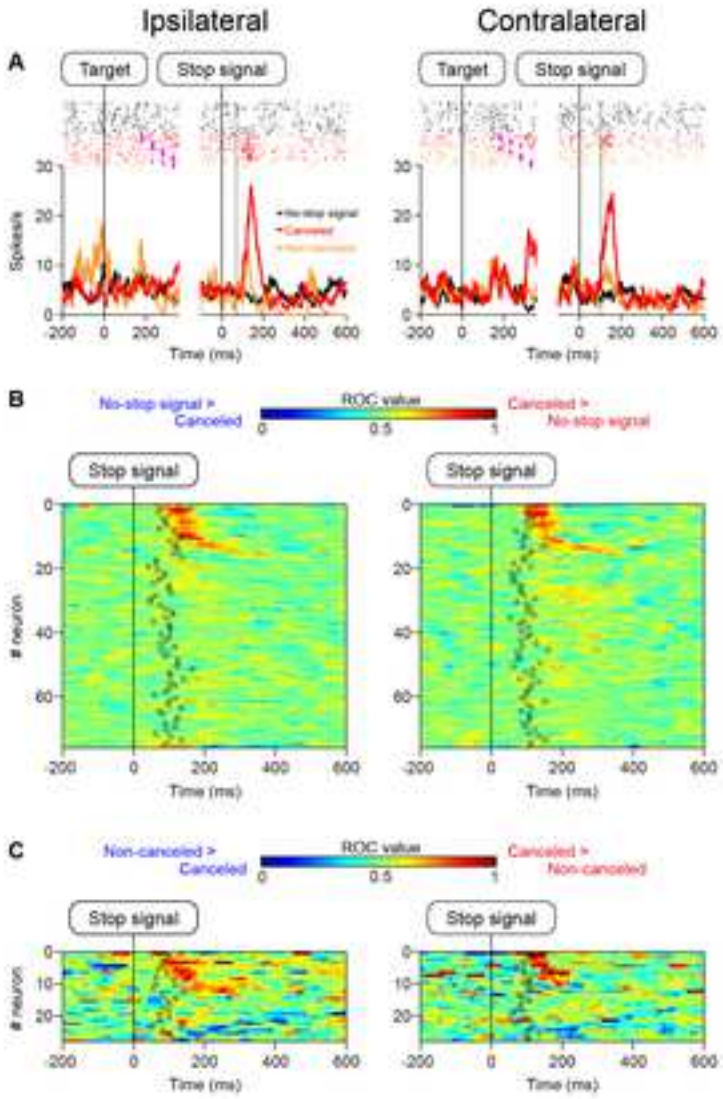

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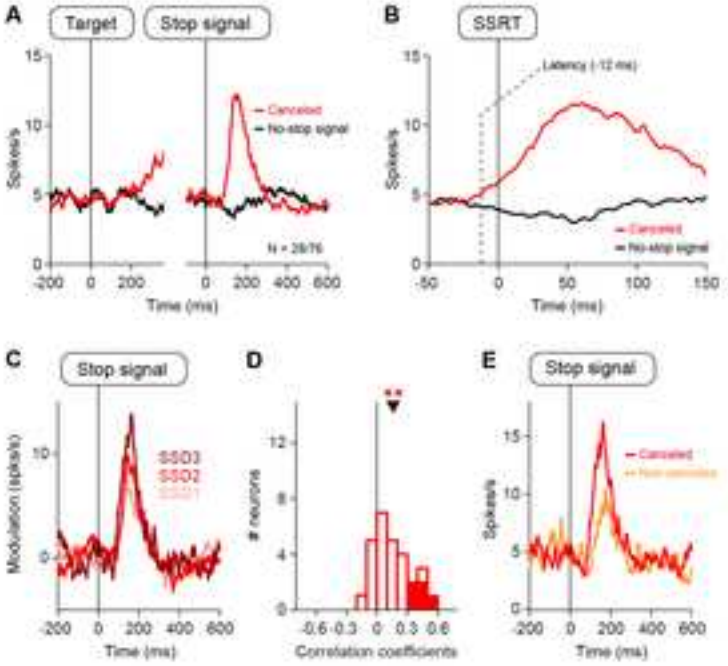

Figure 3 Ogasawara et al.

Figure 4

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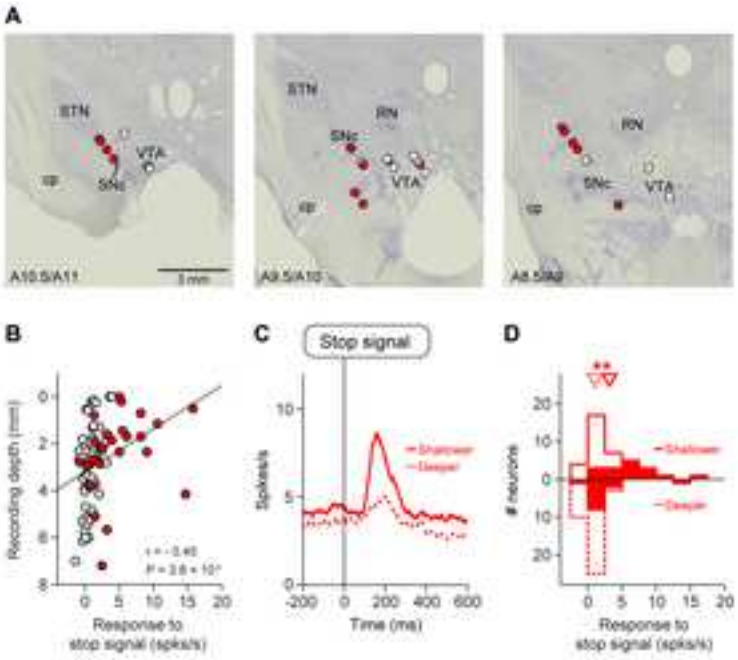

Figure 4 Ogasawara et al.

