Reacquisition Deficits in Prism Adaptation After Muscimol Microinjection Into the Ventral Premotor Cortex of Monkeys

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INTRODUCTION

It has been suggested that the ventral premotor cortex (PMv) plays an important role in visually guided movements (Boussaoud and Wise 1993; Godschalk et al. 1985; Kurata 1993; Kurata and Hoffman 1994; Mushiake et al. 1991, 1997; Okano and Tanji 1987) and in spatial visual information processing (Boussaoud et al. 1993; Fogassi et al. 1996; Graziano et al. 1994, 1997). Linking these two views, it has been hypothesized that the PMv is a site where the transformation of coordinates from visual to motor space takes place (Fogassi et al. 1996; Graziano et al. 1997; see also Alexander and Crutcher 1990; Andersen et al. 1993; Kawato et al. 1988). To test this hypothesis, we injected muscimol, a potent GABA_A receptor agonist, into the PMv of monkeys the neuronal activity of which was recorded. We hypothesized that reversible inactivation of the PMv using muscimol could cause behavioral deficits in performing a visually guided reaching movement requiring coordinate transformation and adaptation to wedge prisms that dissociated visual from motor coordinates (Kitazawa et al. 1995; Martin et al. 1996). The second aim of this study was to compare the behavioral effects of muscimol injection into the PMv and PMd because it has been suggested that a similar transformation of coordinates also takes place in the dorsal premotor cortex (PMd) (Shen and Alexander 1997). In this study, the exact locations for muscimol injection were selected by identifying dense concentrations of neurons with activity related to reaching movements.

METHODS

Subjects and apparatus

Two male Japanese monkeys (Macaca fuscata) weighing 5.5–6.2 kg were trained to perform a visually guided task that involved reaching toward a target with their right arms. All the experiments were conducted following the standards of the Guide for the Humane Care and Use of Animals by the American Physiological Society (http://www.faseb.org/aps/animal.htm). The monkeys sat comfortably in a primate chair facing a 14-in CRT screen covered with a transparent touch panel (PC-9873L, NEC, Tokyo, Japan). The touch panel monitored the position of the monkey’s hand on the screen by detecting local pressure. The hand’s position on the touch screen was sampled at 500 Hz through an eight-channel, 12-bit A/D converter and stored in a laboratory computer (PC-9801RX2, NEC). The screen was placed 30 cm away from the monkey’s eyes, and the vertical centers of the screen and the monkey’s head and body were aligned. Two acrylic armrests were attached to the chair. A switch made of a 5 × 10-cm acrylic plate was placed at the end of the right armrest to serve as a hold key. The monkey’s left arm was immobilized on the armrest by Velcro straps. An apparatus with two pairs of 4 × 4-cm wedge prisms (10° to the left or right) was placed immediately in front of the monkey’s eyes. The apparatus was sized 46 cm wide, 17 cm high, and 8 cm deep and had a 4 × 8-cm hole in which a pair of the prisms were placed. Within the apparatus, each pair of the prisms was aligned horizontally on a mounting frame and was separated by 5 cm, which matched the monkey’s interocular distance. The mounting frame also had a pair of 4 × 4-cm holes without prisms between the left and right 10° wedge prisms. The frame was moved horizontally by a stepping pulse motor under remote control to position the prisms in front of the monkey’s eyes. The monkey’s visual field through the prisms apparatus matched the size of the CRT screen, and the center of the visual field was aligned at target 5 in each prism condition (see Fig. 1). Because of the size and placement of the prism apparatus, the monkey could not see its hand until the hand came within the visual field through the hole of the prism apparatus.

