Dopamine Receptor–Mediated Mechanisms Involved in the Expression of Learned Activity of Primate Striatal Neurons

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INTRODUCTION

To acquire a new behavioral repertoire under new environmental conditions is one of the most important functions of the brain. It is especially true for humans, who have a large variety of behavioral repertoires. It has been suggested that the cerebellum and its related subcortical structures are critically involved in motor learning, such as adaptive learning in the vestibuloocular reflex (Ito 1984; Raymond and Lisberger 1996), conditioned eye blink response (Krupa et al. 1993), and coordination in limb movement control (Thach et al. 1992).

The basal ganglia have recently been suggested to play a major role in the process of behavioral learning (Aosaki et al. 1994a,b; Cools 1980; Hikosaka 1992; Kimura 1995; Schultz et al. 1997). Marsden (1982) drew a conclusion that the basal ganglia are responsible for automatic execution of learned motor plans, based on the observations of motor abnormalities in a large number of Parkinson’s disease patients. The involvement of the basal ganglia in learning seems different from that of the cerebellum. Cools (1980) suggested that the nigrostriatal dopamine (DA) system takes part in sequencing and selecting behavioral strategies. In a positron emission tomography (PET) study using human subjects, Seitz and Roland (1992) demonstrated a selective increase of regional blood flow in the putamen and globus pallidus in the process of learning sequential finger movements. Matsumoto et al. (1994) showed that there is a selective impairment of learning arm movement sequences after destruction of the nigrostriatal DA system by local infusion of dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Two important findings have recently been made on the neuronal mechanisms through which the basal ganglia play a role in behavioral learning. One came from observations of the activity of midbrain DA neurons of monkeys during learning behavioral tasks (Ljungberg et al. 1992; Mirenowicz and Schultz 1996; Schultz et al. 1993). These DA neurons are activated by appetitive but not aversive stimuli (Mirenowicz and Schultz 1996), and thus respond to stimuli predictive of reward. Interestingly, the response of the DA neurons to reward predictive stimuli gradually disappeared as the monkeys became able to execute behavioral tasks automatically through overtraining (Ljungberg et al. 1992; Schultz et al. 1993). The other finding is the modification of striatal neuron activity through behavioral learning and its control by the nigrostriatal DA system. Aosaki et al. (1994b) recorded the activity of a class of striatal neurons of monkeys, tonically active neurons (TANs), which are believed to be cholinergic interneurons in the striatum (Aosaki et al. 1995; Kawaguchi 1992; Kimura et al. 1996; Wilson et al. 1990). The monkeys were trained to associate sensory stimuli with reward. These authors demonstrated that there is a remarkable increase in the number of TANs (from 10–20% to 50–70% of TANs examined) that respond to reward-associated stimuli through the process of...
learning for ~3 wk. Quite importantly, it was found that the acquired responses of TANs almost disappeared when the nigrostriatal DA system was inactivated either permanently by MPTP or reversibly by the DA receptor antagonist, haloperidol (Aosaki et al. 1994a). Thus it was suggested that the nigrostriatal DA system is indispensable for the expression of learned activities of the striatal neurons, TANs.

Molecular cloning studies have shown that there are at least five DA receptor genes in the striatum (D1a, D2, D3, D4, and D1b) (Sibley 1995). These receptors can be grouped into D1 (D1a, D1b) and D2 (D2, D3, and D4) classes. How these DA receptors are distributed among the classes of neurons in the striatum has been the subject of debate. In situ hybridization studies suggested that D1a and D2 mRNA are segregated primarily in the two major projection neuron classes, one projecting to substantia nigra and the other projecting to the globus pallidus (Gerfen 1992; Le Moine and Bloch 1995). Electrophysiological studies revealed that striatal neurons respond not only to D1-class but also to D2-class DA receptor agonists (Cepeda et al. 1993; Uchimura et al. 1986). On the other hand, most cholinergic interneurons in the striatum have D2-class receptors and some of them have D1-class receptors (Le Moine et al. 1991; Levey et al. 1993; Weiner et al. 1991; Yan et al. 1997).

To understand the mechanisms through which the basal ganglia neurons acquire new activities during behavioral learning and retrieve learned activity, in particular behavioral contexts after learning, it is essential to know from where the striatal neurons receive inputs and which classes of DA receptor mechanisms are involved in modifying the activities of the striatal neurons through the process of learning and memory. In the present study, our goal was to identify DA receptor subtypes through which the expression of acquired activities of a class of striatal neurons, TANs, are controlled. The results of the present study have been published in abstract form (Watanabe et al. 1996).

METHODS

We performed two types of experiments using two macaque monkeys (Macaca fuscata) weighing 6.0 kg (monkey A) and 9.0 kg (monkey B). The experiments were carried out in compliance with the guidelines for the care and use of experimental animals by the Physiological Society of Japan. The monkeys were trained to sit in a primate chair in an isolated, electrically shielded room. Each monkey was trained to associate the click sound of a solenoid valve with a drop of water delivered to a spoon in front of its mouth. The monkey’s head was mechanically fixed to the chair through two bolts to record striatal neuron activity and to apply drugs to the recorded neurons during task performance. The drop of reward water on the spoon was not visible to the animals. The behavior of the animals was routinely monitored by a video camera.