Figure 5

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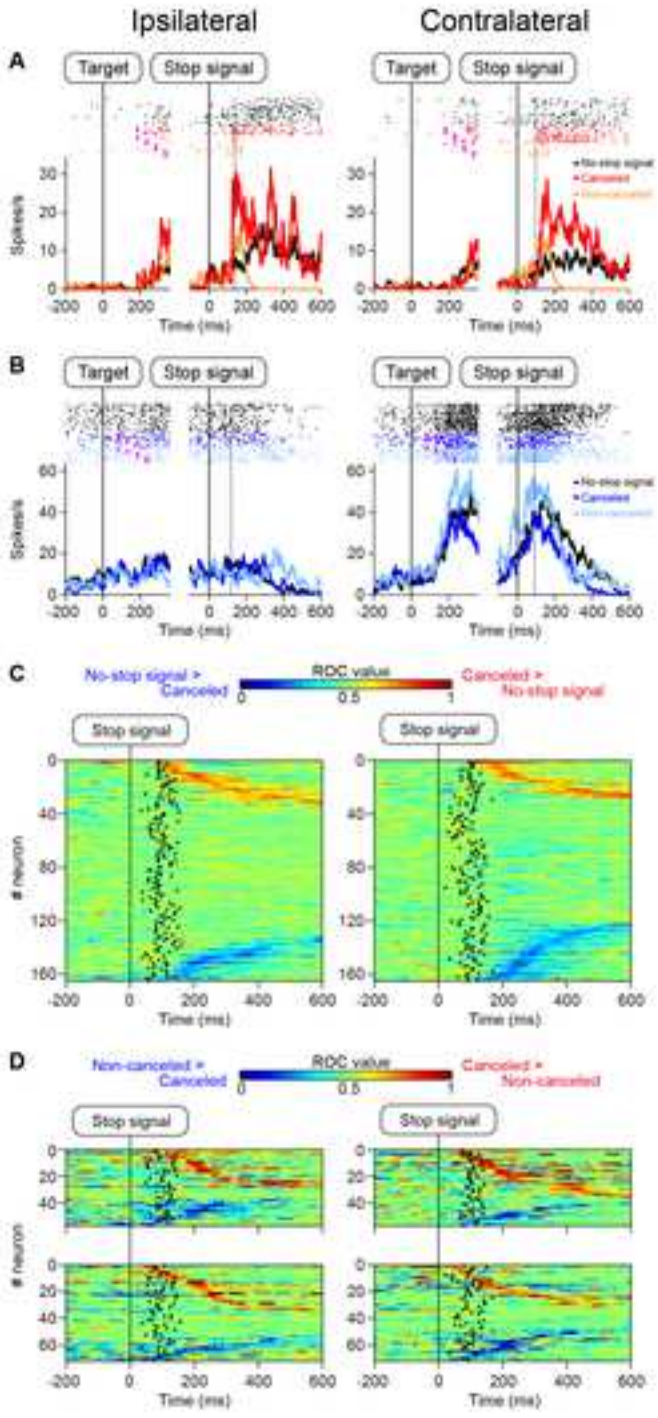

Figure 5 Ogasawara et al.

Figure 6

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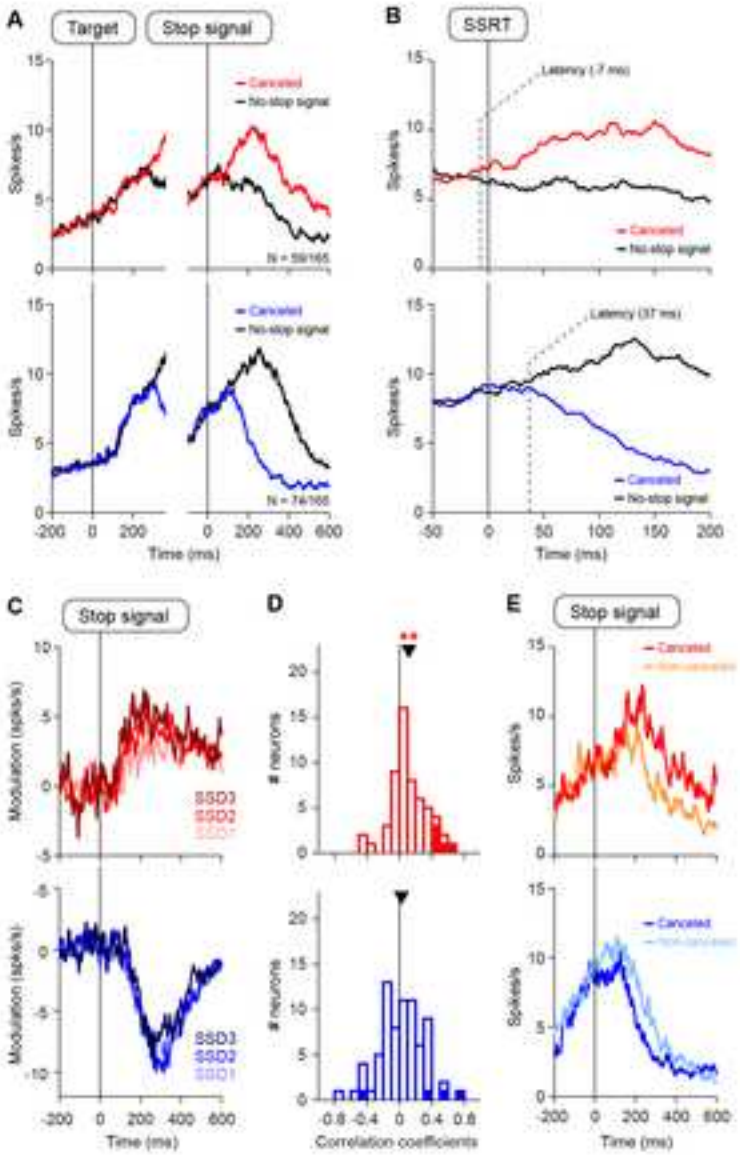

Figure 6 Ogasawara et al.