Behavioral task

In each trial, the monkeys were required to reach for a 5 × 8-mm blue rectangular target randomly appearing in one of nine locations (Fig. 1). The targets were separated by 6.0 cm horizontally and vertically or 10° of visual angle. Throughout the sessions, the targets in the visual frame (visual targets) remained in the same locations, regardless of whether the 10° wedge prisms were present or absent (Fig. 2). A set of nine targets located in the motor coordinates (motor...
A No prism: no shift of motor targets

B Left 10° prism: motor targets shifted right

C Right 10° prism: motor targets shifted left

FIG. 1. Location of the 9 targets (C) on the CRT screen under no-prism (A), left 10° prism (B), and right 10° prism (C) conditions. In left and right 10° prism conditions, the visual locations of the targets were the same as in the no-prism condition.

targets) was shifted horizontally toward the left or right, depending on the presence or absence of the prisms (Fig. 2). When no prism was applied (Figs. 1A and 2A), the locations of the targets in the visual and motor frames were the same. When the left 10° wedge prisms were applied, the set of motor targets was relocated 10° to the right of the visual targets (Figs. 1B and 2B). Conversely, when the right 10° wedge prisms were applied, the set of motor targets was shifted 10° to the left of the visual targets (Figs. 1C and 2C). The conditions in Fig. 1, A—C, were called the “no prism,” “right 10° prism,” and “left 10° prism” conditions, respectively. For each prism condition, the central targets were placed in the midline of monkey’s view, and the number of target locations was used to map neuronal activity in the visual and motor coordinates.

To start each trial, a monkey was required to press the hold key with its right hand. When it pressed the key continuously for a variable period between 1.5 and 3.0 s, a target randomly appeared in one of the nine possible locations on the screen (Fig. 1). If the monkey released the hold key within 500 ms of the target’s appearance (onset of movement), reached the touch screen within 500 ms of the onset of movement, and hit the correct target, 0.1 ml of orange juice was delivered as a reward. The reaction time (RT) was defined as the period between the appearance of the target and the onset of movement. The movement time (MT) was defined as the period between the onset of movement and contact with the screen. We required the monkeys to initiate and execute the movement quickly (see RESULTS) so that they transformed the coordinates during the RT with minimal sensory feedback during the MT. The monkeys had been completely trained for ≥2 wk to adapt to the prisms frequently before data collection. In any prism condition, they were allowed to hit the screen first (not necessarily the target) and then move their hand to the target. However, the first contact point on the screen was generally close to the target irrespective of the prism condition after prism adaptation (see RESULTS). Eye movements were not monitored, and the monkeys were free to move their eyes at any time during a trial.

EMG recording

Electromyographic (EMG) activity was monitored bilaterally with surface and wire electrodes from the following muscles: biceps and triceps brachii, deltoid, extensor carpi radialis, flexor carpi ulnaris, trapezius, supraspinatus, infraspinatus, pectoralis major, rhomboid, thoracic and lumbar paravertebral muscles, gluteus maximus, quadriceps, and tibialis anterior. EMG activity from each muscle was recorded both before chamber implantation and near the completion of data collection from each monkey. The position on the touch screen and EMG data were sampled at 500 and 100 Hz, respectively, through an eight-channel, 12-bit A/D converter and stored in the laboratory computer.

Neuronal recording method

After completion of the behavioral training, a stainless steel recording chamber (27 × 27 mm) and head fixation bolts were implanted on the skull under aseptic conditions. The monkeys were anesthetized with pentobarbital sodium (30 mg/kg im) and after induction with ketamine hydrochloride (8 mg/kg im) with atropine sulfate. During surgery, additional ketamine hydrochloride was given as necessary. Antibiotics and analgesics were used to prevent postsurgical infection and pain.

After complete recovery from the surgery (≥7 days), neuronal activity was recorded in the PMv, PMd, and the primary motor cortex (MI) during task performance, using glass-insulated Elgiloy microelectrodes (1.5–2.0 MΩ at 333 Hz), which were inserted through the dura mater with a hydraulic microdrive (Narishige, MO95). The same microelectrodes were used for intracortical microstimulation (ICMS). Each ICMS consisted of a train of 11 cathodal pulses of 0.2-ms duration at 333 Hz and ±50 μA. ICMS was used to identify the MI physiologically, and within the MI neuronal activity was recorded in the proximal forelimb representation areas but not in the distal forelimb or orofacial areas. When neurons were isolated during recording sessions, only those with an increased discharge rate during the RT were recorded. When an isolated neuron had an increased discharge rate during the reaction time period, the visual response was examined. Two types of visual stimuli were used: the same stimuli that served as targets were presented for 100 ms and then moved to the screen either 10° to the left or right. In any prism condition, the monkeys to initiate and execute the movement quickly (see RESULTS) so that they transformed the coordinates during the RT with minimal sensory feedback during the MT. The monkeys had been completely trained for ≥2 wk to adapt to the prisms frequently before data collection. In any prism condition, they were allowed to hit the screen first (not necessarily the target) and then move their hand to the target. However, the first contact point on the screen was generally close to the target irrespective of the prism condition after prism adaptation (see RESULTS). Eye movements were not monitored, and the monkeys were free to move their eyes at any time during a trial.