Behavioral procedures

In experiment 1, activities of single TANs were recorded in the caudate nucleus and putamen of monkey A, before and after the acquisition of behavioral association of a solenoid click with reward water (auditory click-reward association task). A drop of water was delivered at irregular 5- to 10-s intervals on a spoon in front of the monkey’s mouth 280 ms after the click sound of solenoid valve (Fig. 1A).

In experiment 2, another monkey (monkey B) performed a visually guided push button task. A light-emitting diode (LED) and a push button were positioned on a panel placed 50 cm in front of the monkey. Illumination of the LED triggered a button pushing movement by the monkey. If the button was depressed within 850 ms after the illumination of LED, a drop of reward water was delivered. After the monkey had learned this task (~2 wk), we started to record the activity of TANs in the striatum contralateral to the task-performing hand (Fig. 1B). The activity of TANs was recorded first in the right hemisphere while the monkey was performing the task with its left hand, then in the left hemisphere after switching to the right hand.

Electrophysiological procedures

The activity of single neurons was recorded extracellularly with either glass-insulated elgiloy microelectrodes (Suzuki and Azuma 1976) or Teflon-coated tungsten wire electrodes in an injection-recording device (experiment 1) or carbon fiber electrodes in multi-barreled glass microelectrodes (experiment 2). Electrical signals from the electrodes were amplified and filtered in a conventional manner. Action potentials of single neurons were detected using a time-amplitude window discriminator and were registered in computer memory (NEC PC98BA). Bipolar Teflon-coated stainless steel wire electrodes were chronically and/or acutely implanted in
FIG. 2. Photograph of a coronal histological section at the plane of the recording sites in the right hemisphere of monkey A. Arrow indicates electrolytic lesion mark made by the recording electrode in the putamen. Stars indicate microelectrode tracks directed to the putamen. Recording sites in the striatum covered about the dorsal 2/3s of the nuclei at the level caudal to the anterior commissure. Top and left correspond to dorsal and lateral, respectively. CdN., caudate nucleus; Put., putamen; IC, internal capsule; GPe, external segment of globus pallidus; GPi, internal segment of globus pallidus. Calibration bar: 5 mm.

the prime mover muscles for the task performance to record an electromyogram (EMG). These were digastrics, triceps and biceps brachii. Single-neuron activity and EMG activity were displayed on the computer display in the form of peristimulus time histograms during the experiment. The computer controlled the behavioral tasks.

Surgical procedures

In experiment 1, surgery of monkey A was performed under initial anesthesia with Ketamine (15 mg/kg im) and then pentobarbital sodium (initial 30 mg/kg im, supplement 5 mg·kg⁻¹·h⁻¹). Local anesthetic, 1 and 8% lidocaine hydrochloride, was also used to reduce pain. A stainless steel recording chamber was stereotaxically positioned over the right hemisphere of the cortex and tilted laterally by 45° to avoid damage to the motor cortex and internal capsule. Four T-shape bolts were implanted in the skull with dental acrylic cement to fix the chamber on the skull, and to fix the head to the chair head holder during the experiment.

In experiment 2, after monkey B had achieved a consistent performance of >90% correct in the visually guided push button task, surgery was performed under the same anesthesia used in experiment 1. The skull was exposed, and four T-shape bolts and two stainless steel holders, used for fixation of the head to the primate chair during unit recording, were mounted with dental acrylic cement on the skull. The recording chamber was not used. At each recording, a small hole (3–4 mm diam) was made by drilling the skull over the striatum. Then a small incision (~1 mm diam) was made in both the dura and pia matters for the insertion of a multibarreled glass microelectrode. Local anesthetic, 8% lidocaine hydrochloride, was used to reduce pain during the drilling and dural incision.

Application of DA receptor antagonists

In experiment 1, DA receptor antagonists were administered locally in the striatum by pressure microinjection. We used a stainless steel injection cannula (300 μm ID) through which a Teflon-coated tungsten wire (50-μm base diameter, 75-μm coated diameter) for recording neuronal activity had been threaded with its cut tip protruding 0.7–0.8 mm from the tip of the cannula. The proximal end of the cannula was connected to a microsyringe (1 μl, Hamilton) with Teflon tubing. A guide tube was fixed to a microdrive, and the injection-recording device was positioned inside the guide tube. After the tip of the guide tube was inserted 10 mm below the dura matter into the brain, the injection-recording device was advanced to the striatum while neuronal activity was recorded. Once responses of TANs to sensory cues were recorded through the tungsten wire electrode, either D1- or D2-class DA receptor antagonist was injected (total volume <1 μl, at a rate of 1 μl/min). SCH23390 (10 μg/μl in saline, 31 mM, pH 5.7; RBI) or cis-flupenthixol (30 μg/μl in saline, 59 mM, pH 6.6; RBI) were used as the D1-class antagonists. (-)-Sulpiride (20 μg/μl in saline, 58 mM, pH 6.8; RBI) was used as the D2-class antagonist. As a control experiment, we applied saline (<1 μl) to confirm that the activity of TANs was not significantly influenced.

