

Correlation of Primate Caudate Neural Activity and Saccade Parameters in Reward-Oriented Behavior

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¹Department of Mathematical Engineering and Information Physics, The University of Tokyo, Tokyo 113-8656; ²Laboratory for Mathematical Neuroscience, RIKEN Brain Science Institute, Saitama 351-0198; ³School of Knowledge Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292; ⁴Department of Physiology, Juntendo University, School of Medicine, Tokyo 113-8421; and ⁵Core Research for the Evolutional Science and Technology Program, Japan Science and Technology Corporation, Saitama 332-0012, Japan

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Itoh, Hideaki, Hiroyuki Nakahara, Okihide Hikosaka, Reiko Kawagoe, Yoriko Takikawa, and Kazuyuki Aihara. Correlation of primate caudate neural activity and saccade parameters in reward-oriented behavior. *J Neurophysiol* 89: 1774–1783, 2003; 10.1152/jn.00630.2002. Changes in the reward context are associated with changes in neuronal activity in the basal ganglia as well as changes in motor outputs. A typical example is found in the caudate (CD) projection neurons and saccade parameters. It raised the possibility that the changes in CD neuronal activity contribute to the changes in saccade parameters. To examine this possibility, we calculated the correlation coefficients (CORs) of the firing rates of each neuron with saccade parameters (peak saccade velocity and latency) on a trial-by-trial basis. We then calculated the mean CORs separately for two CD populations: reward-enhanced type neurons (RENs) that showed enhanced activity and reward-depressed type neurons (RDNs) that showed depressed activity when reward was expected. The activity of RENs was positively correlated with the saccadic peak velocity and negatively correlated with the saccade latency. The activity of RDNs was not significantly correlated with the saccade parameters. We further analyzed the CORs for RENs, a major type of CD neurons. First, we examined the time courses of the CORs using a moving time window (duration: 200 ms). The positive correlation with the saccade velocity and the negative correlation with the saccade latency were present not only in the peri-saccadic period but also during the pre- and postcue periods. Second, we asked whether the CORs with the saccade parameters were direction-selective. A majority of RENs were more active before contralateral saccades (contralateral-preferring neurons) and their activity was correlated more strongly with contralateral saccades than with ipsilateral saccades. A minority of RENs, ipsilateral-preferring neurons, showed no such preference. These results are consistent with the hypothesis that CD neuronal activity exerts facilitatory effects on contralateral saccades and that the effects start well before saccade execution. Furthermore, a multiple regression analysis indicated that changes in activity of some, but not all, CD neurons could be explained by changes in saccade parameters; a major determinant was reward context (presence or absence of reward). These results suggest that, while a majority of CD neurons receive reward-related signals, only some of them can make a significant contribution to change saccadic outputs based on expected reward.

INTRODUCTION

According to a recent view, the basal ganglia are involved in the control of behavior based on motivation (Hikosaka et al. 2000; Schultz 1995). Using a one-direction-rewarded (1DR) version of the memory-guided saccade task, we found that caudate (CD) neuronal activity was strongly modulated by the expected presence or absence of reward. In the rewarded trials compared with the nonrewarded trials, task-related activity (anticipatory, visual, sustained, saccadic activity) was enhanced in a majority of CD neurons [reward-enhanced type (REN)] and depressed in a minority of CD neurons [reward-depressed type neurons (RDNs)] (Kawagoe et al. 1998). We also found that the saccade parameters were modulated by the expected presence or absence of reward. Saccades had higher peak velocities with shorter latencies in the rewarded trials than in the nonrewarded trials (Kawagoe et al. 1998; Takikawa et al. 2002). The good neural-behavioral correlation is expected if the CD is a source of the reward-dependent changes in saccade parameters. However, it is also possible that the expected presence and absence of reward affects CD neuronal activity and saccade parameters independently. If this is the case, the CD may not be involved in the saccade control in the reward driven conditions. An objective of this study was to test this null hypothesis.

According to the null hypothesis, there would be no correlation if rewarded trials and nonrewarded trials are analyzed separately. To the contrary, we found small but consistent correlations between CD (especially RENs) neuronal activity and saccade parameters. We further show that the CD neural activity, which can occur well before saccade execution, was correlated with the saccade parameters and that they had clearer relations to contralateral saccades. Furthermore, when we examine a characteristic of each neuron by a multiple regression analysis, we found a variety of modulation by reward and saccade conditions on the CD neural activity. We discuss implications of our results in the DISCUSSION.

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METHODS

General

We used three male Japanese monkeys (*Macaca fuscata*). The monkeys were kept in individual primate cages in an air-conditioned room where food was always available. At the beginning of each experimental session, they were moved to the experimental room in a primate chair. The monkeys were given restricted amounts of fluid during periods of training and recording. Their body weight and appetite were checked daily. Supplementary water and fruit were provided daily. All surgical and experimental protocols were approved by the Juntendo University Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Animals.

The experiments were carried out while the monkey's head was fixed, and its eye movements were recorded. For this purpose, a head holder, a chamber for unit recording, and an eye coil were implanted under surgical procedures. The monkey was sedated by intramuscular injections of ketamine (4.0–5.0 mg/kg) and xylazine (1.0–2.0 mg/kg). General anesthesia was then induced by intravenous injection of pentobarbital sodium (5 mg/kg/h). Surgical procedures were conducted in aseptic conditions. After exposing the skull, 15–20 acrylic screws were bolted into it and fixed with dental acrylic resin. The screws served as anchors by which a head holder and a recording chamber, both made of Delrin, were fixed to the skull. A scleral eye coil was implanted in one eye for monitoring eye position (Judge et al. 1980; Robinson 1963). The recording chamber, which was rectangular (antero-posterior, 42 mm; lateral, 30 mm; depth, 10 mm), was placed over the fronto-parietal cortices, tilted laterally by 35°. The monkey received antibiotics (sodium ampicillin, 25–40 mg/kg, im, each day) after the operation.

Behavioral tasks

The monkey sat in a primate chair in a dimly lit and sound-attenuated room with his head fixed. In front of him was a tangent screen (30 cm from his face) onto which small red spots of light (0.2° diam) were backprojected using two LED projectors. The first projector was used for a fixation point and the second for an instruction cue stimulus. The position of the cue stimulus was controlled by reflecting the light via two orthogonal (horizontal and vertical) mirrors that were servo-controlled by galvanometers.

The monkeys were first trained to perform memory-guided saccades (Hikosaka and Wurtz 1983) (Fig. 1A). A task trial started with onset of a central fixation point on which the monkeys had to fixate. A cue stimulus (spot of light) came on 1 s after onset of the fixation point (duration: 100 ms), and the monkeys had to remember its location. After 1–1.5 s, the fixation point turned off, and the monkeys were required to make a saccade to the previously cued location. The target came on 400 ms later for 150 ms at the cued location. The saccade was judged to be correct if the eye position was within a window around the target (usually within $\pm 3^\circ$) when the target turned off. The correct saccade was indicated by a tone stimulus and reward (drop of water). The monkeys made the saccade usually before target onset based on memory, because, otherwise, the eyes could rarely reach the target window within the 150-ms target-on period. The next trial started after an inter-trial interval of 3.5–4 s.

