The effects of dopamine and its antagonists on directional delay-period activity of prefrontal neurons in monkeys during an oculomotor delayed-response task

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Received 16 February 2001; received in revised form 25 June 2001; accepted 25 June 2001

Abstract

To examine the role of dopamine receptors in the memory field of neurons for visuospatial working memory in the prefrontal cortex (PFC), dopamine and its antagonists (SCH23390 for the D1-antagonist and sulpiride for the D2-antagonist) were applied iontophoretically to neurons of the dorsolateral PFC in monkeys that performed an oculomotor delayed-response task. In this task, the subject made a memory-guided saccade to a remembered target location that had been cued by a visuospatial stimulus (right, up, left, or down; 15° in eccentricity) prior to a 4-s delay period. We focused here on PFC neurons that showed directional delay-period activity; i.e., an increased activity during the delay period, the magnitude of which varied significantly with the target location. Iontophoretic application of SCH23390 (usually 50 nA) decreased or increased the activities of most of these neurons (n = 48/62, 77%); most neurons showed a decrease (n = 43/62, 69%). For the neurons affected by SCH23390, a directional index of directional delay-period activity was attenuated by SCH23390, whereas the preferred direction was not greatly affected. The decreasing effect of SCH23390 was dose-dependent; the extent of the decrease was less with a lower dose (20-nA current) than with the ordinary dose (50-nA current), although the effect of the lower dose of SCH23390 on delay-period activity was similar in nature to that of the ordinary dose of SCH23390. Furthermore, the application of dopamine itself augmented directional delay-period activity in most of the neurons tested (n = 12/16, 75%). Sulpiride did not have any significant effects in most of the neurons tested (n = 15/17). These results suggest that the activation of D1-dopamine receptors play a facilitating role in the memory field of PFC neurons for visuospatial working memory processes. © 2001 Elsevier Science Ireland Ltd and the Japan Neuroscience Society. All rights reserved.

Keywords: Prefrontal cortex; Dopamine; D1-receptor; Memory field; Working memory; Iontophoresis; Rhesus monkey

1. Introduction

In primates, dorsolateral areas of the prefrontal cortex (PFC) are involved in visuospatial working memory (for reviews, see Funahashi and Kubota (1994), Goldman-Rakic (1995)), and dopamine has been implicated in its neuronal processes (Sawaguchi et al., 1988, 1990a,b; Williams and Goldman-Rakic, 1995). Dopamine receptors of the central nervous system are divided into at least five subtypes, which are classified into two major types: D1 (subtypes, D1 and D5) and D2 (D2, D3 and D4). Previous studies have demonstrated that D1-receptors, rather than D2-receptors, are involved in visuospatial mnemonic processes in the primate PFC. For example, when D1-antagonists, such as SCH23390 and SCH39166, are locally injected into the dorsolateral PFC of monkeys, performance in oculomotor delayed-response (ODR) tasks requiring visuospatial memory is impaired (Sawaguchi and Goldman-Rakic, 1991, 1994). In monkeys performing a manual delayed-response task, iontophoretic application of a D1-antagonist attenuated delay-period activities (Sawaguchi et al., 1988, 1990b). The activity of PFC neurons related to ODR performance is also affected by iontophoretic application of D1-antagonists.
2. Materials and methods

2.1. Subjects and surgery

Two male rhesus monkeys (Macaca mulatta, ~ 4.5 kg and ~ 5.5 kg) were used in the present study. Throughout the experiment, the monkeys were treated in accordance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health). Each monkey was trained to perform an ODR task that required the subject to make a memory-guided saccade to a remembered visuospatial target. Before training, each monkey was habituated to a monkey chair, and then preliminary surgery was performed under deep anesthesia with sodium pentobarbital (~ 30 mg/kg, i.v.) and aseptic conditions. Two hollow rods (8-mm i.d.), for restraining the head of the monkey, were implanted on the anterior and posterior portions of the skull with dental acrylic. Small stainless steel bolts (3-mm diameter), for grounding, were anchored to the marginal portions of the skull and fixed with dental acrylic. To prevent bacterial infection, antibiotics were injected intramuscularly on the day of the surgery and daily for 1 week after the surgery.

2.2. Behavioral task

A few weeks after surgery, each monkey began training on the ODR task. During daily sessions, the monkey sat in a monkey chair and faced a multiscan 21 in. cathode ray tube (CRT) monitor placed ~ 60 cm in front of him. The monkey’s head was rigidly fixed by two stainless steel bars (8-mm diameter) to a stereotaxic frame located at the top of the monkey chair, and a water spout was positioned close to the monkey’s mouth. The task and the recordings were controlled by a system consisting of an infrared eye-camera system (R-21C-A, RMS, Hirosaki, Japan), two personal computers which were networked by RS232C and parallel I/O, and other associated peripherals. The eye-camera system was connected to the personal computers via A/D converters to be digitized, and was used for monitoring and sampling eye positions (sampling rate, 4 ms). One of the personal computers controlled the task and generated visual stimuli on the CRT monitor, and the other computer monitored and sampled neuronal activities, eye positions and task events.