In experiment 2, four- or five-barreled glass microelectrodes were inserted into the striatum. The central barrel contained a carbon fiber (7 μm diam) and was filled with 1 M NaCl (1–3 MΩ impedance measured at 1 kHz). This was used for extracellular recording of the activity of TANs. Each DA receptor antagonist was iontophoretically applied through one of the barrels. We used SCH23390 (10 mM in saline, pH 4.5; RBI) as the D1-class antagonist and (-)-sulpiride (10 mM in saline, pH 4.5; RBI) as the D2-class antagonist. During recording, a small retaining current (<10 nA) was applied to prevent the leakage of the DA receptor antagonists from the injection pipettes. When TANs responsive to conditioned cues were encountered, their activity was recorded for >30 successive trials in ~5 min. Then one class of DA receptor antagonist was iontophoretically applied with current of ~50 nA (anodal current) through a Micro Iontophoretic Injector (SEZ-3104, Nihon Kohden) to examine the effects of application of the DA receptor antagonists on the activity of TANs. The effects of both D1- and D2-class antagonists were examined in most of the recorded neurons. Recovery from the effects of DA receptor antagonists of TAN responses to either the LED that triggered button pushing or the click associated with reward was confirmed.

Data analysis

Data analysis was performed off-line using a NEC PC98BA computer. Responses of TANs were defined as increasing or decreasing discharge rate after LED or solenoid click relative to that before each stimulus if they achieved at a significance level of P < 0.05 using a two-tailed Wilcoxon test (Kimura 1986). The onset time of a response was defined as the first of three consecutive 15-ms bins of peristimulus time histogram in which the increase or decrease of activity first became significant.

Histological reconstruction

Several small electrolytic lesions were made in each hemisphere to mark the recording sites by passing a positive DC current through the guide tube. Four T-shape bolts were implanted in the skull with dental acrylic cement to fix the chamber on the skull, and to fix the head to the chair head holder during the experiment.

TABLE 1. Effects of dopamine receptor antagonists on background discharge rate of TANs

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<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
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<tbody>
<tr>
<td>Before</td>
<td>4.2 ± 0.9 (10)</td>
<td>4.3 ± 0.7 (42)</td>
</tr>
<tr>
<td>After D1-class</td>
<td>4.3 ± 1.0 (5)</td>
<td>4.2 ± 0.7 (11)</td>
</tr>
<tr>
<td>After D2-class</td>
<td>4.2 ± 0.9 (5)</td>
<td>4.3 ± 0.8 (31)</td>
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Values are means ± SD of discharges per s; number of neurons is in parentheses. TANs, tonically active neurons.
DA RECEPTOR MECHANISMS IN THE STRIATUM FOR LEARNING

FIG. 3. Specimen records of the effects of micropressure application of dopamine (DA) receptor antagonists on the activities of 2 tonically active neurons (TANs): one was suppressed by D2-class antagonist [(-)-sulpiride, 0.7 µl, A–D], the other was not suppressed by D1-class antagonist (cis-flupentixol, 0.7 µl, E–H). Activity of a TAN is shown before (A), and 9 min (B), 31 min (C), and 81 min (D) after injection of D2-class antagonist. Activity of another TAN is shown before (E), and 16 min (F), 46 min (G) and 107 min (H) after injection of D1-class antagonist. Each row in the raster display indicates a spike train in a single trial, with dots representing single spike discharges. Histograms are constructed by summation of spike discharge in each raster. Neuronal activity is aligned at the time of the click preceding the reward.

CLICK WITH REWARD

The experiments were completed, the animals were then deeply anesthetized with an overdose of pentobarbital sodium (70 mg/kg im) and were perfused transcardially with heparin-containing saline and then 10% formaldehyde. Each brain was cut into several blocks, and frozen sections of 50-µm thickness were made in the coronal plane and stained with cresyl violet. Comparison of electrode tracks and electrolytic lesions with descriptions of depth profiles of electrical activity in the penetrations during the recording sessions allowed us to reconstruct the recording tracks and recording sites in the striatum (Fig. 2).
RESULTS

In experiment 1, monkey A was tested with the auditory click-reward association task, and in experiment 2, monkey B was tested by both the auditory click-reward association task and visually guided push button task.

The activities of 75 TANs in monkey A and 220 TANs in monkey B were recorded extracellularly before (in experiment 1) and after behavioral conditioning (in experiment 1 and experiment 2). TANs were identified based on the shape of extracellularly recorded action potentials and the pattern of background discharges (Aosaki et al. 1994b; Apicella et al. 1991; Kimura 1986; Raz et al. 1996).