The monkeys were then trained to perform the memory-guided saccade task in two different reward conditions: all-directions-rewarded condition (ADR) and IDR (Kawagoe et al. 1998). In ADR, every correct saccade was rewarded with the liquid reward together with the tone stimulus. In IDR, an asymmetric reward schedule was used in that only one of the four directions was rewarded while the other directions were either not rewarded (exclusive IDR) or rewarded with a smaller amount (about 1/5; relative IDR). The correct saccade was indicated by the tone stimulus regardless of the rewarded direction, and the next trial followed. The highly rewarded direction

was fixed for each block of experiments defined by 60 successful trials. Even for the nonrewarded or less-rewarded direction, the monkeys had to make a correct saccade, since the same trial was repeated if the saccade was incorrect. While we noted that the monkeys made more errors in the nonrewarded trials than the rewarded trials (Takikawa et al. 2002), the analysis in the present paper is done only with those correct trials. The amount of reward per trial in a block was set approximately the same between IDR and ADR, i.e., the amount of reward for a rewarded trial in IDR was about four times larger than for that in ADR. The cue was chosen pseudo-randomly such that the four directions were randomized in every sub-block of four trials; thus one block of experiment (60 trials) contained 15 trials for each direction. Other than the actual reward, no indication was given to the monkeys as to which direction was currently rewarded. IDR was performed in four blocks, in each of which a different direction was rewarded highly. The order of blocks was randomized. The behavioral tasks as well as storage and display of data were controlled by computer (PC 9801RA, NEC, Tokyo, Japan).

Recording procedures

Eye movements were recorded using the search coil method (Enzanshi Kogyo MEL-20U) (Judge et al. 1980; Matsumura et al. 1992; Robinson 1963). Eye positions were digitized at 500 Hz and stored into a separate file continuously during each block of trials.

Before the single-unit recording experiment, we obtained MR images (AIRIS, 0.3T, Hitachi) perpendicular to the recording chamber. We then determined the recording sites in the CD based on the chamber-based coordinates (Kawagoe et al. 1998). The recording sites were further verified by MR-imaging a plastic guide tube through which the electrodes were inserted.

Single-unit recordings were performed using tungsten electrodes (diameter: 0.25 mm; 1–2 M Ω ; measured at 1 kHz, Frederick Haer). A hydraulic microdrive (MO95-S, Narishige) was then used to advance the electrode into the brain. We recorded extracellular spike activity of presumed projection neurons that showed very low spontaneous activity (Hikosaka et al. 1989a), but not of presumed interneurons that showed irregular tonic discharge (Aosaki et al. 1994).

Methods

To find CD projection neurons, we let the monkeys perform IDR continuously. If a CD neuron was found, we let the monkeys perform some blocks of IDR with different rewarded directions, each for several trials. Depending on the neuron's preferred direction, we chose a set of four target locations of equal eccentricity, arranged in either normal or oblique angles. The target eccentricity was fixed for each recorded neuron throughout all IDR/ADR blocks and was usually set either 10° or 20°. We then asked the monkeys to perform at least one block of ADR and four blocks of IDR (i.e., four different rewarded directions). The order of the four blocks of IDR was randomized. In addition, we sometimes repeated IDR blocks to confirm the reproducibility of the neuron's behavior.

Data analysis

SACCADIC EYE MOVEMENTS. Time series of eye positions were recorded by a scleral search coil with a 500-Hz sampling rate. The eye velocity at time t was calculated as $V(t) = \sqrt{[x(t+1) - x(t)]^2 + [y(t+1) - y(t)]^2}$, where $x(t)$ and $y(t)$ are the horizontal and vertical eye positions at time t . Then, the velocity was convolved with the vector [1/4, 2/4, 1/4] for smoothing. A saccadic eye movement was detected when the smoothed eye velocity first exceeded a threshold value (40°/s), which we called the onset of the saccade, and second returned to less than another threshold value (28°/s), where the time interval between the onset and end of the saccade should be longer than 25 ms. The saccade onset and peak

velocity were stored for the following analysis. The saccade latency was defined as the duration from the offset of the fixation point until the saccade onset. Trials with premature saccades (<80 ms of the saccade latency) were eliminated from the following analyses.

CORRELATION COEFFICIENTS (CORS). We calculated CORS between the firing rate at a particular time window and the saccade parameters (peak velocity or saccade latency). The CORS were obtained separately with respect to each direction in each reward condition (in the same block), because the saccade parameters may be intrinsically different for different directions (Becker 1989). Thus the data of one IDR block was divided to four sub-blocks, each corresponding to one of four saccade directions and containing ≥ 15 trials. Since there were 5 blocks (1 ADR and 4 IDR blocks) for each neuron, we obtained CORS for 20 (4×5) sub-blocks per neuron. We then pooled all CORS for each neuron type. For a dominant type, REN ($n = 96$), 1,920 (96×20) CORS were calculated. We used two kinds of time windows to calculate the CORS. In Figs. 2–4 and 7–10, we used a larger time window, that is, from fixation onset until saccade start, to study the overall correlation between the CD neural activity and the saccade parameters. In Figs. 5 and 6, we used a 200-ms moving time window (see MOVING WINDOWS), to study the temporal changes of the CORS.

MOVING WINDOWS. In calculating the time courses of the mean CORS, we set a time window (200 ms) for calculating the firing rate that was moved in 100-ms steps throughout a trial. The time windows were set such that one of them started with one of the following events: fixation onset (shown as “fon” in the figures), cue onset (cue), fixation offset (foff), or saccade start (sac). In the figures, there are breaks between cue and foff, or between foff and sac, since their intervals varied from trial to trial.

CELL POPULATIONS. CD neurons were classified in two ways according to reward contingency and spatial preference.

1) Reward contingency: RENs, which showed significantly higher firing rates in rewarded trials than in nonrewarded trials; and RDNs, which showed significantly lower firing rates in rewarded trials than in nonrewarded trials, based on the firing rates in the neuron’s best activity period in IDR trials (ANOVA; $P < 0.01$). There were neurons that showed no significant reward modulation; they were not studied further.

2) Spatial preference: contralateral-preferring neurons (CPNs), which showed higher firing rates before contralateral saccades than ipsilateral saccades; and ipsilateral-preferring neurons (IPNs), which showed higher firing rates before ipsilateral saccades. To determine the preferred direction for each neuron, we first determined the neuron’s best activity period, averaged the activity over the four IDR blocks, and compared the mean activity among the four cue directions. Neurons that preferred the upward or downward direction were eliminated from this analysis. In cases where the preferred direction was oblique (e.g., right-downward), the preferred direction was defined as left or right.

