The role of D1-dopamine receptors in working memory-guided movements mediated by frontal cortical areas

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Abstract

Like the striatum, the frontal motor cortices receive dopaminergic fibers from midbrain dopamine cells and contain high levels of dopamine receptors. Among frontal cortical areas, the dorsolateral PFC (PFd1) and the dorsal premotor cortex (PMd) have strong neural connections and play a major role for working memory-guided directional movements. To reveal the role of dopamine in this cognitive motor function, dopamine antagonists (SCH23390 for D1 receptors and sulpiride for D2 receptors) were applied locally or iontophoretically to the PFd1 and PMd in monkeys that performed delayed-response tasks with memory-guided directional movements. Applications of SCH23390, but not sulpiride, to these areas had significant effects at both the behavioral and neuronal levels. In the PFd1 and at the behavioral level, local injections of SCH23390 induced specific errors for memory-guided saccades, whereas it had no effects on visually guided saccades. In the PMd, local injections of SCH23390 induced directional errors and increased reaction time and movement time in memory-guided reaching movements. At the neuron level, iontophoretic applications of SCH23390 attenuated directional tuning of neurons of the PFd1 and PMd, which showed directional activities during the delay-and/or response-period(s). These findings suggest that the activation of D1-dopamine receptors in these frontal cortical areas plays a facilitating role in a series of neuronal processes of working memory-guided directional movements; the working memory process for guiding motor act in the PFd1 and preparation/control of directional manual movements in the PMd. In addition, our findings may provide insight into symptoms of schizophrenia and Parkinson’s disease; the dysfunction of D1-dopamine receptors in the PMd1 and PMd may contribute to some symptoms, such as bradyphrenia and bradykinesia, in these disorders. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

While it is well known that dopamine in the striatum is involved in the control of movements and its dysfunction produces movement disorders, such as Parkinson’s disease [1], evidence is accumulating that dopamine in the frontal cortex may also play a role in motor functions, in particular, motor control by cognitive functions. Like the striatum, the frontal cortex receives dense dopaminergic fibers from the ventral tegmental area and the substantia nigra pars compacta [2]. In primates, both dopaminergic fibers [3–7] and dopamine receptors [8–12] are prominent in frontal cortical areas, in particular dorsolateral areas of prefrontal cortex (PFC) and the dorsal area of the premotor cortex (PMC) (Fig. 1). The dorsolateral PFC (PFd1) plays a major role in working memory processes [13,14] and the dorsal PMC (PMd) is involved in the preparation and control of directional manual movements [15–18]. Working memory is a brain system that is critical for various cognitive operations, and it also works for guiding goal-directed motor acts [19]. The PFd1 and PMd have strong neuronal connections [20,21], and information flow from the PFd1 to the PMd appears to play a crucial role in controlling of directional movements guided by working memory. Further, dopamine receptors in the mammalian central nervous system are divided into at least five subtypes, and are classified into two major types; D1- and D2-receptors. The frontal cortices of primates, including humans, contain a high level of D1 receptors [8–12]. Based on these findings, we hypothesized that D1-dopamine receptors in the PFd1 and PMd may be involved in working memory-guided directional movements.

To address this hypothesis, we have performed two lines of studies in each of these frontal cortical areas of monkeys; i.e. local injection studies [22–24] and iontophoretic studies [25–27]. In the local injection study, we examined the effects of intracortical local injections of dopamine antagonists into the PFd1 and PMd while monkeys performed behavioral tasks requiring working memory-guided directional movements. In the iontophoretic study, we examined the effects of iontophoretic applications of dopamine antagonists on neurons of the PFd1 and PMd, which showed
activities related to performance of such tasks. For behav-
ioral tasks, an oculomotor delayed-response (ODR) task
was used in the study of the PFd1; the ODR task is known
to be adequate for assessing visuospatial working memory
process [28,29]. For the PMd, we used a delayed-reaching
(DR) task with visuospatial cues, since a critical function of
the PMC is to prepare for directional reaching movements
based on visuospatial cues [15–18,30]. With these studies,
we obtained significant evidence that the activation of
D1-dopamine receptors plays a facilitating role in working
memory-guided directional movements mediated by these
frontal cortical areas.