The vast majority of TANs (36 of 42 recorded) did not respond to the click before conditioning. After behavioral conditioning, 18 of 33 TANs showed responses to the clicks associated with reward. The response consisted of a pause in tonic firing of ~250-ms duration (18 of 18 TANs) and was flanked by brief initial (11 of 18) and late facilitation of firing (12 of 18). The responsiveness of 20 TANs to the click without reward (nonconditioned stimulus) was examined. Only 4 of the 20 TANs responded to the nonconditioned stimulus, and no significant pause in discharge was observed. About a one-half of TANs (122 of 220 TANs examined) became responsive to the LED illumination used as a trigger stimulus for button pushing. The responses consisted of a pause (122/122) with initial (44/122) and late facilitation (76/122) of tonic firing. However, almost no TANs (4/220) responded to the click associated with reward, as if through learning the push button task, the responses to reward had shifted in time to the preceding predictive sensory event, the LED illumination.

Effects of DA receptor antagonists on background discharge rate of TANs

It has been suggested that DA in the striatum would set the baseline firing rate very low, based on the observation of a dramatic decrease of spontaneous discharge rates of striatal neurons of monkeys that were performing behavioral tasks (Rolls et al. 1984). This let us examine the effects of DA receptor antagonists on the background discharge rates of TANs in both experiment 1 and experiment 2. Average background discharge rates of TANs before and after application of DA receptor antagonists are summarized in Table 1. In both experiment 1, in which DA receptor antagonists were applied by micropressure, and experiment 2, in which DA receptor antagonists were iontophoretically applied, the discharge rates of TANs after administration of both D1-class and D2-class antagonists were not significantly different from those before administration (P > 0.05, paired t-test). Therefore neither D1- nor D2-class antagonists affect the mechanisms that set the background discharge rates of TANs.

Effects of topical application of DA receptor antagonists on learned responses of TANs

TANs have been proposed to be cholinergic interneurons in the striatum with large, elongated dendrites extending up to 600 μm on an average (Aosaki et al. 1995; Kawaguchi 1992; Kimura et al. 1996; Wilson et al. 1990; Yelnik et al. 1993). Thus in experiment 1, we locally applied either
selective D1- or D2-class antagonist by micropressure, while recording the activity of TANs, to examine the effects of DA receptor antagonists on the acquired responses of TANs to the conditioned stimuli.

Figure 3 shows sample records of the effects of micropressure application of DA receptor antagonists on the activities of two TANs. A TAN illustrated in Fig. 3, A–D, showed a pause of its tonic firing followed by late excitation at a latency of 135 ms after the click sound of the solenoid valve associated with reward water (Fig. 3A). An application of 0.7 μl of (−)-sulpiride suppressed the pause response 9 min after application (Fig. 3B). The suppression of the pause response continued at least 81 min after the application of the drug. The late excitation, on the other hand, persisted after application of (−)-sulpiride (Fig. 3, B–D). We could test the effects of only a single DA receptor antagonist on the responses of an individual TAN, because the effects of pressure application continued for a few hours. Another TAN illustrated in Fig. 3, E–H, showed no significant sensitivity to D1-class antagonist (cis-flupenthixol). A pause in firing flanked by initial and late excitations triggered by the click associated with reward water continued to appear at least 107 min after application of the drug.

We carried out a total of 10 experiments of pressure appli-
cation of DA receptor antagonists, in which D1-class antagonists were applied in 5 experiments (3 injections of SCH23390 and 2 injections of cis-flupentixol) and D2-class antagonists were applied in another 5 experiments (Table 2). In none of the five applications of D1-class antagonists was a significant effect of the antagonist on the responses of TANs to reward-associated clicks observed. On the contrary, responses of TANs were suppressed in four of five applications of D2-class antagonists.

We recorded activity of 3 TANs beneath the injection sites of DA receptor antagonists in 2 of 10 experiments. The response of a TAN 600 μm beneath the site of injection of the D2-class antagonist, (−)-sulpiride (0.9 μl), was suppressed similarly to the response of the TAN recorded at the injection site, whereas response of another TAN located 1,200 μm beneath the injection site was apparently unaffected.

Because temporal profiles of TAN responses, a pause of tonic discharge flanked by initial and/or late facilitation, recorded in different locations in the striatum were quite similar to each other, we obtained a population response by calculating the ensemble average of responses of multiple TANs to reward-associated click before and after application of DA receptor antagonists.

Population responses of 10 TANs to the reward-associated click are illustrated in Fig. 4. Responses of five TANs that were tested for the effects of D2-class antagonists (Fig. 4A) and those of another five TANs tested for the effects of D1-class antagonists (Fig. 4B) are separately illustrated. The administration of D2-class antagonist almost completely abolished the population pause response of the TANs examined. Responses of four of the five TANs were significantly suppressed by the D2-class antagonist. The initial and late excitation, on the other hand, persisted after administration of D2-class antagonist (Fig. 4A). On the contrary, none of five TANs tested for D1-class antagonists was sensitive to the drug, and thus, the population response histograms obtained before and after administration of D1-class antagonist were quite similar with a pause flanked by initial and late excitation (Fig. 4B). Latencies of initial excitation before and after application of the antagonists were 75 and 78 ms, respectively, those of the pause were 154 and 156 ms, and those of late excitation were 280 and 293 ms, respectively.