Statistics 1. For data analysis shown in Figs. 4 and 6, the mean COR was statistically tested against the null hypothesis of no correlation, using shuffled predictors. The observed (i.e., un-shuffled) mean COR was the mean value of CORS, each of which was calculated after pairing a saccade parameter value and a firing rate value in each sub-block. A single shuffled predictor (a single shuffled mean COR) was calculated in the same way as the observed mean COR, except that the pairing correspondence was randomly shuffled in each sub-block. In each time window, 100 shuffled predictors, using different random seeds, were obtained to form a probability distribution of the null hypothesis. The P value of the observed mean COR was calculated, using the mean and variance of this distribution, which was assumed to be the normal distribution.

In both tests (Figs. 4 and 6), we used Bonferroni correction for multiple tests. Dividing factor of Bonferroni correction for the significance is 4 and 82 in Figs. 4 and 6, respectively. This is because there

were four tests with a single window in Fig. 4 and two tests (both velocity and latency) with 41 windows in Fig. 6. In Figs. 4 and 6, the statistical significance is indicated by * or ** for a 0.05 or 0.01 significance level, respectively (after adjustment by Bonferroni correction; e.g., in Fig. 6, * for $P < 0.05/82$ and ** for $P < 0.01/82$).

To be cautious, we further examined the assumption of the normal distribution for shuffled predictors. It was accepted by both the Jarque-Bera and the Lilliefors tests ($P > 0.05$) after correction of multiple tests (Holm correction, which is more likely to reject the null hypothesis, i.e., the normality, than Bonferroni correction). In addition, we also analyzed the data in Fig. 4 by the t -test for a mean of 0. The results (data not shown) were similar to, but less strict than, those by the shuffling test. We also performed a binomial test, which is a nonparametric test for symmetrical distribution around 0, for data in Fig. 6. Again, the results (data not shown) were similar to, but less strict than, those by the shuffling test.

Statistics 2. For data analysis shown in Fig. 9, we used a paired t -test to examine if there was a significant difference between the mean CORS of the contralateral direction and that of the ipsilateral direction. In this test, we first calculated, for each neuron, two mean CORS over the sub-blocks of contralateral directions and over those of ipsilateral directions. Then, pairing the two CORS of each neuron, we compared the grand mean CORS of the contralateral directions and ipsilateral directions by paired t -test. In this test, Bonferroni correction for multiple tests was made with a dividing factor of 4.

Assumptions of normality for the distribution of CORS were examined and accepted by both the Jarque-Bera and the Lilliefors tests ($P > 0.05$). In addition, we performed Wilcoxon signed-rank test, and its results (data not shown) gave the same conclusions with the above test, given the fixed significance level ($P < 0.05$).

MULTIPLE REGRESSION ANALYSIS. We conducted a multiple regression analysis to study how firing activity of a neuron can be attributed to reward expectation and saccade parameters. We used a linear model

$$[\text{firing rate}] = a_0 + a_{\text{RWD}} \times [\text{isReward}] + a_{\text{VEL}} \times [\text{sac_velocity}] + a_{\text{LAT}} \times [\text{sac_latency}] + [\text{noise}]$$

[isReward] was set to 1 if reward was expected and 0 if not. [firing rate] was taken from the window from fixation onset until saccade start. The coefficients (a_0 , a_{RWD} , a_{VEL} , and a_{LAT}) were the constants that were determined for each neuron to minimize the sum of the squared regression errors ($[\text{noise}]^2$; least-squares method), after the all variables were normalized so that mean = 0 and variance = 1 (z -score). The coefficient a_0 should always be 0 after the normalization, and therefore it was omitted for further analysis. To examine the significance of variables with respect to reward condition and saccade parameters, we tested if the regression error ([noise]) became statistically significantly larger (F -test; $P < 0.05$) when we dropped 1) the reward term ([isReward]) or 2) the saccade terms ([sac_velocity] and [sac_latency]). Based on the analysis, each neuron was classified as follows: “RWD-only” type if it showed a significant regression error when the reward term, but not the saccade term, was dropped, “SAC-only” type if it showed a significant error when the saccade term, but not the reward term, was dropped, and “RWD-SAC” type if it showed a significant error both when the reward term was dropped and when the saccade term was dropped.

RESULTS

According to the null hypothesis we tested in this study, there is correlation between CD neuronal activity and saccade parameters because each of them is contingent on reward condition separately. If so, the correlation would disappear if the correlation analysis is done separately for different reward

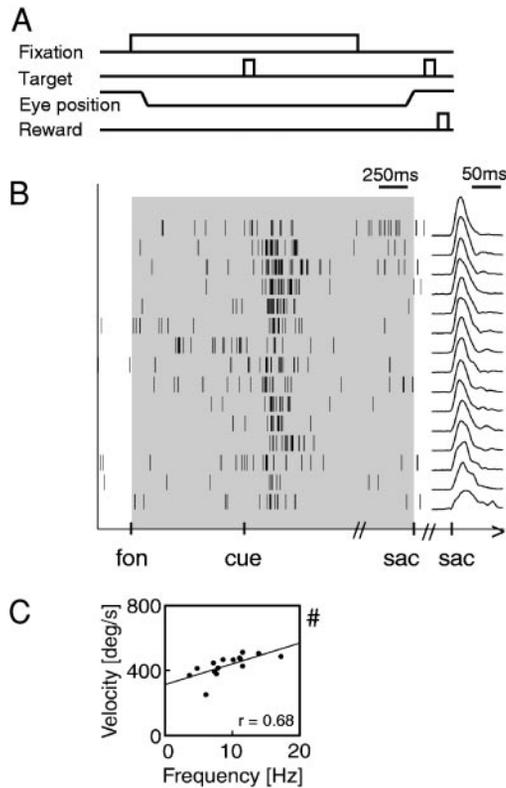


FIG. 1. An example of the correlation between caudate (CD) neural activity and saccade velocity. *A*: schematic display of the memory-guided saccade task (see METHODS). *B*: action potentials of a CD neuron are plotted with the corresponding eye velocity profiles. Shown here are the data for 1 sub-block in ADR in which saccades were made to the right-up direction (see Fig. 2). The neural activity was aligned with the fixation onset (*left*) or with the saccade onset (*right*). The eye velocity profile was aligned with the saccade onset. Trials were sorted from faster to slower saccades, from top to bottom. The faster saccades tended to be accompanied by more spikes. *C*: scatter plot of saccade peak velocity (ordinate) vs. firing rate (abscissa). The firing rate was calculated for the time window from fixation onset to saccade onset, which is the shaded period in *B*. The regression line and the correlation coefficient (r) are also shown in the figure.

conditions. Figure 1 presents first observation against this null hypothesis.

Figure 1*B* shows an example of the trial-by-trial correlation between saccade velocity and activity of a single CD neuron. Shown here are trials in a sub-block of ADR trials in which the saccade was made to the right-up direction. The trials were sorted from faster to slower saccades, from top to bottom. The faster saccades tended to be accompanied by higher firing rates, especially in the postcue period. A scatter plot between the firing rate (in a time window from fixation onset to saccade start) and the peak saccade velocity (Fig. 1*C*) indicates a positive correlation ($r = 0.68$). The result suggests that the correlation (COR) between CD neuronal activity and saccade velocity cannot be explained just by the common effect of reward condition.