The phases of the ODR task are illustrated in Fig. 1(A). The ODR task was started when the monkey fixated on a central spot (a white square, 0.2° × 0.2°) on the CRT. One second later, a visual cue (white square, 0.5° × 0.5°) was presented for 0.5 s, and this was followed by a delay period. The cue was presented randomly at one of four peripheral locations, which were separated by 90° (i.e., right, up, left and down; in polar coordinates, 0°, 90°, 180°, and 270°, respectively) with an eccentricity of 15° (Fig. 1(B)). After the delay period of 4 s, the fixation spot was then extinguished (‘go’ signal), which instructed the monkey to make a memory-guided saccade to the remembered target location that had been cued prior to the delay period. Correct responses were defined as an eye movement, which fell within a diameter of 4° around the target location. A correct response was rewarded by a drop of water 0.2 s after the response. When the eye movements did not fall within the prescribed window, or when the monkey failed to respond within the allotted 0.5 s, the response was defined as an error and was not rewarded. Trials were separated by an intertrial interval of 2 s.
The monkeys performed ~500–1500 trials during daily training or recording sessions at a correct-response rate of more than 95%. During a period of ~30 min in a typical recording session, the latency of the onset of the memory-guided saccade from the onset of the go signal (mean ± S.D.) was 222 ± 52 ms for right trials (N = 43), 209 ± 46 ms for up trials (N = 44), 237 ± 49 ms for left trials (N = 47), and 218 ± 28 ms (N = 41) for down trials for one monkey; and 216 ± 50 ms for right trials (N = 42), 280 ± 40 ms for up trials (N = 41), 221 ± 50 ms for left trials (N = 44), and 274 ± 49 ms (N = 39) for down trials for the other monkey.

2.3. Recording and iontophoresis

Four- to six-barreled glass micropipettes (tip diameter, ~10 μm) were used for the iontophoretic application of drugs and extracellular recording of neuronal activity (Sawaguchi, 1987; Sawaguchi et al., 1990a,b). The central barrel was filled with 0.9% saline, contained a carbon fiber filament (7 μm in diameter; T-300, Toray, Tokyo, Japan), and was used for extracellular recordings (impedance: 0.5–1.5 MΩ measured at 1 kHz). The surrounding barrels were filled with, and used for iontophoretic application of, solutions of the following drugs: SCH23390 (0.01 M, pH 5–6; Research Biochemicals International, Natick, MA); L-sulpiride (0.01 M, pH 7–8; Research Biochemicals International); dopamine (0.01 M, pH 5–6; Research Biochemicals International); SCH23388 (0.01 M, pH 5–6; Research Biochemicals International); 0.9% saline (to balance the current), and other drugs, such as ketanserin, for other studies. The micropipette was inserted, with a pulse-motor-driven micromanipulator (MO-95, Narishige, Tokyo, Japan), into the cortex through a small opening (~< 0.2-mm diameter) in the dura. The opening was made by the transient insertion of a metal electrode into the surface of the cortex via intact dura, as in the conventional technique with metal electrodes for single-neuron recording in behaving monkeys (Sawaguchi and Yamane, 1999). This insertion for the opening was performed in the awake condition without anesthesia and with the same micromanipulator that was used for insertion of the micropipette. The monkeys did not show any evidence of discomfort during this procedure, as in the procedure for ordinary single-neuron recording (Sawaguchi and Yamane, 1999).

While the monkey was performing the ODR task, the extracellular activity of single neurons was recorded with the micropipette. When a task-related neuron was