Effects of iontophoretic application of DA receptor antagonists on learned responses of TANs

We applied DA receptor antagonists to the TANs iontophoretically in experiment 2. Sample records of the effects of iontophoretic application of both D1- and D2-class antagonists on a TAN are illustrated in Fig. 5. After the monkey had learned the visually guided push button task, almost no TAN responded to the reward-associated click, but instead responded to LED illumination (Fig. 5A). Thus it is suggested that TANs respond to the LED as a reward-predictive stimulus. An application of (−)-sulpiride with a current of +30 nA suppressed the pause response of the TAN (P > 0.05, Wilcoxon test, Fig. 5B). The response of the TAN recovered in 16 min after the administration (Fig. 5C). When the response had fully recovered from administration of (−)-sulpiride, the D1-class antagonist, SCH23390, was applied with a current of +30 nA. SCH23390 did not have significant effects on the response of the TAN (Fig. 5, D–F).

FIG. 6. Population response of TANs to LED illumination as a trigger for the button pushing movement task to summarize the effects of iontophoretic application of DA receptor antagonists. A: population activities of 31 of 49 TANs examined that were sensitive to D2-class antagonists. B: population activities of 11 of 41 TANs examined that were sensitive to D1-class antagonists. Population histograms are centered at the time of LED onset. Number of neurons included for each histogram is shown in parentheses.
TABLE 3. Effects of iontophoresis of dopamine receptor antagonists on the responses of TANs

<table>
<thead>
<tr>
<th>Antagonists</th>
<th>Exclusive</th>
<th>D1- and D2-class</th>
<th>Examined</th>
</tr>
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<tbody>
<tr>
<td>D1-class</td>
<td>3</td>
<td>7</td>
<td>40</td>
</tr>
<tr>
<td>D2-class</td>
<td>19</td>
<td>7</td>
<td>40</td>
</tr>
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</table>

In 40 TANs, effects of both D1- and D2-class antagonists were examined. TANs, tonically active neurons.

Figure 6 shows the population responses of TANs that were sensitive to the iontophoretically applied DA receptor antagonists. D2-class antagonists were ionophoretically applied to 49 TANs that showed characteristic responses to LED illumination used as a trigger for the button pushing movement task. In 31 of the 49 TANs, pause responses to LED illumination were suppressed by administration of D2-class antagonists, although an initial excitation remained. The population responses, which are ensemble averages of the activities of 31 TANs, are illustrated in Fig. 6A. Responses of the other 18 TANs were not significantly influenced by iontophoresis of D2-class antagonist.

Effects of D1-class antagonists were examined in 41 TANs that showed responses to LED illumination. Histograms in Fig. 6B illustrate population responses of 11 of the 41 TANs examined. Iontophoretic application of D1-class antagonist to these 11 TANs abolished the pause response to LED illumination (Fig. 6B, middle). The remaining 30 TANs were not sensitive to the iontophoretic application of D1-class antagonist.

We examined the effects of both D1- and D2-class antagonists on 40 TANs. Out of these, responses of 10 TANs to LED illumination were suppressed by the administration of D1-class antagonists, whereas those of 26 TANs were suppressed by D2-class antagonists. The pause responses in 19 of 26 TANs were suppressed exclusively by the application of D2-class antagonists, and those of 3 TANs were suppressed exclusively by the application of D1-class antagonists (Table 3). Seven TANs were sensitive to both D1- and D2-class antagonists.

Of 40 TANs in which the effects of both D1- and D2-class antagonists were examined, 21 TANs were examined 1st by D2-class antagonist, then by D1-class antagonist. For these 21 TANs, responses of 3 TANs were suppressed exclusively by D1-class antagonist, whereas those of 10 TANs were suppressed exclusively by D2-class antago-
níst. Responses of 2 TANs were suppressed by both D1- and D2-class antagonists. On the other hand, 19 TANs were examined 1st by D1-, then by D2-class antagonists. For these cells, responses of nine TANs were suppressed exclusively by D2-class antagonist, whereas none of these TANs were suppressed exclusively by D1-class antagonist. In five of these TANs, both D1- and D2-class antagonists suppressed the responses. Thus D2-class antagonist-dominant effects were observed in both sequences of application of antagonists. Nonetheless, it appears that both D2- and D1-class DA receptor–mediated mechanisms play an important role in the expression of acquired activities of TANs during performance of learned behavioral tasks.