Figure 2 shows the scatter plots of the all sub-blocks for the same neuron as Fig. 1. We performed four blocks of 1DR block and one block of ADR. In each block (1DR or ADR), there were four possible saccade directions. Thus the number of sub-blocks was 20 (5 blocks \times 4 directions). We calculated the correlation between CD neuronal activity (firing rate, Hz) and saccade velocity ($^{\circ}$ /s) for each sub-block.

We emphasize that trials in a given sub-block were done in the same reward condition. Yet, the neuron in Fig. 2 showed a positive correlation in most of the sub-blocks (18 of 20). Note that the COR appears less robust in the rewarded sub-blocks (indicated by a bullseye). We previously found that the saccade velocity is higher and has less variability in the rewarded-trials than in the nonreward trials (Kawagoe et al. 1998; Takikawa et al. 2002). Given this fact, there might be a ceiling effect in the rewarded sub-blocks, yielding somewhat less robust COR in the rewarded sub-blocks.

COR dependency on cell type in relation to reward modulation

We previously found, using 1DR and ADR tasks, that a majority of CD neurons showed enhanced activity when re-

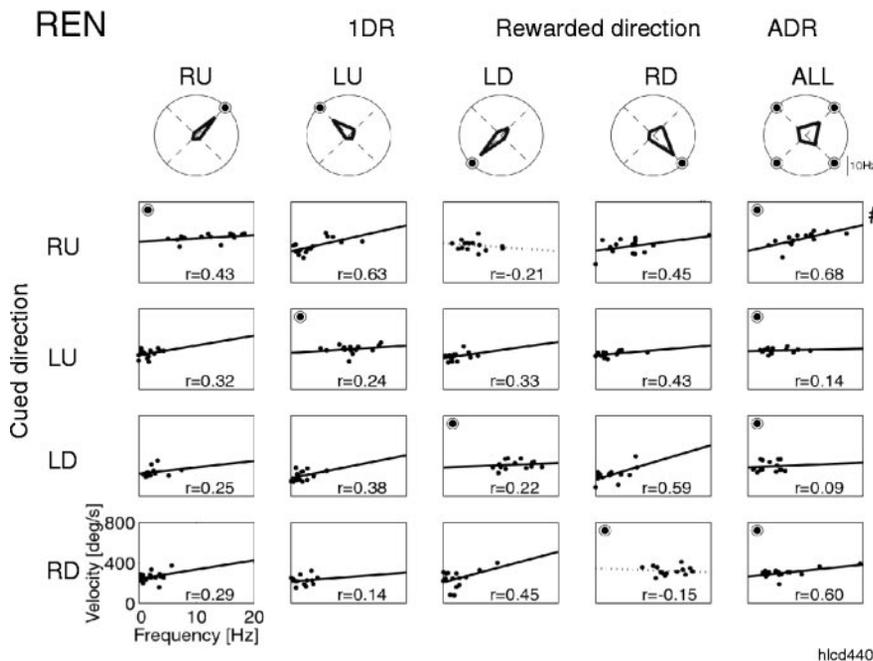


FIG. 2. A standard set of neural-velocity correlations for a reward-enhanced neuron (REN). The neuron was the same as of Fig. 1, recorded in the left CD; the data shown in Fig. 1 are indicated by #. The data for 4 blocks of one-direction-rewarded task (1DR) and 1 block of all-directions-rewarded task (ADR) are shown in columns. For each block, the target was chosen randomly from 4 directions, and scatter plots are shown for individual target directions (called sub-blocks); target direction from *top* to *bottom*: right-up (RU), left-up (LU), left-down (LD), and right-down (RD). One direction was rewarded for each block, which is indicated by a bullseye; rewarded direction from *left* to *right*: RU, LU, LD, and RD. In each scatter plot, the abscissa indicates the firing rate during the task (from fixation onset to saccade onset), and the ordinate indicates the saccade peak velocity. A positive correlation is indicated by a solid regression line, while a negative correlation by a dotted regression line. Correlation coefficient r was calculated for each sub-block, as shown in the figure. A polar diagram at *top* shows the mean firing rate of the neuron for each block with respect to target directions. This neuron showed positive correlations in most of the sub-blocks.

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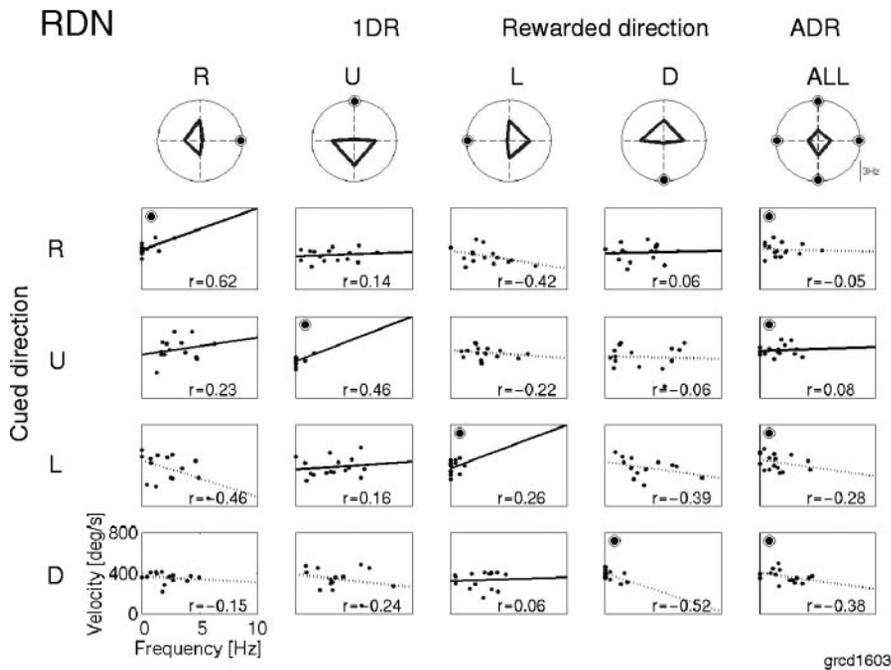


FIG. 3. Neural-velocity correlations for a reward-depressed neuron (RDN) recorded in the right CD. The format is the same as Fig. 2. The target was chosen randomly from right (R), up (U), left (L), and down (D). The correlation coefficients were positive in some sub-blocks and negative in others, apparently without any tendency.

ward was expected (RENs), while the others showed depressed activity (RDNs) (Kawagoe et al. 1998) (see METHODS). For example, the neuron in Fig. 2 was a REN, because its firing rate was above and below 10 Hz, respectively, in most rewarded and nonrewarded trials. CORs were positive for most sub-blocks. An example of RDN is shown in Fig. 3. Its firing rate was close to zero in rewarded trials, while it could be more than 5 Hz in nonrewarded trials. There was no consistent pattern among the CORs.