Fig. 1. Behavioral paradigm used and recording sites in the PFC. (A) Events in the ODR task. After the monkey fixated on a spot on a CRT monitor for 1 s, a visual cue appeared for 0.5 s, and this was followed by a delay period. After the delay period (4 s), the fixation spot was extinguished (‘go’ signal), instructing the monkey to make a memory-guided saccade (arrow) to the location which had been indicated by the cue prior to the delay. A correct response was rewarded by a drop of water 0.2 s after the response. Trials were separated by an intertrial interval (ITI) of 2 s. (B) The four cue locations and central fixation spot. Eccentricity was 15°. (C) Recording sites on the surface of the left frontal cortex. The sites were reconstructed from three hemispheres, and the sites on the right hemisphere were homotopically transferred to the left side. PS, principal sulcus; and AS, arcuate sulcus.
located while the micropipette was advanced into the cortex, its activity was initially recorded for more than 20 successive trials without any drugs, to examine its task-related activity. Drugs were then applied iontophoretically through the barrels of the micropipette using a constant current source (DPI-30, DIA Medical, Tokyo, Japan) for more than 20 successive trials of the task. The intensity of the current was varied between 20 and 90 nA, but was usually 50 nA, since previous studies showed that 50 nA induced responses of PFC neurons to drugs such as noradrenaline, dopamine, acetylcholine and dopamine antagonists (Sawaguchi and Matsumura, 1985; Sawaguchi, 1987; Sawaguchi et al., 1990a,b). In the present study, this intensity of the applied current was sufficient to induce responses in most of the PFC neurons that responded to the drugs. Drug applications were separated by more than 2 min to allow for the recovery of neuronal activity. To prevent leakage of the drugs, a backing current (2–5 nA) was applied continuously, during the predrug control period and between drug applications, through the tip of each barrel containing drug solution. Neuronal activity could be recorded stably for several trials and there were no obvious changes in the shapes of spikes that appeared during drug application (Sawaguchi et al., 1990a). Furthermore, to differentiate the effects of a drug from the effects of electrical stimulation, a current of 50 nA was passed through a barrel filled with 0.9% saline; we did not find any neurons that were affected by the current itself.

No behavioral changes were observed following drug application; the monkeys performed the ODR task at a similar frequency and correct-response rate as before application of the drug. In addition, the latency of the onset of the memory-guided saccade from the onset of the go signal was similar in predrug control trials and in trials with drug application (e.g., mean ± S.D. of the onset latency for a ~10-min period during a typical recording session, 219 ± 28 ms, N = 29, during predrug control trials vs. 210 ± 33 ms, N = 28, during trials with SCH23390, U-test, P > 0.05, NS). This lack of a difference might be because the amount and spread of the applied drug were so limited that only a few neurons around the tip of the micropipette were affected.

2.4. Intracortical microstimulation

At the end of each experiment session, intracortical microstimulation (ICMS; a train of 22 cathodal pulses of 0.2-ms duration at 333 Hz, up to 100 μA) was applied through the tip of the recording barrel to examine whether or not the recording site was in the frontal eye field (FEF). When ICMS induced eye movements while the monkey fixated on the central spot, the recording site was considered to be located in the FEF (Bruce et al., 1985). Data obtained at such sites were excluded from the present study, since our target was neurons in the dorsolateral PFC outside the FEF. A DC current (15 μA) was then passed through the tip of the recording barrel for 10 s at one or two recording sites to make a mark (a small deposit of carbon) to reconstruct the recording sites by histological examination (Sawaguchi et al., 1986, 1989).

2.5. Data analysis

In the on-line analysis during the recording sessions, data for neuronal activity were digitized with a window discriminator (DIS-I, BAK Electronics, Germantown, MD) for analysis by a personal computer (sampling rate, 1 ms), and histograms and raster displays of neuronal activity (50 ms/bin) were generated by the personal computer on-line. The data for task events and eye positions were also digitized to be sampled by the computer. These digitized data were recorded on-line on the hard drive of the computer and eventually transferred to a magneto-optical diskette at the end of daily recording sessions for further off-line analyses. Furthermore, all of the analogue data (i.e., neuronal activities, eye positions and task sequences) during each recording session were recorded on digital audio tape (DAT) using an eight-channel DAT recorder (PC-108M, Sony, Tokyo, Japan).

Off-line analysis was carried out with a personal computer. The digitized data were analyzed, and raster displays and averaged histograms of neuronal activities were generated (usually 50 ms/bin). For the statistical analysis, the discharge rate (spikes/s) of each neuron during the 1-s period prior to the onset of the cue (pre cue period) was examined, and the data were pooled for all successive task trials and taken as a control ‘background’ activity for statistical comparison. When the discharge rate (spikes/s) of a neuron during at least one task-period differed significantly from the control discharge rate (Mann–Whitney U-test, P < 0.05), the neuron was considered to be ‘task-related’. Since the aim of the present study was to examine the effect of iontophoretic application of drugs on delay-period activity with directional tuning and a preferred direction, only task-related neurons that showed an increase in activity during the delay period, the magnitude of which varied significantly with the target location (i.e., neurons with directional delay-period activity) were considered in the present study. When the discharge rate of a neuron was significantly greater than the control discharge rate during both the early and latter halves (2 s, respectively) of the delay period, the neuron was considered to show delay-period activity. In addition, when the delay-period activity of such a neuron varied significantly with the target direction (one-way ANOVA, P < 0.05), it was considered to show directional delay-period activity.
The comparison of activity in predrug trials and trials during drug application was based on overall activities during all of the periods of the task. When the activity was found to be significantly affected by the drug (Mann–Whitney U-test, \( P < 0.05 \)), the neuron was judged to be affected by application of the drug. The activities during each of the task periods (for all and each direction(s)) during predrug trials were compared to those during trials with drug application (Mann–Whitney U-test).