2. Studies in the prefrontal cortex

2.1. Local injection study

In our local injection study of the PFd1, monkeys were
trained to perform an ODR task (Fig. 2A). The subject
was seated in a monkey chair and faced a computer moni-
tor. The ODR task started when the monkey fixated on a
central spot of the monitor. One second later, a visual cue
was presented for 0.3 s, followed by a delay period. The
cue was presented randomly at one of the 16 peripheral
locations, which were separated by 45° and whose eccen-
tricities were 10 and 20° (Fig. 2B). After the delay period
(2–6 s, but usually 4 s), the fixation spot was extinguished
(‘go’ signal), which instructed the monkey to make a
memory-guided saccade to the location that had been
cued prior to the delay period. A correct response was
rewarded by a drop of juice 0.2 s after the response. Trials
were separated by an intertrial interval of 2 s. As a control
task, the monkey was also trained on an oculomotor
visually guided response (OVR) task (Fig. 2A). The
OVR task was exactly the same as the ODR task except that the target continued to be presented during the “delay” period, thus providing sensory guidance for saccade in the go period.

While the monkey was performing the ODR and OVR tasks, SCH23390 (a selective D1-receptor antagonist), SCH23388 (an inactive analog of SCH23390), ketanserin (an antagonist for 5-HT2 receptor), or sulpiride (a selective D2-receptor antagonist) was locally injected into the PFdl with a microsyringe (Fig. 2C). The dose was usually 30 μg, which was dissolved in 3 μl of 0.9% saline. A micromanipulator and a plastic grid with numerous holes (0.7 mm i.d., 1.5 or 2.0 mm apart from each other) on a cylinder mounted on the skull were used to insert the syringe into the cortex and control its precise localization and relocalization in subsequent sessions. The experimental sessions consisted of blocks of trials of 5 min in duration. In each block, the monkey performed the ODR or OVR task, and the blocks associated with the OVR task were intermixed with the blocks associated with the ODR task. The monkeys performed two to four OVR and two to four ODR blocks before, and at least six blocks of each task after injection. The following behavioral parameters were examined: discrepancy between the target location and the end-point of the saccade during the go period; the onset latency of the response after the onset of the go signal; trajectories of saccades; and amplitude and mean velocity of saccades.

The local injection of SCH23390 induced reversible deficits in ODR performance at several injection sites of the PFdl. The impairment was quantified as an increase in errors, as measured by the discrepancy of the position of the to-be-remembered target and the saccade performed by the monkey at the end of each trial, as an example shown in Fig. 3. The trajectories of the saccades were abnormal (Fig. 3C), although their velocities did not change. These deficits usually occurred within 10 min after the injection (in most cases, within 1–3 min after the injection), reached a peak at 20–40 min, and recovered at 60–90 min after the injection (Fig. 3D). The change in performance was restricted to a few specific target locations, which varied with the injection site and were usually contralateral to it. In contrast to the ODR task, no significant changes were observed in performance of the control OVR task after the injection of SCH23390 during the same experimental session in which performance on the ODR task was impaired (Fig. 3). The accuracy and trajectories of sensory-guided saccades in the OVR task were similar before and after the injection for all target locations. Furthermore, the deficit in the ODR task induced by SCH23390 was sensitive to the duration of the delay period.
and longer delays were associated with greater errors. Moreover, the degree of the deficit in the ODR task depended on the dose of the injected drug; higher doses of SCH23390 produced larger errors in the memory-guided saccades.

Although SCH23390 is a selective antagonist of D1 receptors [31], it also appears to bind to serotonin receptors [32]. Thus, it might be argued that the effect of SCH23390 was due to its effects on 5-HT2 receptors. Therefore, we examined the effects of a selective antagonist of 5-HT2 receptor, ketanserin, and an inactive analog of SCH23390, SCH23388, on ODR performance. Local injection of ketanserin or SCH23388 near the same sites at which the injection of SCH23390 induced deficits in the ODR task did not produce any clear changes in the performance of either the ODR or OVR task. In addition, the injection of 0.9% saline (3 μl) into sites that were associated with deficits in ODR performance by SCH23390 failed to induce any significant changes in the performance of either the ODR or OVR task. Thus, the effect of SCH23390 does not appear to be a consequence of either non-specific effects or any effect on 5-HT2 receptors.