To test if RENs and RDNs are related to saccade parameters differently, we performed the correlation analysis separately for these groups of neurons (Fig. 4). The mean COR was calculated as the average of CORs for individual sub-blocks. RENs ($n = 96$) showed significantly positive and negative CORs with the saccade velocity and latency, respectively ($P < 0.0001$ for both; taking $P = 0.01/4 = 0.0025$ for judging

significance according to Bonferroni correction of multiple tests). RDNs ($n = 19$) showed no significant correlations ($P = 0.88$ for velocity, $P = 0.81$ for latency). Hence, further analyses focus on only RENs.

Time course of the CD-saccade correlation

We then asked about the time course of the CD-saccade correlation for RENs. For this purpose, we used a moving time window (200 ms with 100-ms step) to obtain the CD firing rates (see METHODS). For each time window, we calculated 1,920 CORs (20 sub-blocks \times 96 RENs). Figure 5 shows the distribution of the CORs for four different time windows. It appears that the COR distribution was not skewed before the fixation onset (Fig. 5A), was slightly positively skewed in the precue period (Fig. 5B), and was more positively skewed in the postcue and presaccade periods (Fig. 5, C and D). These observations suggest that the CD-saccade correlation changed over time in a trial.

The suggestion was confirmed by a statistical analysis shown in Fig. 6. The mean COR for saccade velocity (Fig. 6A, solid thick line) gradually increased from the precue period and stayed at a high sustained level after the cue onset until the saccade onset. The statistical significance of the mean COR examined against shuffled predictors is indicated at the top of the figure (see METHODS). The result showed that the CD-saccade velocity correlation was present not only during or just before the saccade, but also during the precue, postcue, and delay periods.

Figure 6B shows the correlation between the firing rate and the saccade latency. There were significant negative correlations distributed over the precue, postcue, and saccade periods. These results together showed that activity of RENs started to correlate with the saccade parameters well before the execution of the saccade.

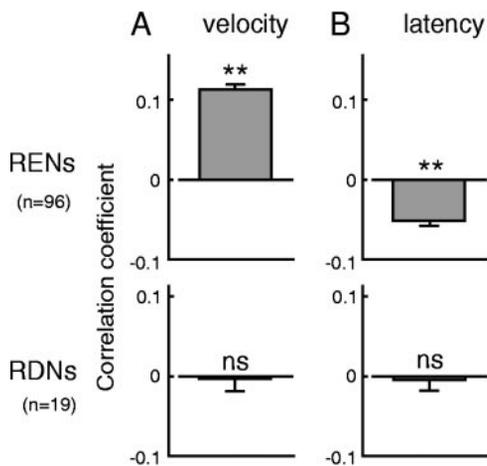


FIG. 4. Correlation coefficients (CORs) of neural activity with velocity (A) and latency (B) for RENs and RDNs. The mean CORs are plotted with the SE. RENs ($n = 96$) showed significantly positive and negative CORs with the saccade velocity (A) and latency (B) ($P < 0.0001$ for both). RDNs ($n = 19$) showed no significant correlations ($P = 0.88$ for velocity, $P = 0.81$ for latency).

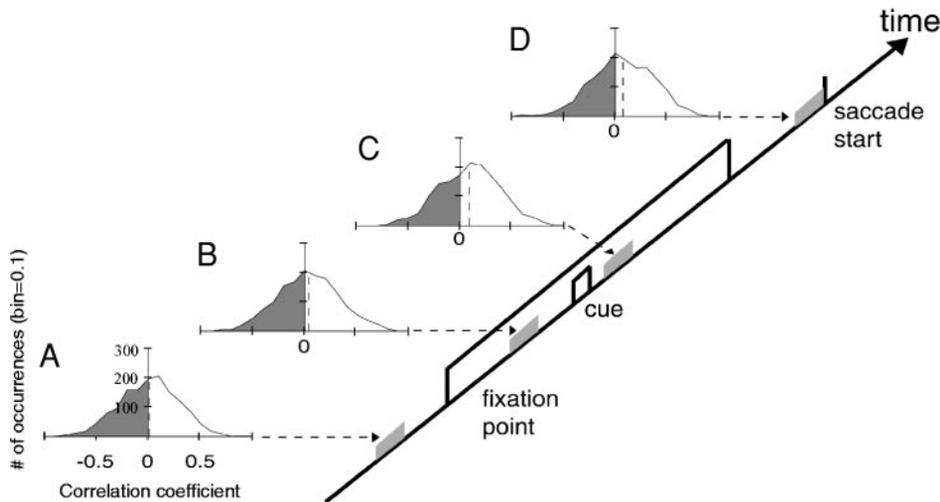


FIG. 5. Task-related change in the COR between saccade velocity and REN activity. COR distributions (i.e., histograms of the COR) are shown for 4 different time windows (each duration of which is 200 ms, indicated by broken arrows and gray quadrilaterals). Each histogram was based on 1,920 CORs (20 sub-blocks per neuron \times 96 RENs). Positive and negative correlations are indicated by white and gray portions of the histograms, respectively. Vertical broken line in each histogram indicates the mean value of the CORs. The closer to the saccade onset (from A to D), the more positively the mean COR tended to shift.

COR dependency on the direction selectivity

We so far have analyzed CORs between CD neural activity and saccade parameters regardless of the saccade directions. However, it is important to ask whether such a correlation is direction-selective, especially because a majority of CD neurons show a spatial preference (Hikosaka et al. 1989a–c). To answer this question, we first divided CD neurons into two groups, CPNs and IPNs.

Figures 7 and 8 show examples of CPN and IPN, respectively. Both neurons were recorded in the right CD. As depicted in the polar plots, the neuron in Fig. 7 tended to be more active when leftward (contralateral) saccades were required, whereas the neuron in Fig. 8 tended to be more active when rightward (ipsilateral) saccades were required. For the CPN in Fig. 7, CORs for the contralateral direction (row L; mean r is 0.28) were generally higher than those for the ipsilateral direction (row R; mean r is -0.088). For the IPN in Fig. 8, CORs were mostly positive and no asymmetry was discerned.

To examine overall tendency, we calculated the mean CORs for the contralateral- and ipsilateral-target directions, separately for CPNs and IPNs (Fig. 9). The results showed interesting differences between CPNs and IPNs. CPN activity was

correlated with the parameters of contralateral saccades more strongly than those of ipsilateral saccades: velocity ($P = 0.001$, Fig. 9A; METHODS) and latency ($P = 0.036$, Fig. 9B). In contrast, IPN activity was correlated with both contralateral and ipsilateral saccades, but with no significant difference in laterality: velocity ($P = 0.51$, Fig. 9A) and latency ($P = 0.89$, Fig. 9B). These results may suggest different functions of CPNs and IPNs (see DISCUSSION).

Relation to the reward expectation and/or the saccade parameters

In a previous paper (Kawagoe et al. 1998), we indicated that activity of CD neurons was modulated by the expected presence or absence of reward. The present study so far indicated that the activity of CD neurons (especially RENs) was correlated with saccade parameters even when the reward condition was the same. What then is the relationship between these types of correlation? Could the reward correlation be accounted for by the saccade correlation entirely or partially?