Figure 7

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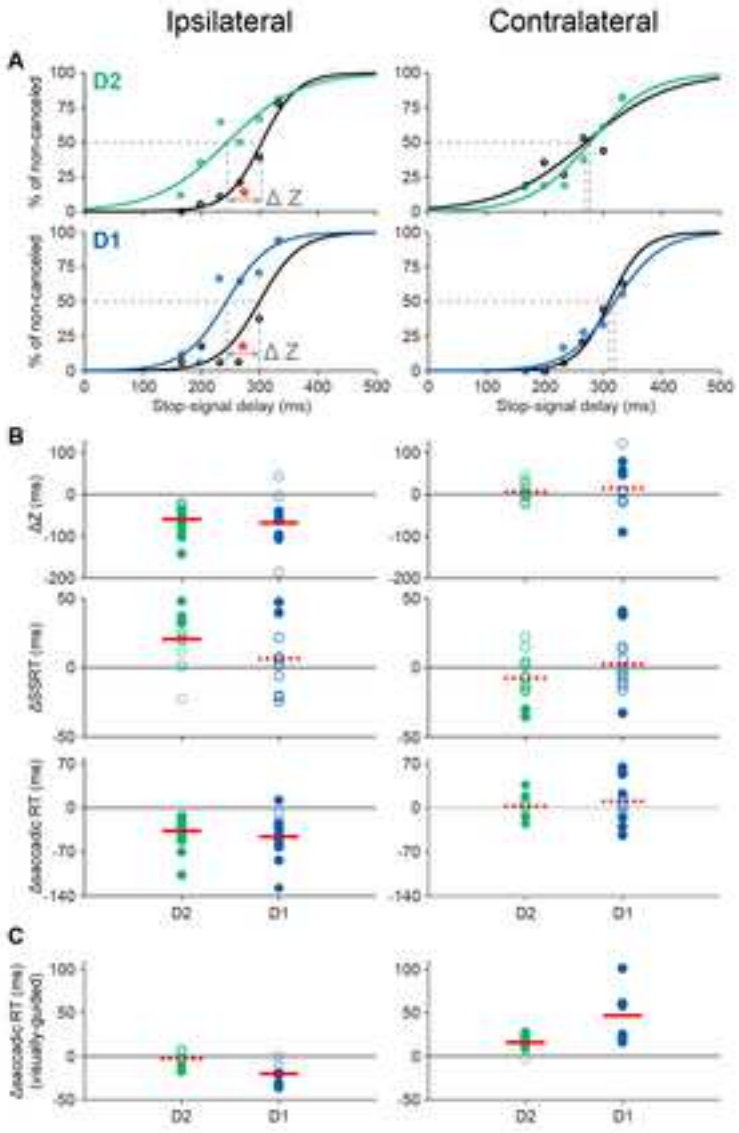

Figure 7 Ogasawara et al.

SUPPLEMENTAL INFORMATION

Primate nigrostriatal dopamine system regulates saccadic response inhibition

Takaya Ogasawara, Masafumi Nejime, Masahiko Takada, Masayuki Matsumoto

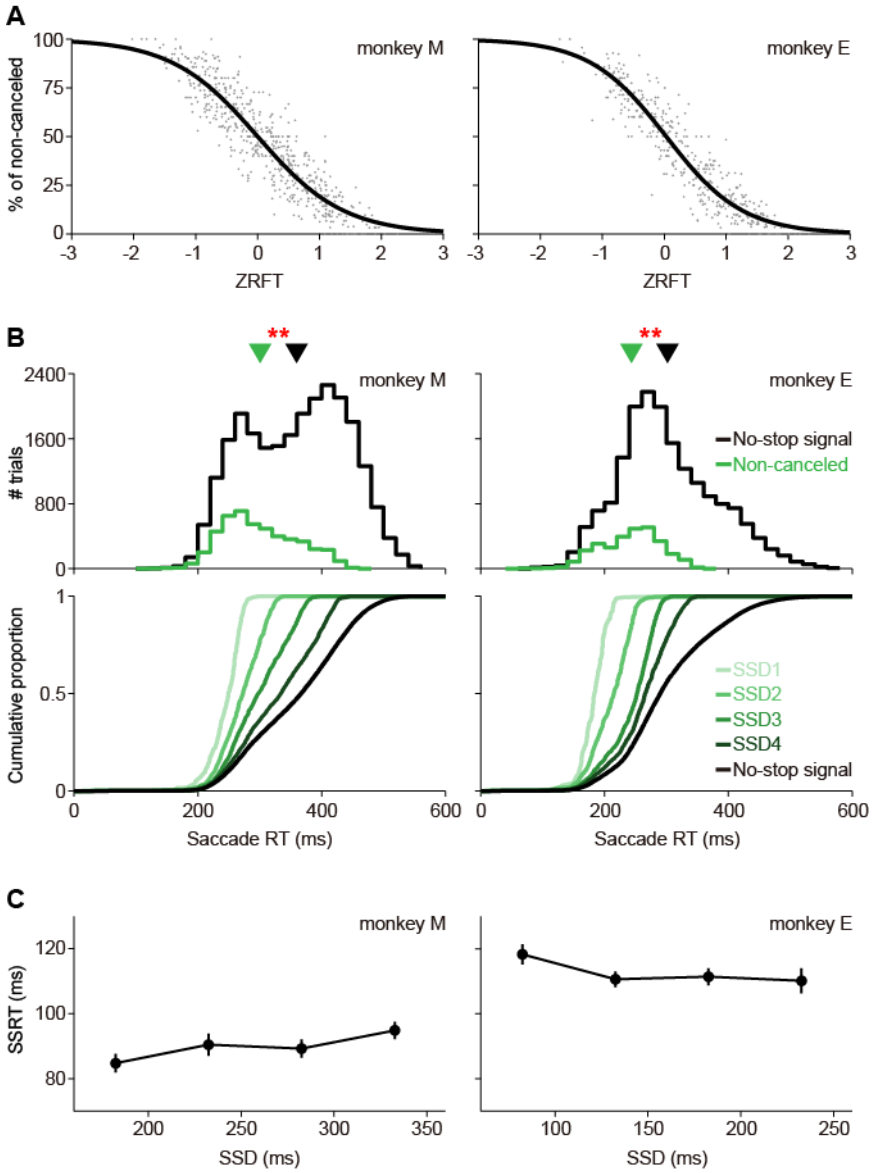

Figure S1. Additional data analyses on behavioral performance, Related to Figure 1.

(A) Normalized probability of non-canceled trials from all sessions for each monkey. Abscissa indicates the relative finishing time Z-score (ZRFT). ZRFT = (mean saccadic reaction time - stop-signal delay – SSRT) / SD of saccadic reaction time. This quantity represents the time relative to the finish times of the GO and STOP processes normalized by the SD of the saccadic reaction times in no-stop signal trials. Each dot indicates the probability of non-canceled trials as a function of ZRFT for each session.

Curve indicates the fitted logistic function. See Stuphorn et al. (2010) for details. (B) Upper; distributions of saccadic reaction times in no-stop signal (black) and non-canceled (green) trials. Arrowheads denote the mean saccadic reaction times. Double asterisk represents a significant difference between the distributions ($P < 0.01$, Wilcoxon rank-sum test). Lower; cumulative distributions of saccadic reaction times shown for each stop-signal delay in non-canceled trials. (C) Mean SSRT across sessions as a function of the stop-signal delay. Error bars indicate SEM. The SSRT was not influenced by the stop-signal delay (monkey M, $P = 0.12$, $n = 141$ sessions; monkey E, $P = 0.20$, $n = 100$ sessions; one-way ANOVA).