**Effects of DA receptor antagonists on learned behavior**

To examine the effects of iontophoresis of DA receptor antagonists on learned behavior, reaction times of button pushing movements after LED onset were measured in the visually guided push button task in experiment 2. The average reaction time was 663.3 ± 109.1 (SD) ms before administration of drugs, whereas it was 661.2 ± 96.0 ms during administration of D1-class antagonists and 674.8 ± 109.2 ms during administration of D2-class antagonists. The average reaction times after the administration of D1-class and D2-class antagonists were not significantly different from those before the administration (P > 0.05, paired t-test). It was concluded that the reaction time of button pushing after administration...
LED onset was not affected by the application of either class of DA receptor antagonists. Thus the observed effects of DA receptor antagonists on the responses of TANs were not because of altered task performance.

Control experiments

As a control, saline was iontophoretically applied to confirm that the observed suppression of learned responses of TANs by the application of DA receptor antagonists was not induced by the electric current itself. In one neuron, the iontophoretic administration of D2-class antagonist with a current of +30 nA abolished the conditioned response of the TAN (Fig. 7B, P > 0.05). The conditioned response of the TAN recovered in 14 min after the administration of D2-class receptor antagonist (Fig. 7C). In the same neuron, saline was subsequently applied with a current of +30 nA, but the response of the TAN was not influenced (Fig. 7, D and E, P < 0.05). In all TANs examined in this way (n = 4), iontophoretic administration of saline with <50 µA had no significant effects on the responses of TANs to conditioned stimuli. From these observations, it was concluded that the very strong suppressive effects of DA receptor antagonists on the responses of TANs were not artificially induced by ejecting current but directly caused by the DA receptor antagonists.

Localization of recording sites

The TANs were usually encountered at intervals of 300–700 µm along the recording microelectrode tracks both in the caudate nucleus and in the putamen. We sampled TANs in both nuclei in the right hemisphere of monkey A in experiment 1, and in both nuclei in both hemispheres of monkey B in experiment 2. The recording sites in the striatum covered about the dorsal two-thirds of the nuclei at the level caudal to the anterior commissure. The locations at which responses of TANs to sensory cues were influenced by the application of D1-class and/or D2-class antagonists are indicated on the electrode tracks with different symbols in Fig. 8 (experiment 1) and Fig. 9 (experiment 2). No clear difference in the distribution in the striatum was observed between TANs sensitive to D1-class antagonist and those sensitive to D2-class antagonist in either monkey.

Discussion

Nigrostriatal DA system enables TANs to express learned activity primarily through D2-class receptor–mediated mechanisms in the striatum

In the present study, the pause responses of 65% (26/40) of TANs examined with iontophoretic application of DA receptor antagonists were abolished by D2-class antagonist, whereas micropressure application of the D2-class but not D1-class antagonist abolished the responses of TANs. In 2 of 10 experiments using micropressure injection, we recorded 3 TANs beneath the injection site of DA receptor antagonist in the same electrode track. After the application of 0.7 µl of the D2-class antagonist, (-)-sulpiride, the response of a TAN 600 µm beneath the injection site was suppressed similarly to the response of the TAN at the injection site, whereas the response of another TAN 1,200 µm beneath the same injection site remained.

These observations using alert behaving monkeys are entirely consistent with the results of recent pharmacological and neurobiological studies on the striatal neurons. Dopaminergic afferents end directly on both the spiny projection neurons and the cholinergic interneurons in the striatum (Chang 1988; Freund et al. 1984; Kubota et al. 1987). Yelnik et al. (1993) reported that the cholinergic interneurons in the primate striatum have large elongated dendrites extending up to 600 µm on an average. TANs are thought to be the striatal cholinergic interneurons, based on their slow tonic firing, morphology of cell soma and dendritic arbors (Kawaguchi 1992; Wilson et al. 1990), and preferential distribution at striosome/matrix borders in the striatum (Aosaki et al. 1995).

Striatal cholinergic interneurons have been reported to contain D2-class DA receptors and to express the D2 DA receptor gene (Zhou et al. 1993). In situ hybridization studies have shown that cholinergic interneurons express D2- and/or D1-class receptor mRNA (Le Moine et al. 1991; Weiner et al. 1991). Recent investigation by the use of reverse transcription-polymerase chain reaction analysis (RT-PCR) of dissociated striatal cells provided evidence that cholinergic interneurons express primarily D2 and D1b receptor mRNAs (Yan et al. 1997). These studies indicate that most cholinergic interneurons in the striatum possess D2-class DA receptors and some of them have both D1-class and D2-class DA receptors.

Recently, Aosaki and Kawaguchi (1997) investigated the actions of DA receptor agonists on the striatal cholinergic interneurons in the slice preparation using whole cell patch-clamp technique. Application of the D1-class agonist, SKF38393, almost always induced an inward current, and thus caused burst discharges of these neurons. On the other hand, a D2-class agonist, quinpirole, induced an outward current and suppressed spike discharges in one-half of them and induced inward current and burst discharges in the other half of them. In a study that used an acute dissociated cell preparation (Yan et al. 1997), it was reported that activation of D2 DA receptors in cholinergic interneurons reduces N-type Ca2+ current. This D2 receptor-mediated reduction of N-type Ca2+ current should not only attenuate the dendritic invasion of initial segment spikes (Spruston et al. 1995) but also attenuate the active augmentation of excitatory synaptic events arising from cortical or thalamic sources (Bernander et al. 1994; Kim and Connors 1993; Wilson 1993).