To answer this question, we conducted a multiple regression analysis. We focused on the preferred direction of RENs. We used a linear model: [firing rate] = $a_0 + a_{\text{RWD}} \times [\text{isReward}] +$

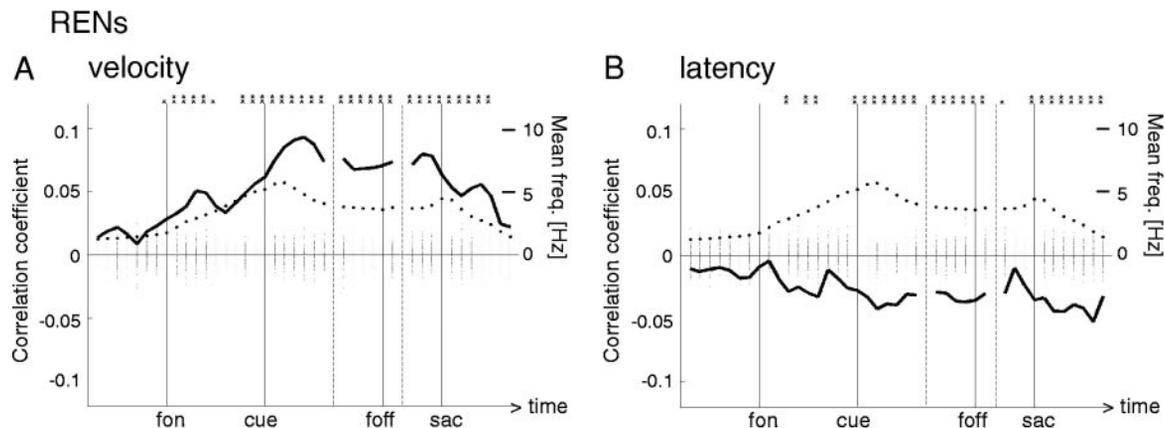


FIG. 6. Time course of mean CORs for saccade velocity (A) and latency (B). Mean CORs were calculated for the RENs ($n = 96$). A thick line indicates the mean COR, while a dotted line indicates the mean firing rate. The abscissa indicates the time within a trial (fon: fixation onset, cue: cue onset, foff: fixation offset, sac: saccade start). The 200-ms time window was set for calculating the firing rates and was moved in 100-ms steps throughout the trial. Statistically significant correlations based on a shuffling test (see METHODS) are shown at the top of the figure as * or ** for a 0.05/82 or 0.01/82 significance level, respectively (divided by 82 for correction of multiple tests; see METHODS). The tiny dots around the horizontal centerline are shuffled predictors.

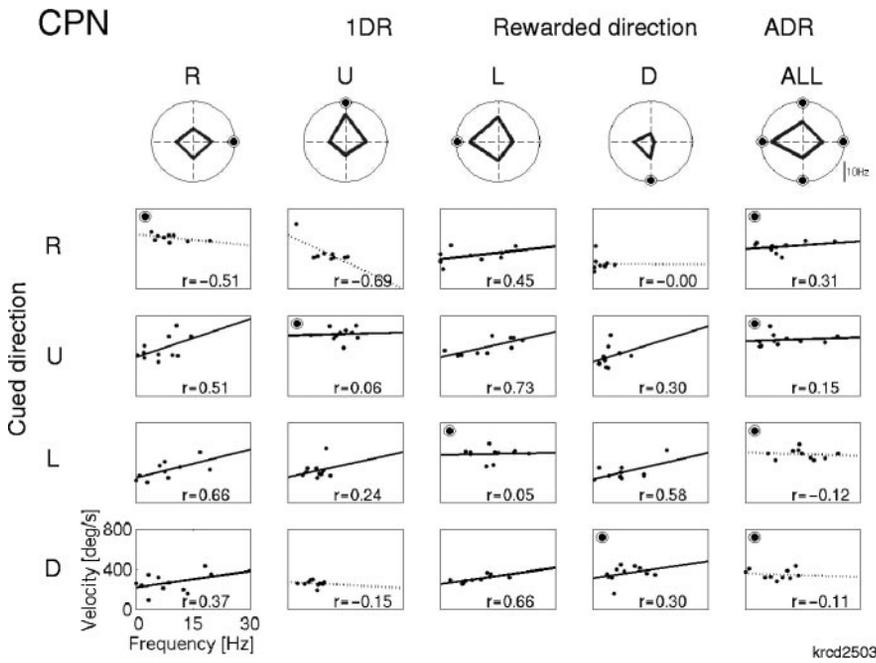


FIG. 7. Neural-velocity correlations for a contralateral-preferring neuron (CPN) recorded in the right CD. The format is the same as Fig. 2.

$a_{VEL} \times [sac_velocity] + a_{LAT} \times [sac_latency] + [noise]$ (see METHODS). More than one-half of RENs (64.6%, $n = 62$) showed a significant error when the reward term was dropped, indicating that their activity was modulated by reward condition (“RWD” type, Fig. 10A). About one-third of RENs (32.3%, $n = 31$) showed a significant error when the saccade term was dropped, indicating that their activity was modulated by saccade (“SAC” type). Some of these RENs (16.7%, $n = 16$) were modulated by both reward and saccade (“RWD-SAC” type). Accordingly, 47.9% ($n = 46$) of neurons showed only reward modulation (i.e., “RWD-only”), while 15.6% ($n = 15$) of neurons showed only saccade modulation (“SAC-only”).

Figure 10B shows the distribution of the estimated coefficients. The coefficients for reward (a_{RWD}) and saccade velocity

(a_{VEL}) were positively skewed, while the coefficients for saccade latency (a_{LAT}) were negatively skewed. The tendency for positive correlation of REN activity with reward was expected by the definition of REN. The positive correlation of REN activity with saccade velocity is consistent with the COR analysis (Figs. 4–6).