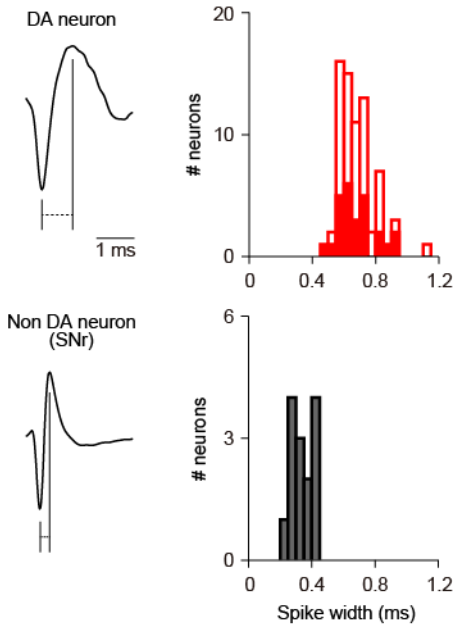

Figure S2. Spike width of dopamine neurons, Related to Figure 2.

Distributions of the spike widths of dopamine neurons (upper, n = 72) and non-dopamine neurons (n = 14) in the substantia nigra pars reticulata (lower). Of the 76 recorded dopamine neurons, we recorded the spike shape of 72 neurons. These neurons constitute the data used in this analysis. Filled red bars indicate dopamine neurons showing a significant increase in their activity in canceled trials compared with latency-matched no-stop signal trials ($P < 0.05$, Wilcoxon rank-sum test). The spike widths of the stop-signal responsive dopamine neurons were not significantly different from those of the non-responsive dopamine neurons (stop-signal responsive dopamine neurons, mean ± SD = 0.68 ± 0.12 ms, n = 26; non-responsive dopamine neurons, mean ± SD = 0.68 ± 0.12 ms, n = 46; $P = 0.95$, Wilcoxon rank-sum test). Example spike shapes for each neuron type are shown on the left. Two vertical lines indicate how the spike duration was measured.

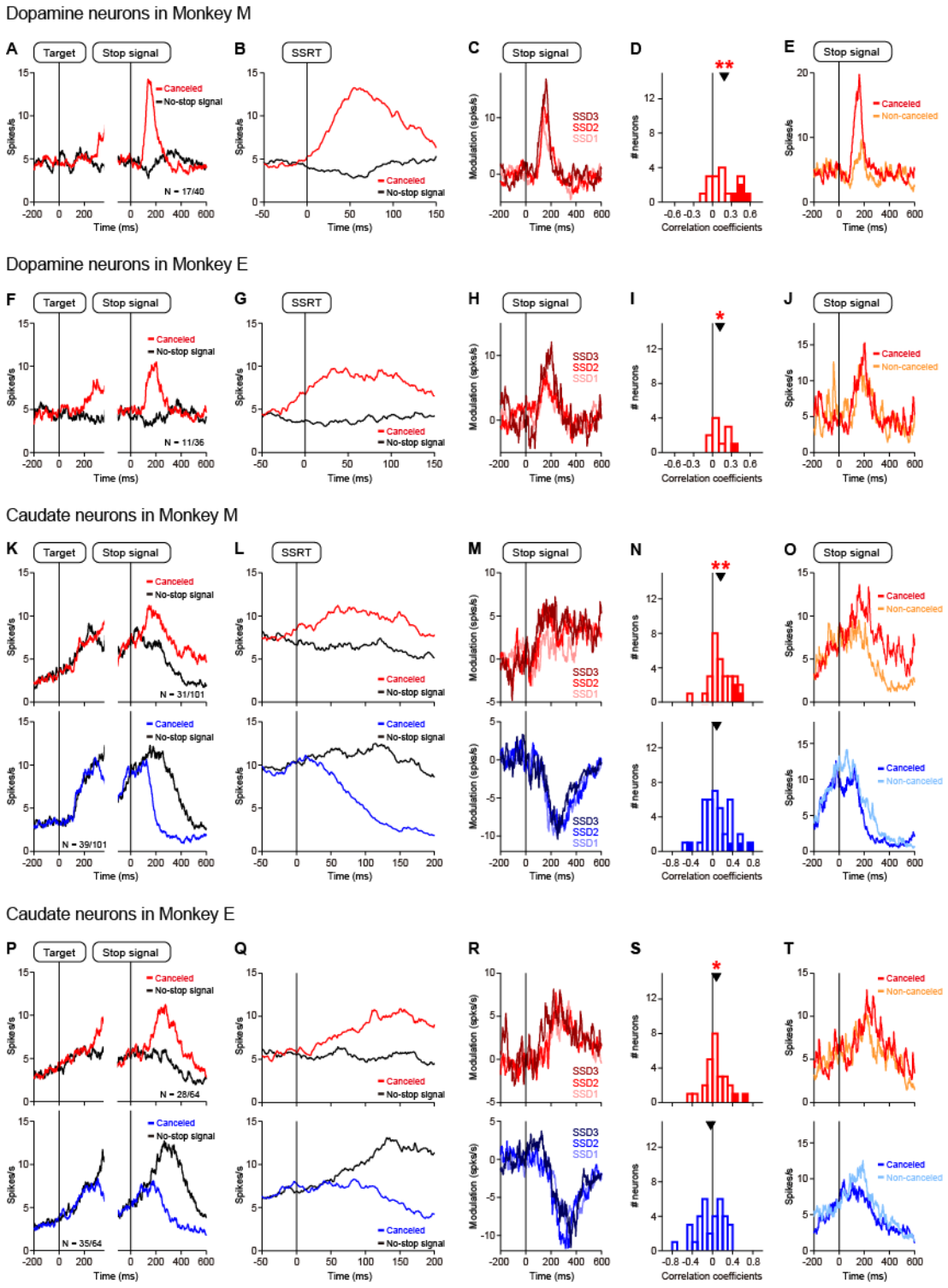

Figure S3. Population analyses in individual monkeys, Related to Figures 3 and 6.

Population analyses on dopamine neurons in monkey M (A - E) and monkey E (F - J),

and caudate neurons in monkey M (K - O) and monkey E (P - T). All conventions are as in Figures 3 and 6.