2.6. Histology

After the experiments were complete, the monkeys was deeply anesthetized with an overdose of sodium pentobarbital and perfused with 0.9% saline (\( \sim 200 \text{ ml} \)) followed by 10% formalin (\( \sim 500 \text{ ml} \)). The cortical surface was examined to detect the points of penetration. Fig. 1(C) illustrates the sites of penetration on the surface of the left hemisphere. The sites on the right hemisphere were homotopically transferred to the left side. Overall, 27 penetrations were made in the dorso-lateral PFC of three hemispheres in the two monkeys. The points were scattered throughout the caudal half of the peririncipal sulcal area and the immediately adjacent cortex in the PFC, which has cytoarchitectural features of area 9 and Walker’s area 46 (Walker, 1940). Furthermore, ICMS during the recording sessions did not induce eye movements for any of these 27 penetrations in both monkeys, which confirmed physiologically that the penetration sites were outside the FEF (Bruce et al., 1985).

3. Results

3.1. Neuronal database

A total of 92 neurons recorded in the dorsolateral PFC during the ODR task performance showed an increase in activity during the delay period (i.e., delay-period activity). Of these neurons, we focused here on 71 neurons that showed directional delay-period activity, i.e., delay-period activity whose magnitude varied significantly with the direction of the memory-guided saccade (ANOVA, \( P < 0.05 \)), since such activity has been demonstrated to have a memory field (Funahashi et al., 1989). Among these 71 neurons, we tested the effect of iontophoretic application of SCH23390 with a 50-nA current for a total of 62 neurons, and such treatment decreased or increased the activities of most of the neurons tested (\( n = 48/62, 77\% \)); most neurons showed a decrease (\( n = 43/62, 69\% \)), as shown in Table 1. SCH23390 was also applied with a smaller current intensity (20 nA) for 15 neurons, and again this most often produced a decrease in activity (\( n = 11/15, 73\% \)), whereas the results with other drugs are described only briefly.

3.2. Directional tuning of delay-period activity

Before examining the effects of drugs on directional delay-period activity of PFC neurons during ODR task performance, we first analyzed its directional tuning. Fig. 2 shows an example of a neuron in which delay-period activity showed a preferred direction. In Fig. 2, raster displays and averaged histograms, aligned at each task event, for this activity are shown separately according to the location of the cue (i.e., right, 0°; up, 90°; left, 180°; and down, 270°). The neuron showed an increase in activity during the delay period, particularly in left trials (180°). The average discharge rate during the delay period varied significantly with the location of the cue (ANOVA, \( P < 0.001 \)). The left direction was associated with the maximal delay-period activity and the opposite direction (right) was associated with the minimal activity, as shown in the polar plot in the inset of Fig. 2, where the percent change in delay-period activity, compared to the pre-cue period (control) activity, is illustrated for the four different directions of the cue. Thus, this neuron showed directional delay-period activity with a preferred direction on the left (180°) side.

To quantitatively examine the directional tuning, a directional index (tuning index, TI) was calculated as follows:

\[
\text{TI} = \frac{\text{(preferred} - \text{unpreferred)}}{\text{(preferred} + \text{unpreferred})},
\]

Table 1

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<th>Decrease</th>
<th>No effect</th>
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<td>0</td>
<td>4</td>
<td>16*</td>
</tr>
<tr>
<td>Sulpiride (50 nA)</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>17*</td>
</tr>
<tr>
<td>SCH23388 (50 nA)</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>11*</td>
</tr>
</tbody>
</table>

* The activity of these neurons was significantly decreased by iontophoretic application of SCH23390 with a 50-nA current.
Fig. 2. An example of delay-period activity with a preferred direction (i.e., directional delay-period activity). Raster displays and averaged histograms for the neuronal activity are shown according to the cue location (right, 0°; up, 90°; left, 180°; and down 270°). F, fixation period; C, cue period; D, delay period; GS, onset of the go signal. Vertical scale, 10 spikes/s. Inset: percent change in delay-period activity, compared to the precue period (control activity) is illustrated for four different directions as a polar plot (solid cross, baseline activity; i.e., 100%).

where ‘preferred’ is the average discharge rate during the delay period for the preferred direction associated with the maximal delay-period activity and ‘unpreferred’ is that for the unpreferred direction (usually opposite the preferred direction) associated with the minimal delay-period activity. Thus, TI represents the sharpness of tuning of directional delay-period activity; it ranges from 0 to 1, and larger values indicate greater sharpness of such tuning. The same index was used in our previous study for PFC neurons, using an ODR task (Sawaguchi, 1998). For the particular neuronal activity shown in Fig. 2, the preferred direction was toward the left side, the unpreferred direction was on the opposite side (right), and TI was 0.34.