These findings indicate that the activation of D1-dopamine receptors is normally involved in the working memory processes mediated by the PFdl. Since the injection site of SCH23390 and the target direction affected showed a gross topographic relationship (Fig. 4A), the PFdl may contain a memory map for representing visuospatial working memory processes and the activation of D1-dopamine receptors may modulate the activity of this cognitive map, as schematically illustrated in Fig. 4B.

2.2. Iontophoretic study

The ODR task for the iontophoretic study in the PFdl was exactly the same as that used in the local injection study. However, for simplification, the delay period was fixed at 4 s, and the cue was presented at only four locations (right, upper, left and down; 15° eccentricity) (Fig. 5A). The details of the iontophoretic technique have been described previously [33,34]. Briefly, multibarreled glass micropipettes were used for extracellular recording of neuronal activity and iontophoretic application of drugs (Fig. 5B). The central barrel of the micropipette, which contained a carbon-fiber filament (7 μm in diameter), was filled with 0.9% saline and used to record neuronal activity. The surrounding barrels were filled with and used for iontophoretic application of solutions of the following drugs: SCH23390 (0.01 M, dissolved in saline); sulpiride (0.01 M, dissolved in saline); SCH23388 (0.01 M, dissolved in saline); ketanserin (0.01 M, dissolved in saline); 0.9% saline (to balance the current). While the monkey performed the task, the task-related activity of a single neuron was recorded by the micropipette for more than 20 successive trials. Each of the drugs was then applied with a current of
30–90 nA (usually 50 nA) for more than 20 successive trials to examine the influence of the drug on the task-related neuronal activity.

In the iontophoretic study, we focused on neurons that showed directional delay-period activity, i.e. delay-period activity whose magnitude varied significantly with the direction of the memory-guided saccade, since such activity has been demonstrated to represent a central process of visuospatial working memory [28,29]. Among these neurons, iontophoretic application of SCH23390 with a 50-nA current decreased or increased the activities of most of the neurons tested (~80%); most neurons showed a decrease (~70%). SCH23390 was applied also with a smaller current intensity (20 nA) for some neurons, and the main effect was also decreasing. In contrast, iontophoretic (50 nA) application of dopamine increased the activities of most of the neurons tested (~75%). Sulpiride, ketanserin, or SCH23388 did not have any significant effect in most of the neurons tested.

An example of the effect of SCH23390 on directional delay-period activity is shown in Fig. 6. 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 of 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. 6A, 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. Iontophoretic (50 nA) application of SCH23390 significantly decreased this activity, and particularly that for the preferred direction of 180° (Fig. 6B). However, the preferred direction (i.e. left side) did not change.

To better examine the effect of SCH23390 on directional tuning, a directional index (DI) was calculated as follows:

$$ DI = \frac{\text{preferred} - \text{unpreferred}}{\text{preferred} + \text{unpreferred}} $$

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 to the preferred direction) associated with the minimal delay-period activity. Thus, DI quantitatively represents the sharpness of the tuning of directional delay-period activity; its ranges from 0 to 1, and larger values indicate greater sharpness of such tuning. For the particular neuron shown in Fig. 6, its DI value was decreased from 0.32 to 0.07 (~78%) during the SCH23390 application.

Fig. 7 summarizes the results of analysis of the directional tuning for a total 48 neurons that showed directional delay-period activity and were significantly affected by iontophoretic (50 nA) application of SCH23390. We first examined the discrepancy in the preferred direction between predrug trials and trials with iontophoretic application of SCH23390 to see how the preferred direction was
affected by application of SCH23390. The data are shown in Fig. 7A, which illustrates the number of neurons with different discrepancies for the preferred direction. As shown in Fig. 7A, the discrepancy for the preferred direction was 0° for most of the neurons (70%); the preferred direction of delay-period activity was the same for these neurons between predrug trials and trials with SCH23390. In Fig. 7B, the DI for delay-period activity during the application of SCH23390 is plotted against those 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.