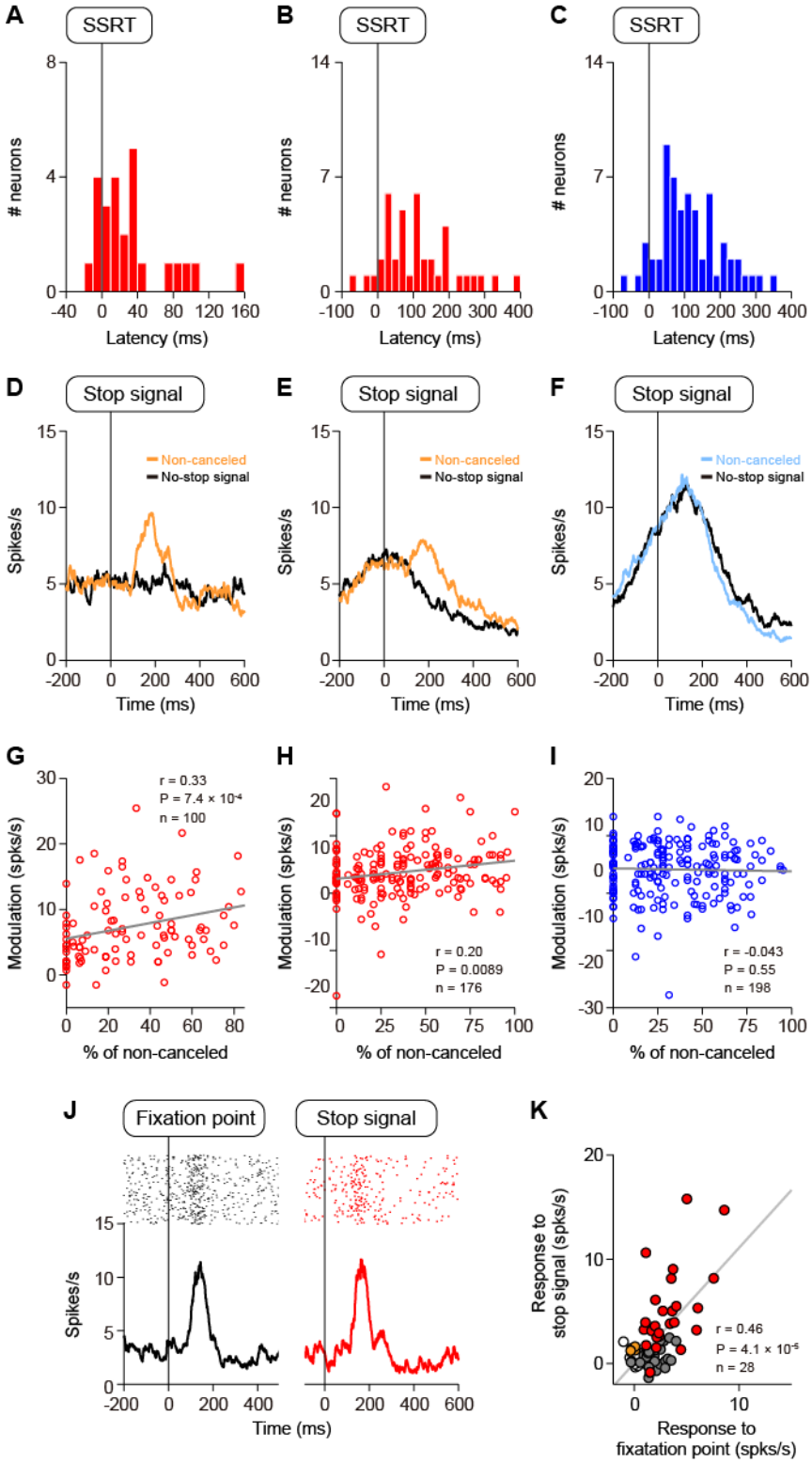

Figure S4. Additional data analyses on neuronal activity, Related to Figures 3 and 6.

(A - C) SSRT-aligned latency of neuronal modulation evoked by the stop signal for each dopamine neuron (n = 25) (A), each increase-type caudate neuron (n = 40) (B), and each decrease-type caudate neuron (n = 61) (C). For 3 dopamine neurons, 19 increase-type caudate neurons, and 13 decrease-type caudate neurons, their latencies were not detected. These neurons were excluded from the analysis. (D - F) Averaged activities of the 28 dopamine neurons (D), 59 increase-type caudate neurons (E), and 74 decrease-type caudate neurons (F) in non-canceled (colored curve) and latency-matched no-stop signal (black curve) trials. SDFs are aligned at stop signal onset. (G - I) Relationship between the probability of non-canceled trials and the magnitude of the neuronal modulation evoked by the stop signal in canceled trials. The data are shown for the 28 dopamine neurons (G), 59 increase-type caudate neurons (H), and 74 decrease-type caudate neurons (I). Each plot indicates data obtained for each stop-signal delay in each neuron with sufficient canceled trials (4 trials or more). Black lines indicate the regression lines. (J) Activity of an example dopamine neuron aligned at fixation point onset (left) and stop signal onset (right). (K) Relationship between the responses of each dopamine neuron to the stop signal and fixation point. Light red and gray circles indicate dopamine neurons showing a significant response to the stop signal and fixation point, respectively ($P < 0.05$, Wilcoxon signed-rank test). Red circles indicate dopamine neurons showing significant responses to both of them ($P < 0.05$, Wilcoxon signed-rank test), whereas open circles denote neurons with no significance ($P > 0.05$, Wilcoxon signed-rank test).