Similar analyses were performed for the 71 neurons that showed directional activity. Fig. 3(A) illustrates the distribution of TI for these neurons. The TI value ranged from 0.08 to 0.66 (mean ± S.D., 0.24 ± 0.13) with a median of 0.20, and there was no obvious boundary or segregation in the distribution of the TI value. In Fig. 3(B), the number of neurons is plotted for each preferred direction. As shown, half of the neurons (n = 31, 44%) showed a preferred direction contralateral to the recording site, while the smallest percentage was seen for the ipsilateral direction (n = 9, 12%). This contra-ipsilateral bias in the preferred direction was statistically significant (one-sample $\chi^2$ test, $P < 0.001$). Equal percentages of neurons had preferred directions on the up and down sides (n = 17, 24%; n = 14, 20%, respectively).

3.3. The effect of SCH23390 on delay-period activity

Iontophoretic application of SCH23390 with a 50-nA current affected the activities of 48 of the 62 neurons tested with this drug, and directional delay-period activity was attenuated during drug application. An example is shown in Fig. 4(A). Raster displays and averaged histograms for the activity before (control) and during the application of SCH23390 with a 50-nA current are shown separately. The activities associated with different target locations (right, 0°; up, 90°; left, 180°; and down, 270°) are also illustrated separately. In the polar plot in the inset, the percent change in delay-period activity, compared to the baseline activity, is illustrated for the four different directions of the cue. As shown in Fig. 4(A), this neuron showed activity during the delay period, particularly for trials on the left (180°) side. The discharge rate during the delay period varied significantly with direction (ANOVA,
Fig. 4. The effect of iontophoretic application of SCH23390 on directional delay-period activity of two PFC neurons (A and B). In both (A) and (B), raster displays and averaged histograms for the activity before (control) and during the application of SCH23390 with a 50-nA current are shown. The activities associated with different cue locations (right, 0°; up, 90°; left, 180°; and down 270°) are illustrated separately. Conventions are as in Fig. 2. Inset: percent change in delay-period activity, compared to the baseline activity, is illustrated for four different directions as a polar plot (shaded cross, baseline activity; i.e., 100%). TI, tuning index.

$Iontophoretic (50 \text{ nA}) application of SCH23390 significantly decreased this activity, and particularly that for the preferred direction of 180° (average discharge rate for all directions, mean ± S.D. spikes/s, 8.43 ± 6.19 for predrug control trials vs. 6.05 ± 4.03 for trials with SCH23390, U-test, $P < 0.01$ mean discharge rate during the delay period for 180° direction, 6.90 ± 3.36 for predrug control trials vs. 10.79 ± 5.95 for trials with SCH23390, $P < 0.01$). However, the directional preference, i.e., the variation in discharge rate during the delay period with direction, was still significant during SCH23390 application (ANOVA, $P < 0.01$) and the preferred direction (i.e., left side) did not change. To better examine the effect of SCH23390 on directional tuning, we examined the effect of SCH23390 on the TI. The TI value of this neuron decreased from 0.34 to 0.21 ($−38.2\%$) during SCH23390 application.

In some cases, the directional tuning of delay-period activity was completely abolished by the iontophoretic application of SCH23390; i.e., the variation in the discharge rate during the delay period with the cue direction became insignificant during drug application. Fig. 4(B) shows such data. This neuron showed a sustained activation during the delay period, particularly for trials on the right (0°) side. The discharge rate during the delay period varied significantly with direction (ANOVA, $P < 0.001$). Iontophoretic (50 nA) application of SCH23390 decreased this activity (average discharge rate for all directions, mean ± S.D. spikes/s, 10.03 ± 6.30 for predrug control trials vs. 3.28 ± 3.50 for trials with SCH23390, $U$-test, $P < 0.01$). At the same time, the difference in the direction of delay-period activity became insignificant (ANOVA, $P > 0.05$, NS). Indeed, TI decreased from 0.32 to 0.07 ($−78.1\%$) with drug application, although the preferred direction (right side) did not change.

Fig. 5((A) and (B)) summarizes the results of an analysis of directional tuning. We first examined the discrepancy in the preferred direction between predrug trials and trials with iontophoretic (50 nA) application of SCH23390 to see how the preferred direction was affected by the application of SCH23390. The data are shown in Fig. 5(A), which illustrates the number of neurons with different discrepancies for the preferred direction. As shown in Fig. 5(A), the discrepancy for the preferred direction was 0° for most of the neurons ($n = 32/48$, 67%); the preferred direction of delay-period activity was the same for these neurons in predrug trials and trials with SCH23390.