Taken together, the regression analysis indicates that modulation of REN neuronal activity was accounted for by both the two factors, reward condition (presence and absence of reward) and saccade parameters, not by either one. That the reward factor was more common confirmed the results of a previous study from our laboratory (Kawagoe et al. 1998). The saccade factor played a significant role in more than 30% of REN neurons but was less in proportion than the reward factor. The

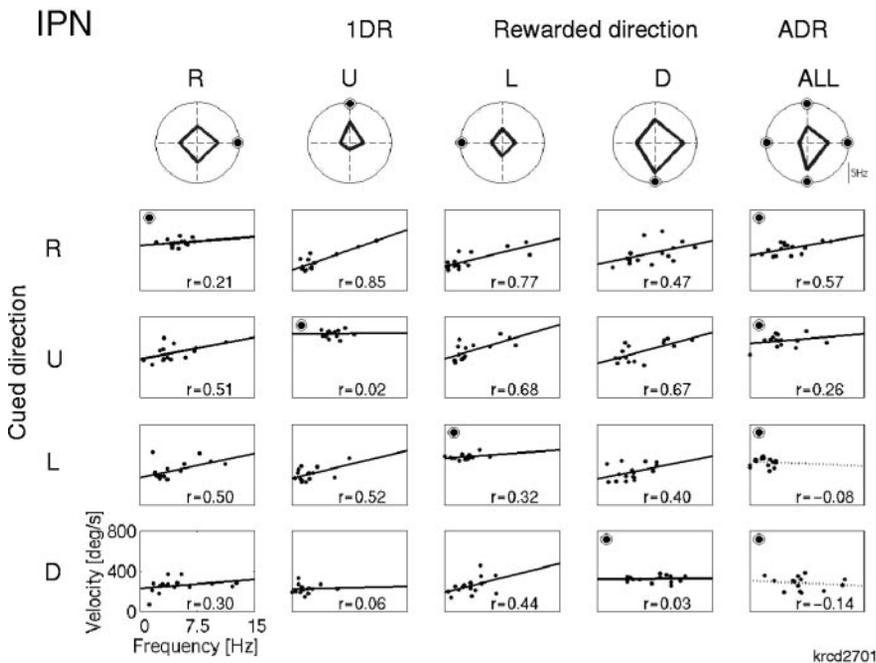


FIG. 8. Neural-velocity correlations for an ipsilateral-preferring neuron (IPN) recorded in the right CD. The format is the same as Fig. 2.

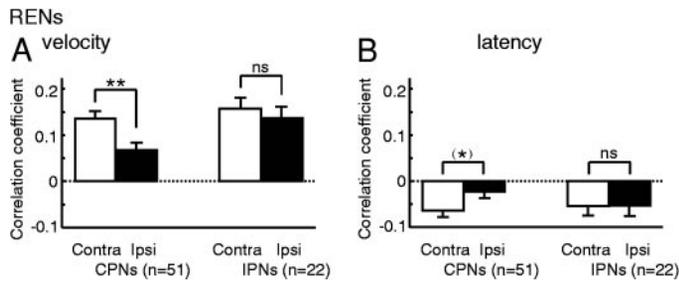


FIG. 9. Laterality of the correlations of CD activity with saccade velocity (A) and with saccade latency (B). The RENs were first divided into CPNs and IPNs. For each of the CPNs (left) and IPNs (right), the mean COR with the SE is plotted separately for saccades to the contralateral direction (white) and the ipsilateral direction (black). A wide time window (from fixation onset until saccade start) was used. A: for CPNs (left), the mean COR for the contralateral direction was significantly larger (more positive) than that for the ipsilateral direction ($n = 51$, $P = 0.001$). For IPNs (right), the difference was not significant ($n = 22$, $P = 0.51$). B: for CPNs (left), the mean COR for the contralateral direction was significantly larger (more negative) than that for the ipsilateral direction ($n = 51$, $P = 0.036$). For IPNs (right), the difference was not significant ($n = 22$, $P = 0.89$). Note, however, that we repeated 4 tests and hence we take $P = 0.05/4 = 0.0125$ for 5% confidence level (Bonferroni correction of multiple tests). Thus $P = 0.036$ for latency in CPN would not be highly significant (indicated by (*)).

relation of the regression analysis with the COR analysis is discussed in the DISCUSSION.

DISCUSSION

Findings in this study can be summarized as follows: 1) the activity of RENs in the CD was correlated with the saccade velocity positively and with the saccade latency negatively, while the activity of RDNs showed no significant correlation; 2) the correlation of RENs started to be evident in the pre- and postcue periods, far before the saccade; 3) The correlation was direction-selective among CPNs of REN such that it was stronger for contralateral saccades, but was direction-nonspecific among IPNs of REN; and 4) the modulation of most REN activity could be attributed to reward expectation and/or saccade parameters.

Significance of correlation between CD neuronal activity and saccade parameters

We previously have shown that changes in reward condition were associated with changes in saccadic eye movements (Takikawa et al. 2002) and CD neuronal activity (Kawagoe et al. 1998). This correlation was observed by comparing the mean values of saccade parameters and CD neuronal activity for individual reward states. Therefore the correlation could be explained by the hypothesis that the changes in reward state induced changes in both saccades and CD neuronal activity, independently. To test this “independent” hypothesis, we excluded the possible contribution of the reward state by calculating the neural-behavioral correlation separately for different reward states (i.e., presence and absence of reward). This method would eliminate pseudo-correlation due to the common effect of the reward state, and we still could detect significant correlations between CD neuronal activity and saccade parameters. The results are consistent with the “dependent” hypothesis that CD neuronal activity contributes to changes in saccade parameters. With this “dependent” hypothesis, changes in reward condition would induce changes in CD neuronal activity, consequently resulting in changes in saccadic eye movements. This is along with the previous studies on basal ganglia’s contribution to the arm movements (DeLong 1973; Georgopoulos and DeLong 1983).

However, the present study has not proved the causal relationship between CD neuronal activity and saccade parameters. Even within each sub-block with the same reward condition and cued direction, there may exist common factors, such as general arousal, that modulate both CD neuronal activity and saccade parameters. However, general arousal may not be sufficient to account for the correlation for the following reasons. First, activity of CPNs (of RENs) was correlated with contralateral saccades more strongly than with ipsilateral saccades (Fig. 9). This laterality is unlikely to be caused by general arousal because general arousal should affect saccades in both directions equally. Second, RDNs did not show significant correlation as a population. It seems unlikely that general arousal affects RENs, for which it induces the lateral effect as for CPNs, but not RDNs.

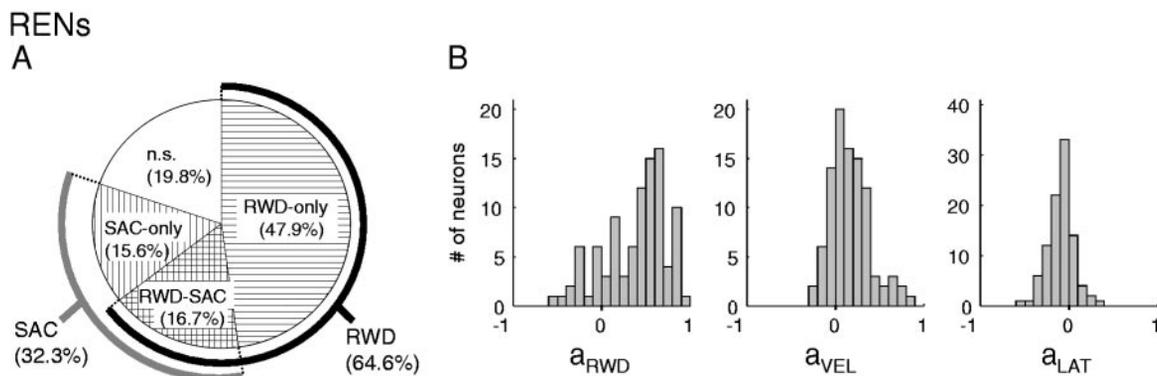


FIG. 10. Results of a multiple regression analysis. A linear regression model, $[\text{firing rate}] = a_0 + a_{\text{RWD}} \times [\text{isReward}] + a_{\text{VEL}} \times [\text{sac_velocity}] + a_{\text{LAT}} \times [\text{sac_latency}] + [\text{noise}]$, was used for each of RENs ($n = 96$) with trials for its most preferred direction. The coefficients (a_0 , a_{RWD} , a_{VEL} , and a_{LAT}) were calculated that minimize the sum of the squared errors ($[\text{noise}]^2$), after all variables were normalized as mean = 0 and variance = 1. A: relation to the reward expectation and/or the saccade parameters. RENs were classified based on how the modulation of their activity could be attributed to the reward expectation and/or the saccade parameters (F -test; $P < 0.05$; see METHODS). B: distribution of the coefficients in the linear regression model.