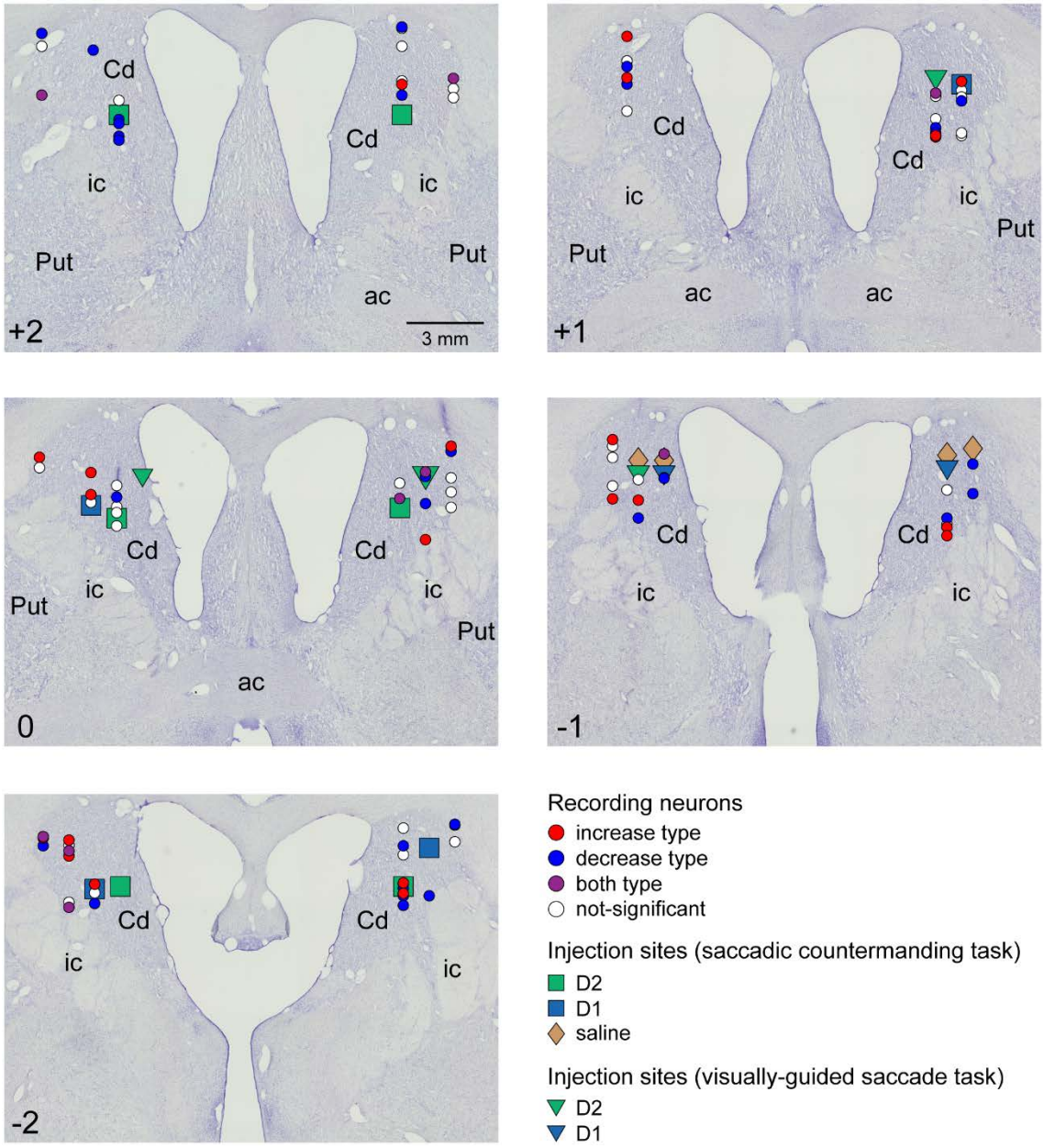

Figure S5. Histological reconstruction of the recording and injection sites in the caudate nucleus, Related to Figures 5, 6 and 7.

The recording sites of 101 caudate neurons and the injection sites in the pharmacological experiments in monkey M are plotted. Red and blue circles indicate neurons showing a significant increase (i.e., increase type) and decrease (i.e., decrease

type), respectively, in their activity in canceled trials compared with latency-matched no-stop signal trials in either or both of the ipsilateral and contralateral conditions ($P < 0.05$, Wilcoxon rank-sum test). Purple circles denote neurons showing a significant but opposite modulation of their activity in the ipsilateral vs. contralateral conditions ($P < 0.05$, Wilcoxon rank-sum test). Open circles indicate neurons with no significant modulation ($P > 0.05$, Wilcoxon rank-sum test). Green and blue rectangles and light brown diamonds represent the injection sites of the D2 and D1 antagonists and saline, respectively, in the saccadic countermanding task. Green and blue triangles indicate the injection sites of the D2 and D1 antagonists, respectively, in the visually-guided saccade task. The approximate anteroposterior distance (mm) from the anterior commissure is shown at the left-bottom corner of each panel. ac, anterior commissure; Cd, caudate nucleus; ic, internal capsule; Put, putamen.

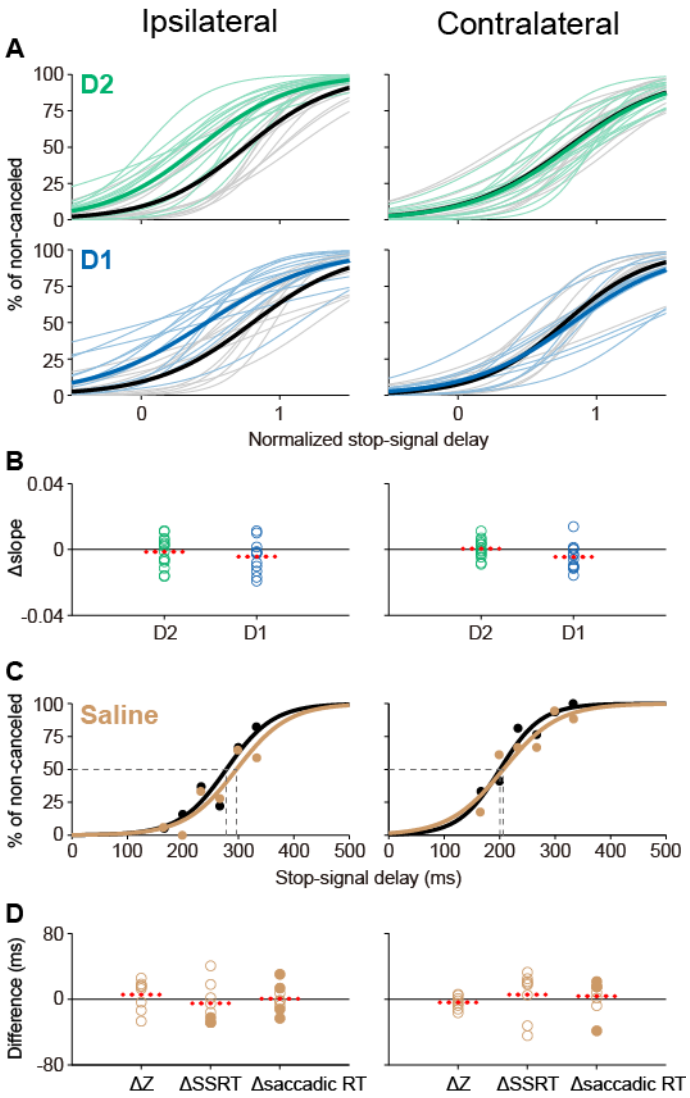

Figure S6. Additional data analyses in the pharmacological experiments, Related to Figure 7.

(A) Probability of non-canceled trials as a function of the normalized stop-signal delay in each injection experiment. Fitted logistic functions are shown for the pre-injection condition (gray curve) and the post-injection condition (D2, green curve; D1, blue curve), and for the ipsilateral condition (left) and the contralateral condition (right). Bold curves indicate the averaged logistic functions across each injection experiment. The stop-signal delay was normalized as it ranges from 0 to 1. (B) Change in the slop of the

fitted logistic function in the pre- vs. post-injection conditions. All conventions are as in Figure 7B. (C) Effect of saline injection on the performance of canceling saccade eye movements in a representative injection experiment. All conventions are as in Figure 7A. (D) Changes in the stop-signal delay incurring 50% failed (non-canceled) trials (ΔZ), SSRT (ΔSSRT), and the reaction time of saccadic eye movements (Δsaccadic RT) in the pre- vs. post-injection conditions. All conventions are as in Figure 7B. None of the mean values showed a significant deviation from zero ($P > 0.05$, Wilcoxon signed-rank test).