Thus it would be reasonable to assume that DA receptor antagonists applied by micropressure in the present study covered a spherical volume of striatal tissue with a radius of >600 µm, and thus covered not only the whole cell body but also distal dendrites of TANs. Of 40 TANs that were examined with iontophoresis of both D1- and D2-class antagonists, pause responses of 26 TANs to conditioned stimuli were almost completely suppressed by the administration of D2-class antagonist, whereas those of 10 TANs were abolished by D1-class antagonists. Therefore the nigrostriatal DA system appears to play a fundamental role in the expression of acquired activities of TANs, primarily through D2-class receptors and partly through D1-class receptors.

It has been demonstrated that local infusion of the dopa-
minergic neurotoxin MPTP into the striatum abolishes pause responses of TANs to reward-predictive stimuli that were acquired through behavioral conditioning (Aosaki et al. 1994a). But an initial excitation preceding the pause remains after MPTP infusion. Quite similarly, our study demonstrates that application of specific DA receptor antagonists largely reduced pause responses of TANs to stimuli that were predictive of reward, but the initial excitation remained. The initial excitatory response of TANs thus must be mediated through mechanisms that are not related or poorly related to DA receptors. Aosaki et al. (1994a) demonstrated that application of the DA receptor agonist, apomorphine, reinstated responses of TANs in striatum previously infused with MPTP. The present results explain, at least partly, the mechanisms of action of the nigrostriatal DA system on TANs that are working when animals are performing learned behavioral tasks. First, the nigrostriatal DA system does not seem to convey specific, sensory inputs to TANs, but rather to supply control signals for TANs to express responses to inputs. Second, the control of responsiveness of TANs by the nigrostriatal DA system seems to be mediated primarily through D2-class, but partly through D1-class receptor mechanisms. Therefore it can be concluded that the nigrostriatal DA system enables TANs to express learned activities primarily through D2-class and partly through D1-class receptor-mediated mechanisms in the striatum.

Although we examined responses of striatal neurons to conditioned stimuli in most neurons, we examined responses of the neurons to nonconditioned stimuli as well in a considerable number of neurons. Only 4 of 20 TANs examined responded to the nonconditioned stimulus, and there is no significant pause response, although the initial excitation is present, consistent with the results of Aosaki et al. (1994b) that 10–20% of TANs respond to a solenoid click before conditioning. Responses of most of the TANs to the conditioned stimulus thus must have been acquired through the conditioning process. Therefore the present results strongly suggest that tonic DA receptor stimulation enables the striatal neurons to express learned activities, although activity in a small percentage (10–20%) of neurons may depend on DA receptor stimulation independent of learning.

In the present study, the acquired responses of 11 of 41 (27%) TANs were almost abolished either exclusively by D1- or by both D1- and D2-class antagonists applied with iontophoresis. On the other hand, an effect of D1-class antagonist was observed in none of five microinjection applications, although the results of the two experiments were otherwise relatively consistent. This difference might at least partly be explained either by the use of different D1-class antagonists (both SCH23390 and cis-flupenthixol in experiment 1; only SCH23390 in experiment 2) or by different concentrations of the antagonists applied. Recent findings showed that D1-class receptor agonists selectively facilitate γ-aminobutyric acid (GABA)–mediated inhibitory transmission of the strioentopeduncular neurons containing substance P (SP) (Ferre et al. 1996). This observation was supported by other studies (Girault et al. 1986; Reid et al. 1990). On the other hand, the most consistent response of TANs to sensory stimuli is the pause of tonic discharge, which is presumably mediated by either inhibitory mechanisms or resetting of tonic background discharges (Aosaki et al. 1995). If GABAergic striatal projection neurons containing SP send their axon collaterals to TANs that are supposed to be cholinergic (Bolam et al. 1983, 1986), administration of D1-class antagonist would diminish inhibitory GABAergic neurotransmission to TANs. This could lead to the suppression of the pause response of TANs by D1-class antagonist. This mechanism may be responsible for the observation that sensory response of some TANs were significantly reduced by D1-class antagonist.

In this investigation, it was revealed that TANs express learned activities primarily through D2-class and partly through D1-class receptor–mediated mechanisms in the striatum. In this relevance, it is interesting to note the enabling effects of D1 rather than D2 receptor stimulation on task-related activity in the cerebral cortex. Williams and Goldman-Rakic (1995) showed that D1 antagonists can selectively potentiate the “memory fields” of prefrontal neurons that subserve working memory. This might reflect specificity in the cellular mechanisms of DA receptors involved in learning and memory of actions in different brain areas. On the other hand, it remains to be studied which class of DA receptors is involved in the acquisition of new activities in both TANs and the other class of striatal neurons, phasically active neurons (PANs), which are believed to be projection neurons to the globus pallidus and substantia nigra.