In Fig. 5(B), TI for delay-period activity during the application of SCH23390 is plotted against that for
predrug control activity for the 48 neurons. As shown, most of the plots fell below a 45° line, indicating that this index decreased with the application of SCH23390 for most of the neurons analyzed. The average percent decrease was \(-46\%\), and this decrease was statistically significant (mean ± S.D., \(0.28 ± 0.13\) for predrug control vs. \(0.15 ± 0.16\) with SCH23390 application; paired t-test, \(P < 0.001\)).

To further examine the characteristics of the effect of SCH23390 on delay-period activity, we compared the percent changes in the discharge rate during the delay period (relative to predrug control activity) during SCH23390 application with the changes in the background activity for each neuron (\(n = 48\)) whose activity was significantly decreased with the iontophoretic (50 nA) application of SCH23390. In Fig. 5(C), the percent changes in the discharge rates during the delay period (preferred direction) are plotted against the percent changes in the background activity for the neurons affected by SCH23390. As shown, most of the data points fell below the 45° line, indicating that the application of SCH23390 had a greater attenuating effect on delay-period activity than on background activity. In some of the neurons (\(n = 7\)), background activity was either increased (\(n = 3\)) or not affected (\(n = 4\)) by SCH23390, even though it significantly decreased delay-period activity. On average, the percent decrease in delay-period activity was significantly greater than the percent change in background activity (mean ± S.D., \(-65.3 ± 22.2\%\) vs. \(-36.7 ± 28.1\%\), paired t-test, \(P < 0.001\)).
Thus, the iontophoretic (50 nA) application of SCH23390 attenuated the sharpness of the tuning of directional delay-period activity, but did not greatly affect the preferred direction. Further, SCH23390 had a greater attenuating effect on delay-period activity than on background activity.

3.4. The effect of a low dose of SCH23390

A previous study, which used the ODR paradigm and the iontophoretic technique, examined the effect of D1-dopamine antagonists on directional delay-period activities of PFC neurons during the performance of an ODR task (Williams and Goldman-Rakic, 1995). This study reported that the application of D1-dopamine antagonists with smaller currents (i.e., 20–50 nA) increased delay-period activity, whereas application with larger currents (> 50 nA) decreased such activity. To test this point, we applied SCH23390 with a current intensity (20 nA) smaller than the ordinary 50-nA on 15 neurons, which showed directional delay-period activities. Fig. 6 illustrates a typical example of the results. In Fig. 6(A), raster displays and averaged histograms of the neuronal activity only the preferred and unpreferred directions are illustrated for simplicity, and Fig. 6(B) illustrates the average discharge rate (spikes/s, mean ± SE) during the delay period for the preferred direction (bar charts) and TI (line chart) for predrug control trials and trials with drugs for the neuronal activity shown in (A).

As shown in Fig. 7(A), for most of the neurons tested with a 20-nA current (> 13/16, 81%), their delay-period activity was significantly decreased by a low dose of SCH23390. We tested both 20- and 50-nA currents in six neurons, and for all of these neurons the decrease with a 50-nA current was greater than that with a 20-nA current (Fig. 7(A)). TI decreased for all of the neurons tested with a 20-nA current (TI = 0.08, −79%), and this decrease was greater than with a 50-nA current (TI = 0.03, −57%), as shown in Fig. 6(B). Further, for all of the neurons tested with both 20- and 50-nA currents, TI with 20 nA was significantly smaller than that with a 50-nA current (0.08 ± 0.03 for SCH23390 with a 20-nA current vs. 0.06 ± 0.04 for SCH23390 with 50 nA; paired t-test, P < 0.001).
0.03 ± 0.02 for SCH23390 with a 50-nA current; paired t-test, $P < 0.05$). Thus, both delay-period activity and TI were decreased by lower doses of SCH23390, in a dose-dependent manner.

For properties other than TI, the low dose (i.e., 20-nA current) of SCH23390 had effects similar to those of the ordinary dose (50-nA current) of SCH23390. The preferred direction did not change with the lower dose of SCH23390 for most of the neurons examined ($n = 15/16$). While the background activity decreased with a low dose of SCH23390, this decrease was significantly less than that for delay-period activity ($−14.2 ± 29.1\%$ vs. $−34.5 ± 23.9\%$, paired $t$-test, $P < 0.05$).