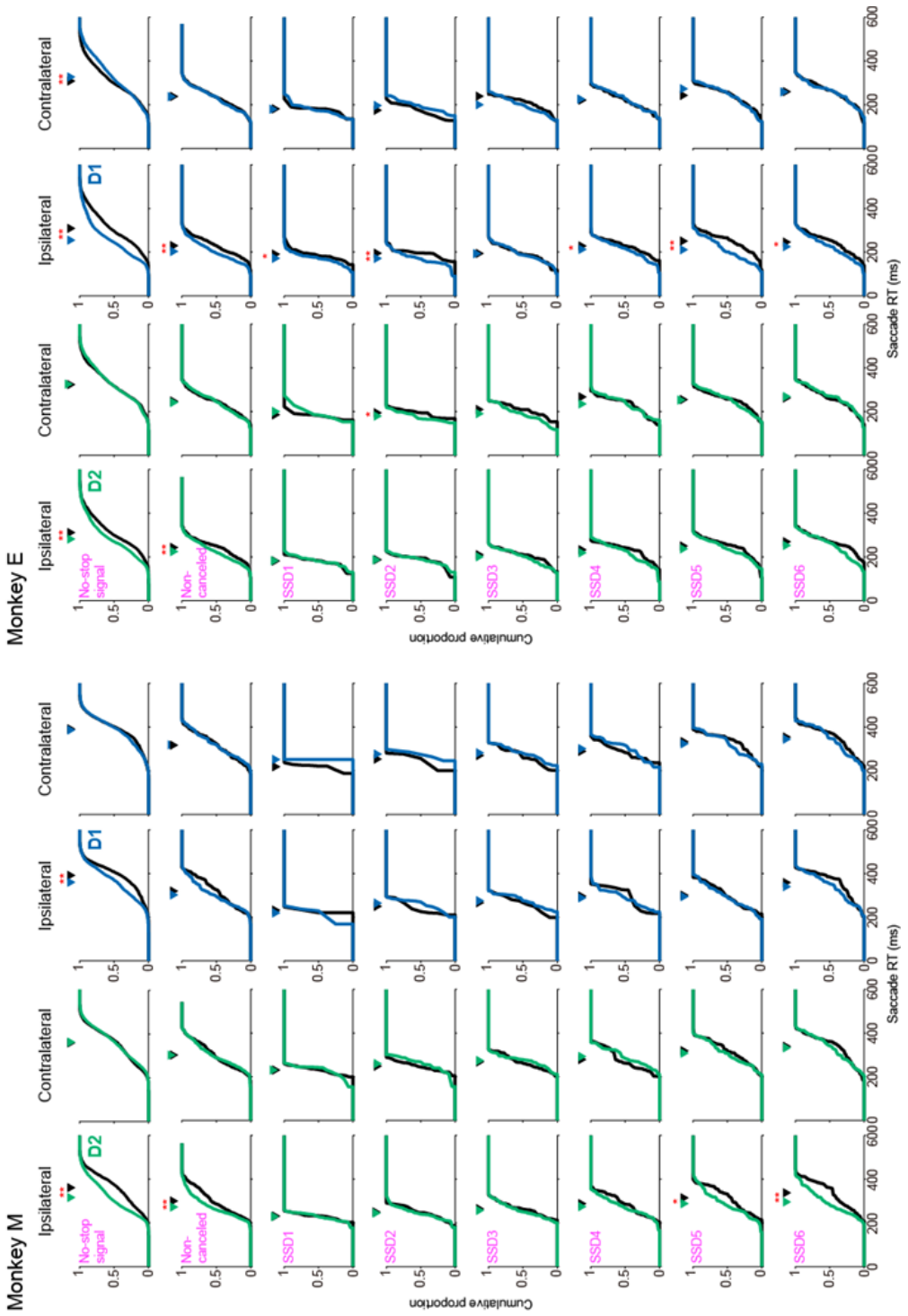

Figure S7. Cumulative distributions of saccadic reaction times in no-stop signal and non-canceled trials in the pre- and post-injection conditions, Related to Figure 7.

The first row exhibits reaction times in no-stop signal trials. The second to eighth rows

exhibit reaction times in non-canceled trials (the second row, all stop-signal delays; the third to eighth rows, each stop-signal delay). Black and colored lines indicate reaction times in the pre- and post-injection conditions, respectively (green, D2; blue, D1). Arrowheads indicate mean reaction times. Single and double asterisks indicate a significant difference in reaction times between the pre- and post-injections ($P < 0.05$ and 0.01, respectively, Wilcoxon rank-sum test).