Neuronal data analysis

For each of the nine targets, a raster display of recorded neuronal activity was aligned at the movement onset, and its peri-event histogram with 20-ms binwidth was created. Then the mean discharge rate and its standard deviation (SD) during the 0.5- to 1.5-s interval before
target presentation (premovement period) was calculated. If the neuronal activity change exceeded 2.56 SD ($P < 0.01$) during the RT in at least two consecutive bins of any of the nine histograms, it was defined as movement-related activity. The first bin that exceeded 2.56 SD was defined as neuronal onset of the movement-related activity. The mean discharge rate between the neuronal onset and the onset of movement was used for subsequent statistical analyses. The movement-related neuron may not show a statistically significant increase in some of the nine histograms. In that case, the mean discharge rate during the RT was sampled. The mean discharge rates during sampling times were compared using an ANOVA with repeated measures (SPSS for Windows, ver. 6.1, Chicago, IL) to judge whether the activity was directionally selective toward one of the nine targets.

**Muscimol microinjection**

After recording the neuronal activity from the PMv, PMd, and MI of the monkey’s left (contralateral) hemisphere, 1.0 $\mu$l of muscimol was injected into the PMv or PMd. The density of movement-related neurons with direction selectivity was used to determine the injection sites (see RESULTS). The method of muscimol injection was essentially the same as that used in our previous study (Kurata and Hoffman 1994). For injection, we prepared muscimol (Sigma, concentration: 5 $\mu$g/$\mu$l) dissolved in 0.1 M phosphate buffer at pH 7.4. Using an electric microdialysis pump (CMA/100, Carnegie Medicin, Sweden), muscimol (1.0 $\mu$l) was injected at a rate of 0.1 $\mu$l/min through a thin stainless-steel tube that was inserted into the cerebral cortex by the same hydraulic microdrive used for the microelectrodes. The system enabled us to inject muscimol at known coordinates within the recording chamber.

**Collection and analysis of behavioral data**

In each daily session, a single injection of muscimol was made at one location in the cerebral cortex. First, the tube for the injection was inserted into the cerebral cortex. Then the monkey’s behavior was recorded for three blocks of trials (no prism, right 10° prism, and left 10° prism) without a muscimol injection. Throughout the experiment, the prism conditions were alternated by the no-prism condition. In addition, under the no-prism condition, the two sets of targets used for the right and left 10° prism conditions (Fig. 1, B and C) also were presented to study effects of the muscimol injection when the monkeys reached for the left- and rightmost targets (targets 3, 6, and 9 in Fig. 1B and targets 1, 4, and 7 in Fig. 1C). In each block during the preinjection period, ~200 trials were recorded. Immediately after the behavioral data from the preinjection period had been collected, muscimol was injected into the cerebral cortex. During and after muscimol injection (postinjection period), we switched the prisms in blocks of ~50 trials to examine behavioral deficits. We switched the prisms more frequently in the postinjection than in the preinjection periods, because the behavioral effects of a muscimol injection were observed for ~30 min after starting the injection (see also Kurata and Hoffman 1994). The effective period of 30 min contained at least two recording blocks of each prism condition.