In our previous studies, we have demonstrated that iontophoretic application of a D1 antagonist attenuated delay-period activity of PFdl neurons during a manual delayed-response task [35], whereas dopamine itself enhanced it [34,36]. The present data are in line with
these previous studies and indicate that activation of D1-dopamine receptors is critical for maintaining directional delay-period activity for delayed-response performance. Since such activity has been demonstrated to represent “memory field” for visuospatial working memory processes [28,29]. Therefore, it is suggested that the activation of D1-dopamine receptors play a role in facilitating memory field of PFdl neurons. This conclusion is also supported by the above finding that local injections of D1 antagonists into the PFC induced a specific error in the ODR task, but not in the OVR task that requires only a visually guided response.

3. Studies in the premotor cortex

3.1. Local injection study

In our local injection study for the PMd, we trained monkeys to perform the delayed-reaching (DR) task (Fig. 8). The DR task was initiated when the monkey pressed a central hold lever. After a 1 s waiting period, a warning cue (green square, $2^\circ \times 2^\circ$) appeared at the center of a computer monitor. One-half second later, one of three visual target cues (left, upper or right, green square, $2^\circ \times 2^\circ$) appeared for 0.5 s, followed by a delay period of 2–5 s. The color of the central cue then changed from green to red: i.e. the go signal. Within 1.2 s after the appearance of the go signal, the monkey released the hold lever to reach toward one of three target levers (right, upper and left) above which the cue had been presented (go period). A correct response was rewarded by a drop of water 0.2 s after the response.

While the monkeys were performing the DR tasks with their left hands, SCH23390, SCH23388, ketanserin, or sulpiride was locally injected into the right PMd. The dose was usually 30 µg, and was dissolved in 3 µl of 0.9% saline. The procedure of the injection was similar to that for the PFdl. The experimental sessions consisted of several 10-min blocks, and the monkeys performed the task for 3 blocks (i.e. 30 min) before the injection of drug and for more than 10 blocks after the injection. The following behavioral parameters were examined and compared between blocks before and after the injection: percent directional errors, in which the monkey reached an incorrect target lever; percent omission errors, in which the monkey did not reach to any of the target levers during the go period; reaction time from the go signal to movement onset; and movement time from movement onset until the monkey reached toward a target lever. To examine the effect of injections on muscle activities during task performance, electromyograms (EMGs) in muscles of the arms and shoulders were recorded with fine wires that were inserted into the muscles (biceps, triceps, deltoid, radial extensor, radial flexor and/or ulnar flexor) during experimental sessions.

Local injections of SCH23390 at several sites in the PMd affected the DR task performance. The effect was characterized by significant increases in directional errors, reaction time and movement time, as an example shown in Fig. 9. The effects of SCH23390 occurred within 10 min after the injection, reached a peak at 30–60 min, and then gradually recovered. The impairment occurred in every direction reached in each case, although the greatest impairment sometimes involved reaching in a specific direction. Furthermore, the muscle activity in the arms during the reaching movement was attenuated after injection. The effect of SCH23390 was dose-dependent: low doses (10 or 20 µg) of SCH23390 induced significant but smaller declines in DR performance. In addition, the effect of SCH23390 was pharmacologically specific: injections of sulpiride, SCH23388, or ketanserin into the sites that had been affected by SCH23390 did not have clear significant effect on the DR task performance. These findings indicate that activation of D1-dopamine receptors in the PMd is normally involved in controlling of memory-guided directional reaching movements.

3.2. Iontophoretic study

The DR task for the iontophoretic study in the PMd was exactly the same as that used in the local injection study, except that the delay period was fixed at 4 s. The methods for the iontophoretic study in the PMC were the same as those in the PFdl. While the monkey performed the task with either its left or right hand, the task-related activity
of a single neuron was recorded by the micropipette for more than 15 successive trials from the hemisphere contralateral to the hand used for task performance. Each of the following drugs was then applied with a current of 30–90 nA (usually 50 nA) for more than 15 successive trials: SCH23390 (0.01 M, dissolved in saline); sulpiride (0.01 M, dissolved in saline); SCH23388 (0.01 M, dissolved in saline); ketanserin (0.01 M, dissolved in saline); and 0.9% saline (to balance the current).