To summarize, our results lend support to the dependent hypothesis that CD neurons contribute to the reward-contingent changes in saccade parameters, although there remain other possibilities and other factors. In the next section, we discuss implications of our findings, assuming that the dependent hypothesis is correct.

Neural circuits from CD to saccades

We consider two pathways as the responsible mechanism for CD influences on saccades: direct pathway and indirect pathway (Smith et al. 1998). The direct pathway is considered to be facilitatory on saccades because it contains two inhibitory connections (CD-SNr and SNr-SC) before reaching the SC, a common mediator of voluntary saccades (Sparks and Hartwich-Young 1989). On the other hand, the indirect pathway is considered to be inhibitory on saccades because it contains three inhibitory connections (CD-GPe, GPe-STN or GPe-SNr, and SNr-SC). Consistent with these anatomical data, electrical stimulation in the CD induced inhibitions and excitations of SNr neurons (Chevalier et al. 1985; Hikosaka et al. 1993).

Result 1 (positive correlation with saccade velocity and negative correlation with saccade latency) suggests that the effect of CD neural activity is largely conveyed by the "direct" pathway. Our analysis further revealed that significant correlations were present for RENs, but not for RDNs. If so, SNr neurons should be more suppressed by RENs in rewarded trials than in nonrewarded trials. This is exactly what Sato and Hikosaka (2002) found in a prominent group of SNr neurons. However, there also were a variety of neurons in the SNr whose activity were suppressed or enhanced either in rewarded or nonrewarded trials.

Early effects of CD neural activity on saccades

Result 2 suggests that CD neuronal activity starts to influence the parameters of a memory-guided saccade far before its execution, before an instruction (cue stimulus) is given. We propose two explanations: 1) accumulation of information in single neurons, and 2) transmission of information through loop circuits.

According to the information accumulation theory, intermediary neurons would accumulate information by time before they emit a functionally meaningful signal. It could then take a long time for information to be transmitted across one synapse. This type of phenomenon has been demonstrated in various brain areas, such as the cortical area MT in relation to motion perception (Shadlen et al. 1996) and the frontal eye field in relation to saccade initiation (Schall et al. 1995). Particularly relevant to our study is that SC saccadic neurons tend to accumulate information before emitting a saccadic burst (Munoz and Wurtz 1995). It is also shown in a gap paradigm that the pretarget level of SC neural activity was correlated negatively with the saccade latency (Dorris and Munoz 1998). What is unique about our case would be that information is transmitted largely by inhibitory signals, both in the SNr and the SC. It would be interesting to examine whether and how such accumulation process occurs for inhibitory signals.

According to the loop circuit theory, the output of CD neurons may be directed to the thalamus, perhaps in addition to

the SC, which constitutes various loop circuits, such as the basal ganglia-thalamo-cortical circuits (Alexander et al. 1986) or the basal ganglia-thalamus circuits (Parent 1990). The information related to the instruction cue stimulus could be sent to the cerebral cortex thereby contributing to or being modified by the processes for attention (Damasio et al. 1980; Hikosaka et al. 1989b,c) or working memory (Hikosaka et al. 1989c; Levy et al. 1997), which would then be sent back to the CD. Such cortico-basal ganglia interaction could occur repeatedly during the delay period before a memory-guided saccade.

Laterality of CD effects on saccades

Most CD neurons have some spatial preference for their visual, memory, or saccadic motor activity (Hikosaka et al. 1989a–c). A majority of them preferred the contralateral side, while a minority preferred the ipsilateral side. Result 3 suggested functional differences between CPNs and IPNs.

Our finding that CPNs had larger CORs with contralateral saccades than with ipsilateral saccades may be explained by the presumed laterality of the CD efferent connections: ipsilateral for CD-SNr and SNr-SC connections (Williams and Faull 1985) and basal ganglia-thalamo-cortical circuits and contralateral for the SC output (Robinson 1972; Sparks 1986). Taking the laterality strictly, a given CD neuron would contribute only to saccades directed to the contralateral side of the CD neuron. The above finding fits this scheme qualitatively.

How could the CORs of IPNs have nearly equal effects on ipsilateral (preferred) and contralateral (anti-preferred) saccades? Their effects, on the average, were no weaker than those of CPNs. An obvious possibility is that their efferents are bilateral, as a population, somewhere downstream of the CD. IPNs tend to have a larger motor field, sometimes ranging over the bilateral hemifields (Hikosaka et al. 1989a–c). It has also been known that the SNr-SC connection in rodents or cats is largely ipsilateral but partially contralateral (Beckstead 1983). Interestingly, Jiang et al. (2000) have shown that a small number of SNr neurons have ipsilateral response fields and may project to the contralateral SC.

Reward-driven behavior: reward expectation to saccade

A previous study from our laboratory (Kawagoe et al. 1998) showed that activity of CD neurons was heavily dependent on the expected presence or absence of reward. Our next study (Takikawa et al. 2002) showed that the same manipulation of reward led to changes in saccade parameters. These results, taken together, raised an important question: can the reward-contingent changes in CD neuronal activity be explained solely by the changes in saccade parameters? The results of the COR analysis in the present paper indicated that changes in CD neuronal activity, as a whole, were indeed correlated with the changes in saccade parameters. However, the results of our multiple regression analysis indicated that activity of some, but not all, CD neurons can be explained by changes in saccade parameters; the other CD neurons might not be involved in oculomotor control. Consistent with this idea, the outputs of the CD may be fed back to the cerebral cortex through the SNr or the globus pallidus internal segment (GPi) and the thalamus (Middleton and Strick 2000). Alternatively, other brain areas may contribute to the changes in saccade parameters robustly

and independently so that contribution of a CD neuron, if it was weak, may not have reached a statistical significance. In fact, the SC receives inputs independently from the basal ganglia (originating from the CD) and from the cortical eye fields (Hikosaka et al. 2000). The stronger dependency of CD signals on reward context revealed by the multiple regression analysis is consistent with the reward-dependent nature of CD neuronal activity as shown before (Kawagoe et al. 1998), but the reward-contingent CD signals would affect saccade parameters only partially due to the presence of the non-CD effect.

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