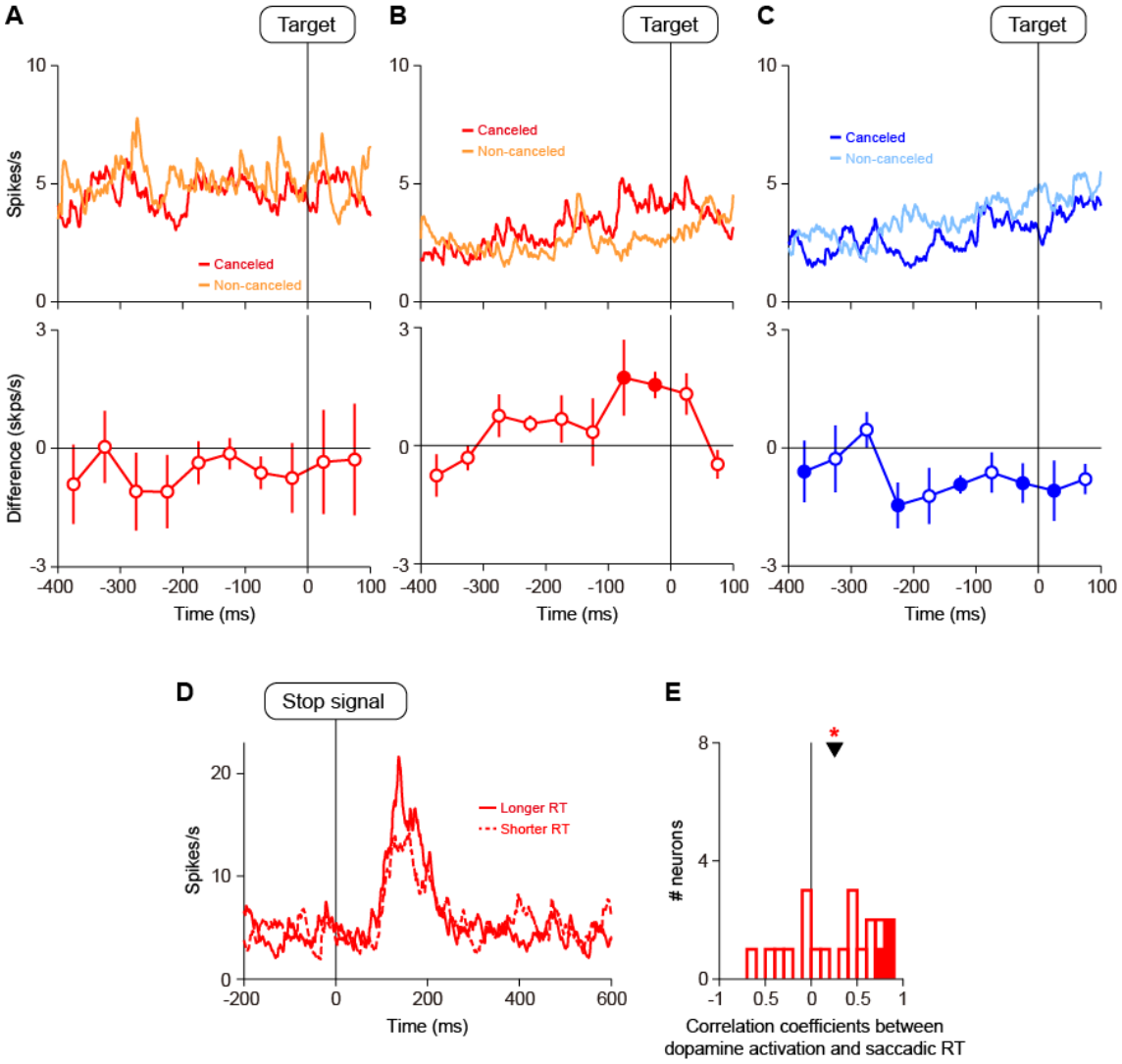

Figure S8. Proactive regulation of saccadic eye movements by dopamine neurons and the caudate nucleus, Related to Figures 3 and 6.

(A - C) Upper; averaged activity of the 28 dopamine neurons (A), 59 increase-type caudate neurons (B), and 74 decrease-type caudate neurons (C) in canceled trials (red and blue curves) and non-canceled trials (orange and cyan curves) with the third shortest stop-signal delay (SSD3, 284 and 184 ms in monkeys M and E, respectively). In this stop-signal delay, canceling saccadic eye movements was equally successful and failed, and we were able to collect enough data to compare neuronal activities in

canceled vs. non-canceled trials. SDFs are aligned at target onset. Lower; difference between the averaged activities in canceled and non-canceled trials calculated for each 50-ms bin. Filled circles indicate differences with a significant deviation from zero ($P < 0.05$, bootstrap test with 1,000 repetitions). Open circles indicate differences with no significance ($P > 0.05$, bootstrap test with 1,000 repetitions). Error bars indicate SEM. (D) Averaged activities of 20 of the 28 dopamine neurons in canceled trials. In order to examine the relationship between the dopamine neuron activation evoked by the stop signal and the reaction time of saccadic eye movement in the next trial, we analyzed dopamine neuron activity in canceled trials followed by a no-stop signal trial in which the monkey properly executed a saccadic eye movement. We excluded 8 of the 28 dopamine neurons in which the number of canceled trials followed by a no-stop signal trial was smaller than 5 trials. We split the canceled trials into two groups according to the saccadic reaction time in the next no-stop signal trial for each neuron. The SDFs are shown for canceled trials followed by a no-stop signal trial with a longer saccadic reaction time (solid red curve) and those followed by a no-stop signal trial with a shorter saccadic reaction time (dotted red curve). (E) Distribution of the correlation coefficients of the 20 dopamine neurons between the magnitude of activation evoked by the stop signal and the saccadic reaction time in the next no-stop signal trial. Arrowhead denotes the mean correlation coefficient. Single asterisk represents a significant deviation from zero ($P < 0.05$, Wilcoxon singed-rank test).

In order to investigate proactive influences of dopamine neurons and the caudate nucleus on saccade promotion, we examined the relationship between their neuronal activities before the presentation of saccadic target and the performance of response inhibition. We found that the increase-type caudate neurons exhibited a significantly

stronger activation in canceled trials than in non-canceled trials during 100 ms before target onset (**Figure S8B**). Since the increase-type caudate neurons were also more strongly activated in canceled trials than in non-canceled trials in their response to the stop signal (upper in **Figure 6E**), these neurons seem to consistently suppress saccadic eye movements even before the presentation of the saccadic target. On the other hand, the decrease-type caudate neurons exhibited a significantly stronger activation in non-canceled trials than in canceled trials for several 50-ms bins before target onset (**Figure S8C**). Since the decrease-type neurons were also more strongly activated in non-canceled trials in their response to the stop signal (lower in **Figure 6E**), these neurons seem to consistently facilitate saccadic eye movements during the task. These results suggest that the activity of caudate neurons represented whether the monkey would successfully cancel or erroneously execute a saccadic eye movement even before the presentation of saccadic target.

Although the dopamine neurons did not exhibited a significant difference between canceled and non-canceled trials before target onset (**Figure S8A**), we found that the magnitude of the dopamine neuron activation evoked by the stop signal was correlated with the reaction time of saccadic eye movement in the next trial (**Figure S8D** and **E**). On average, the dopamine neuron activation evoked by the stop signal was significantly stronger in canceled trials followed by a no-stop signal trial with a longer reaction time than those followed by a no-stop signal trial with a shorter reaction time (canceled trials followed by a longer reaction time, mean ± SD = 14.8 ± 10.0 spikes/s; canceled trials followed by a shorter reaction time, mean ± SD = 11.4 ± 8.4 spikes/s; $P = 0.044$, $n = 20$, Wilcoxon signed-rank test) (**Figure S8D**). The correlation coefficients between the magnitude of the dopamine neuron activation and the reaction time of saccadic eye

movement in the next trial were significantly larger than zero (r, mean ± SD = 0.25 ± 0.46, n = 20, P = 0.030, Wilcoxon signed-rank test) (**Figure S8E**). Thus, as dopamine neurons were more strongly activated by the stop signal, the eye movement in the next trial more largely delayed. Taken together, our findings suggest that dopamine neurons and the caudate nucleus may proactively regulate saccade promotion by biasing the balance between the cancel and the execution of saccadic eye movement.