3.5. The effect of dopamine

For a total of 16 neurons with directional delay-period activity, we tested the effect of the iontophoretic (50 nA) application of dopamine itself on such activity. Fig. 8 shows an example of such data, where neuronal activities for only the preferred and unpreferred directions are shown for simplicity. Again, iontophoretic (50 nA) application of SCH23390 decreased delay-period activity (average discharge rate during the delay period for the preferred direction, mean ± S.D. spikes/s, 4.69 ± 3.67 for predrug control trials; 3.96 ± 3.69 with SCH23390, $U$-test, $P < 0.01$). In contrast, iontophoretic (50 nA) application of dopamine increased delay-period activity (10.96 ± 3.67, $P < 0.01$, compared to the control). When SCH23390 (50 nA) and dopamine (50 nA) were applied simultaneously, the enhancing effect of dopamine was antagonized (6.04 ± 6.39 with both dopamine and SCH23390, $P < 0.01$, compared to with dopamine alone). Furthermore, whereas TI decreased during the application of SCH23390 (TI $= 0.09$ for predrug control vs. 0.02 with SCH23390, $−78\%$), it increased with dopamine (TI $= 0.39$, 333%). The enhancing effect of dopamine on TI was antagonized by the simultaneous application of SCH23390 (TI $= 0.11$).

Fig. 9 summarizes the effect of dopamine on directional delay-period activity, where the discharge rate during the delay period (Fig. 9(A)) and TI (Fig. 9(B)) are plotted for predrug control activity and the activity with dopamine. As shown in Fig. 9(A), in most of the neurons tested with dopamine ($n = 12/16$, 75%), delay-period activity was significantly increased by the application of dopamine. The TI value also increased in most of the neurons; the average increase was statistically significant (mean ± S.D., 0.11 ± 0.04 for predrug control vs. 0.27 ± 0.07 with dopamine; paired $t$-test, $t = 8.147$, d.f. $= 15$, $P < 0.001$). These data indicate that the iontophoretic application of dopamine augmented directional delay-period activity.

3.6. Lack of effect of sulpiride and SCH23388

To examine the pharmacological specificity of SCH23390, we further applied sulpiride and SCH23388 iontophoretically to some of the neurons that showed directional delay-period activity and whose activity was significantly decreased by SCH23390 (Table 1). Sulpiride was tested in 17 neurons with directional delay-period activity, and it affected the activity of almost none of the neurons tested ($n = 2/17$), despite the fact that their activities were decreased by SCH23390. For all of the neurons whose delay-period activity was not affected by sulpiride, the magnitude of the background activity also did not significantly change. In addition, to test possible non-specific effects of SCH23390, such as a local anesthetic effect, we examined the effects of an inactive analogue of SCH23390, SCH23388, on 11 neurons that showed directional delay-period activity that was significantly decreased by SCH23390. Iontophoretic application of SCH23388 had no significant effect on the activities of
4. Discussion

4.1. General

Dopamine and its antagonists were iontophoretically applied to neurons in the monkey PFC that showed directional delay-period activity during an ODR task associated with saccades directed to memorized target locations. Iontophoretic application of SCH23390, a selective antagonist for D1-receptors, decreased or increased the activities of most of the neurons tested; most neurons showed a decrease. For most of the neurons affected by SCH23390, the directional tuning of directional delay-period activity was attenuated by SCH23390, without significant changes in the preferred direction. Furthermore, iontophoretic application of dopamine augmented directional delay-period activity. These results suggest that the activation of D1-receptors play a facilitating role in the memory field of PFC neurons for visuospatial working memory processes.

4.2. Involvement of D1-receptors in delay-period activity

Iontophoretic application of SCH23390 significantly affected the directional delay-period activity of PFC neurons during ODR task performance, whereas sulpiride did not have a significant effect in most of the neurons tested. SCH23390 is a selective antagonist of D1-receptors. Sulpiride is a selective antagonist of D2-receptors (Hyttel, 1983; Christensen et al., 1984; Memo et al., 1986; Sokoloff et al., 1990). Furthermore, the effect of SCH23390 was dose-dependent; iontophoretic application of SCH23390 with stronger currents had a greater effect on delay-period activity. In addition, SCH23388, an inactive analogue of SCH23390, did not have a significant effect on delay-period activity. Together with biochemical findings that the PFC of primates contains a high level of D1-receptors (Cortes et al., 1989; Rischfield et al., 1989; Goldman-Rakic et al., 1990; Smiley and Goldman-Rakic, 1993; Bergson et al., 1995), these findings indicate that the effect of SCH23390 on directional delay-period activity is due to the blockage of D1-receptors; i.e., the activation of D1-receptors is involved in directional delay-period neuronal activities of the dorsolateral PFC.