Several neurons recorded in the PMd showed increase in activity during the delay and/or go period(s) of the DR task, whose magnitude differed significantly with the direction of reaching; i.e. directional delay-period and go-period activities. Iontophoretic (50 nA) application of SCH23390 decreased the activity of most of these neurons (~85%), as examples shown in Fig. 10A and B. In contrast, application of sulpiride affected the activities of only a few of the neurons tested (~5%), despite the fact that their activities were decreased by SCH23390 (Fig. 10). Furthermore, iontophoretic application of SCH23388 or ketanserin had no any clear effects on the activities of most of the neurons tested.

We further examined the effect of SCH23390 on the directional tuning of directional delay- and go-period activities. A directional index (DI) was calculated by the equation described above; i.e. DI = (preferred − unpreferred)/(preferred + unpreferred). Larger values of DI reflect sharper directional tuning during the delay or go period. In Fig. 11, the DI’s for the delay- or go-related activities during the application of SCH23390 are plotted against those for the pre-drug control activity. Most plots fell below the 45° angle, indicating that the DI’s for the delay- and go-related activities were decreased by the application of SCH23390 for most of the neurons tested.

These data indicate that D1-dopamine receptors in the PMd are activated during DR performance and that this activation facilitates neuronal activity in the PMd for...
directional reaching movements. This conclusion is also supported by the above finding that local injections of a D1 antagonist into the PMd slowed memory-guided directional movements and/or induced directional errors in the DR task.

4. General discussion

In summary, application of the D1-dopamine antagonist SCH23390 to the frontal cortical areas had a significant effect on both behavior and neuronal activity. In the PFdl, local injection of SCH23390 induced errors in the ODR task requiring memory-guided saccades, whereas it had no effects on visually guided saccades. In the PMd, SCH23390 induced directional errors and increased reaction time and movement time for the memory-guided reaching task. At the neuron level, iontophoretic applications of SCH23390 decreased delay- and go-related activities, and also decreased directional tuning of the activities in both of these areas. As mentioned earlier, the PFdl and PMd have strong direct and indirect neuronal connections to be involved in working memory-guided directional manual movements, and these areas receive dense dopaminergic fibers and contain high levels of D1-receptors. Based on these findings, it is concluded that the activation of D1-dopamine receptors in the PFdl and PMd plays a role in facilitating a series of processes for working memory-guided directional movements: the working memory process for guiding motor acts in the
Thus, a critical role of D1-dopamine receptors in the PFdl and PMd appears to be to facilitate, by activating D1-receptors, “cognitive motor functions” mediated by these frontal cortical areas. In particular, their activation may play an important role for facilitating directional tuning for working memory-guided directional movements. It is unlikely that directional tuning is generated by dopamine or its D1-receptors. Instead, directional tuning itself may be generated by other transmitters/receptors, such as amino acids and their receptors. Using with 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 [37]. In addition, D1-receptor immunoreactive terminals form asymmetrical synapses on dendritic spines of pyramidal neurons in the primate cerebral cortex [12], which also suggests that D1-receptors play a modulatory role in excitatory neurotransmission, probably, glutaminergic transmission. Taken together, these findings suggest that the directional tuning of neuronal activities itself may be generated by neurotransmission with amino acids like glutamate and that the activation of D1-receptors in neurons modulates this excitatory neurotransmission, thereby facilitating the directional tuning of neuronal activity. A study in vitro with the rat PFC demonstrated, using SCH23390 and other related drugs, 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 [38]. It is likely that directional tuning itself may be generated by such a signal integration and the activation of D1-receptors has a facilitating role in the directional tuning for working memory-guided directional movement.

Finally, our studies might provide an insight into symptoms of schizophrenia and Parkinson’s disease. Deficits in working memory mediated by the PFdl as well as dysfunction of D1-dopaminergic transmission in this area are evident in schizophrenia [39,40]. Parkinson’s disease is associated with not only a lesion on the substantia nigra pars compacta but also a lesion on the ventral tegmental area [41], and dopamine cells in both of these structures send their fibers to the frontal motor cortices in primates [2]. The concentration of dopamine and its metabolites are reduced in the frontal cortex of Parkinson’s patients [42], and cognitive deficits, in addition to movement disorders, are also evident in Parkinson’s patients [43–45]. Therefore, it is likely that the dysfunction of D1-dopamine receptors in the PFdl and PMd may contribute to some symptoms, such as bradyphrenia and bradykinesia, in these disorders. This possibility is worth considering for the development of improved therapeutic approaches to these diseases.

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