**Midbrain DA neurons and TANs may have contrasting response plasticity during behavioral learning**

Schultz and his colleagues reported a systematic reward-related response plasticity of midbrain DA-containing neurons in behaving monkeys (Ljungberg et al. 1992; Schultz 1986; Schultz et al. 1993). DA neurons were activated by primary food and fluid rewards. But when the rewards were predicted by a sensory stimulus used as a behavioral cue, the DA neurons became responsive to visual or auditory stimuli predictive of reward (Ljungberg et al. 1992). In the striatum, the majority of TANs responded to a reward-associated click, when animals were trained to associate sensory stimuli with reward. But when animals learned a visually guided push button task, TANs responded to the visual trigger stimulus and showed no significant responses to the reward-associated click. This suggests the interesting possibility that the TANs in the striatum exhibit responses to behavioral events predictive of reward under the strong influence of inputs from the DA-containing neurons in the substantia nigra pars compacta (SNC).

Nevertheless, the acquired activities of these two sets of neurons have some contrasting profiles. The response of DA-containing neurons, once acquired, seems to diminish progressively with overtraining (Ljungberg et al. 1992; Schultz et al. 1993). By contrast, the TANs, once having acquired responses to the reward-predictive stimuli, maintained the responses even with prolonged overtraining, when the conditioned behavior became highly automatized (Aosaki et al. 1994b). This contrasting property in activity suggests that nigrostriatal DA neurons and TANs are involved in different aspects of mechanisms in behavioral learning. That is, DA-containing neurons may transmit motivation- or reinforcement-related information to the striatum with phasic release of DA in the striatum through which TANs acquire
responses to behavioral events predictive of reward. When DA neurons lose their responsiveness through overtraining, TANs might express activities encoding reward predictability in terms of the baseline release of DA in the striatum. Because the main targets of TAN axons are nearby projection neurons in the striatum (Aosaki et al. 1995), the projection neurons would receive innervation by both the nigrostriatal DA neurons and TANs. Therefore the contrasting response plasticity of nigrostriatal DA neurons and TANs in the striatum may constitute fundamental role in basal ganglia mechanisms involved in acquisition and retrieval of purposeful behavior.

Possible origins of characteristic responses of TANs in the striatum

Because the present study has revealed that the responsiveness of TANs is controlled primarily through D2-class and partly through D1-class receptors, it is very important to know which afferent inputs to TANs are modulated by the nigrostriatal dopaminergic system. It is known that striatal cholinergic interneurons receive direct innervation from the cortex and the thalamus. Lapper and Bolam (1992) showed that cholinergic interneurons of the striatum receive strong inputs from the intralaminar thalamic nuclei. The latencies and electrophysiological properties of the excitatory postsynaptic potentials of striatal cholinergic interneurons evoked by electrical stimulation of the cerebral cortex and thalamus were consistent with monosynaptic inputs from both structures (Wilson et al. 1990). In addition to the above evidence, recent studies in our laboratory have revealed that centromedian-parafascicular (CM-Pf) nuclei of thalamus are candidates for supplying the conditioning input to TANs (Matsumoto et al. 1996, 1997). Neurons in CM-Pf nuclei were found to respond not only to reward-associated but also to nonreward-associated stimuli. Inactivation of CM-Pf nuclei by local injection of muscimol almost completely abolished responsiveness of TANs in the ipsilateral striatum to reward-associated stimuli. This evidence suggests the possibility that striatal TANs receive two characteristic inputs, one from the nigrostriatal DA system and the other from CM-Pf thalamus, and that CM-Pf nuclei supply signaling inputs, whereas the nigrostriatal DA system modulates conditioning input mainly through D2- and partly through D1-class receptors.

Background discharges of TANs before and after application of DA receptor antagonists

Background discharge rate of TANs was affected by neither D1- nor D2-class antagonists. In both experiments 1 and 2, the average discharge rates of TANs before administration of both D1-class and D2-class antagonists were not significantly different from those after and during administration. These results are concordant with previous observations showing that there were no apparent changes of spontaneous discharge rates of TANs both in chronically MPTP-infused striatum and in acutely haloperidol-injected striatum (Aosaki et al. 1994a). On the other hand, Rolls et al. (1984) observed a decrease in spontaneous firing rates of primate striatal and prefrontal cortex neurons in response to iontophoretically applied DA, and drew their conclusion that DA sets the baseline firing rate very low so that the DA can control the signal-to-noise ratio of processing in the striatum. It is not possible to compare directly the results of Rolls et al. with the present observations, because they recorded the activity of striatal projection neurons, but not TANs, and because they did not examine effects of specific D1- and D2-class antagonists.

The results of our study demonstrate that the nigrostriatal DA system does not affect significantly the mechanisms for characteristic tonic discharges of TANs in the striatum, but specifically modulates responsiveness to conditioned inputs mainly through D2- and partly through D1-class receptors.

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