4.3. Facilitating directional tuning of delay-period activity by D1-receptors

In the present study, iontophoretic application of SCH23390 attenuated directional delay-period activity, whereas dopamine augmented this activity. In their previous study with the ODR paradigm and the iontophoretic technique, Williams and Goldman-Rakic (1995) examined the effect of D1-dopamine antagonists on directional delay-period activities of PFC neurons during ODR task performance. They reported that the application of the D1-receptor antagonist SCH39166 with smaller currents (20–50 nA) increased delay-period activity, whereas application with larger currents
(> 50 nA) decreased such activity. In the present study, although we usually used a current intensity of 50 nA, we also tested a smaller current intensity (i.e., 20 nA) for several neurons. However, no obvious increase was found with the lower dose of SCH23390; rather, the decreasing effect was as usual and the effect appeared to be dose-dependent. Further, the effect of the lower dose of SCH23390 on delay-period activity was similar in nature to that of the ordinary dose (50-nA current) of SCH23390. Since we could not obtain SCH39166, and since Williams and Goldman-Rakic (1995) did not describe the details of their methodology, the reason for the discrepancy between their results and ours is unknown. However, a recent study by Henze et al. (2000) demonstrated that dopamine generally increases the excitability of pyramidal neurons in the monkey PFC. Their findings seem to support the present result that SCH23390 and dopamine generally decreased and increased the delay-period activity of PFC neurons, respectively.

A major finding of the present study is that SCH23390 attenuated the directional tuning, as estimated by the directional index (TI), of directional delay-period activity. In contrast, dopamine augmented this tuning. Since the preferred direction itself was not greatly affected by SCH23390, it is unlikely that directional tuning is generated by dopamine or its D1-receptors. Instead, directional tuning may be generated by other transmitters/receptors, such as amino acids and their receptors. Using SCH23390 and other related drugs, it has been shown in vitro that D1-receptors modulate synaptic transmission of the excitatory amino acid glutamate in the PFC of rats (Law-Tho et al., 1994). Furthermore, Smiley et al. (1994) demonstrated that D1-receptor immunoreactive terminals form asymmetrical synapses on dendritic spines of pyramidal neurons in the primate cerebral cortex, which also suggests that D1-receptors play a modulatory role in excitatory neurotransmission, probably, glutaminergic transmission. In line with these studies, the in vitro study by Henze et al. (2000) showed that dopamine increases the excitability of pyramidal neurons in the monkey PFC; this effect is mediated by D1-receptors. Taken together, these findings suggest that the directional tuning of delay-period activity itself may be generated by neurotransmission with amino acids like glutamate in the PFC and that the activation of D1-receptors in PFC neurons modulates this excitatory neurotransmission, thereby facilitating the directional tuning of delay-period activity during ODR task performance. A study in vitro with the rat PFC showed that the activation of D1-receptors restricts inputs to apical dendrites of pyramidal neurons and potentiates the influence of local inputs from neighboring neurons, thereby modulating dendritic-somatic signal integration of pyramidal neurons (Yang and Seamans, 1996). It is likely that directional tuning itself may be generated by such signal integration and the activation of D1-receptors may play a facilitating role in directional tuning.

4.4. Facilitating role of D1-receptors in the memory field for visuospatial working memory

A subset of neurons in the dorsolateral PFC of primates have been implicated in visuospatial working memory that guides goal-directed behaviors (Funahashi and Kubota, 1994; Goldman-Rakic, 1995). The ODR paradigm used in the present study is useful for accessing this function, since a high level of performance in the ODR task requires the subject to retain visuospatial information during the delay period prior to a response (Funahashi et al., 1989). Directional delay-period activity of PFC neurons during ODR task performance is considered to represent a visuospatial memory field for memory-guided movements (Funahashi et al., 1989). The present findings indicate that tuning of the memory field is attenuated by D1-dopamine antagonism and augmented by dopamine. Thus, the activation of D1-receptors appears to play a facilitating role in the memory field of PFC neurons for visuospatial working memory.

Fig. 9. The effect of the iontophoretic (50 nA) application of dopamine in a total of 16 neurons. Conventions are as in Fig. 7.
memory processes to guide goal-directed behaviors. This conclusion is also supported by previous findings that D1-antagonism in the monkey PFC induces an error in a memory-guided saccade task but not in a visually guided saccade task (Sawaguchi and Goldman-Rakic, 1991, 1994) and that treatment with a non-specific D1/D2-agonist, but not a D2-agonist, facilitates visuospatial working memory performance in humans (Muller et al., 1998). In addition, since deficits in working memory mediated by the PFC as well as dysfunction of dopaminergic transmission in this area are evident in some mental disorders, particularly schizophrenia (Knable and Weinberger, 1997; Okubo et al., 1997; Yang et al., 1999), the present findings may provide a cellular basis for understanding the nature of working memory deficits in schizophrenia; dysfunction of a facilitating role of D1-receptors in PFC neuronal activities for mnemonic processing may be involved in such deficits.

Acknowledgements

This work was supported by Grants-in Aid for Scientific Research (no. 05780621 and 06780688) and a Grant-in-Aid for Scientific Research on Priority Areas (no. 08279105) from the Japanese Ministry of Education, Science and Culture), and by funds provided by the Human Frontier Science Program RF-16/93. The author thanks K. Watanabe-Sawaguchi for her assistance with the animal care.

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