Throughout the pre- and postinjection periods, we measured the RT, MT, and horizontal reaching errors under the three prism conditions before and after muscimol injection. The horizontal reaching error was defined as the horizontal distance between the center of the target and the point of first contact on the screen. The horizontal reaching errors on the left and right sides of the targets were indicated by the positive and negative values, respectively, throughout this study. For the data collected in each injection session, RT, MT, and the horizontal reaching error in each prism condition were compared statistically with a two-way ANOVA (SAS/STAT). Target location and recording period (pre- or postinjection period) were selected as factors for ANOVA. It was judged that muscimol was effective when the difference in the horizontal reaching errors between pre- and postinjection periods was statistically significant ($P < 0.05$) (see RESULTS).

Then the horizontal errors of all the effective sites were combined to specify location of targets where the monkeys showed deficits. Because behavioral data were recorded in block of 50 trials during the postinjection periods, each recording block contained approximately five trials to each target. Accordingly, the horizontal reaching errors of the five trials each before and after switching the prisms during the pre- and postinjection periods were compared by a three-way ANOVA (SAS/STAT) (see Figs. 8 and 9). Target location, recording period (pre- or postinjection period), and prism condition were selected as factors for ANOVA. Scheffé test was used to produce post hoc multiple comparisons between target locations. In addition, using the data obtained in the effective sites, prism adaptations during the pre- and postinjection periods were statistically compared by ANOVA in the following two ways. First, in each prism condition, horizontal reaching errors of 10 trials (regardless of target location) immediately after switching the prisms were compared with examine differences in adaptation curves by ANOVA with repeated measures (SAS/STAT). In the analysis, statistical difference in horizontal reaching errors in a recording block (within subjects) will reflect adaptation effects, whereas statistical difference in the errors between pre- and postinjection (between subjects) periods will reveal injection effects. An interaction between adaptation and injection effects obtained in the analysis (within subjects) will show statistical difference between

![Fig. 2. Schematic diagrams showing the target locations in visual and motor frames. Visual location of a target was kept in the same position regardless of the presence or absence of shift prisms. Consequently, when right (B) and left 10° shift (C) prisms were applied to the monkeys, the location of the target was relocated 10° to the left and right, respectively.](image-url)
adaptation curves before and after injection. Accordingly, the 10 trials in each recording block were numbered to examine adaptation and injection effects and their interaction. If both probabilities of injection effect and the interaction were <0.05, we judged that the difference in adaptation curves before and after muscimol injection was statistically significant. Second, horizontal reaching errors of the last 10 trials in subsequent two recording blocks (1 in the no-prism condition and the other in the left- or right-prism condition) during the pre- and postinjection periods were compared with examine accomplishments of prism adaptation (postadaptation baselines) by a two-way ANOVA (SAS/STAT). In the analysis, prism condition and recording period (pre- or postinjection) were selected as factors. The prisms were switched frequently in daily experimental sessions throughout the whole recording periods (6 mo), and the monkeys adapted to the prisms in each occasion. To examine possible long-term changes of prism adaptation, the horizontal errors during the first and second halves of the recording periods (data from the preinjection periods only) were compared statistically by ANOVA in the same ways as described above for the data of the pre- and postinjection periods.

RESULTS

Prism adaptation before muscimol injection

Figure 3 shows the learning curves for horizontal reaching errors (see Methods for definition) before and after switching prisms. The figure also shows the overall accuracy of movement before and after adaptation to the prism. The data are for monkey 1 at two different recording stages, one in the first half of the experiment and the second at a later stage in the whole recording period, which spanned 6 mo of neuronal recording and subsequent muscimol injections. In the two stages, the monkeys adapted to the prisms within ~10 trials after the prism was switched regardless of target location, and the adaptation curves were similar. The difference in horizontal reaching errors of the 10 trials after prism application between the two stages was not significant by ANOVA (P > 0.05). Regardless of prism conditions and of prism applications and removals, differences in adaptation curves (10 trials after switching prisms) and in baselines (the last 10 trials in subsequent 2 recording blocks of different prism conditions, see Methods) between the two stages were not statistically significant by ANOVA (P > 0.05) in the two monkeys. These observations suggest that the monkeys adapted to the prisms quickly and used the same strategy throughout the recording period.

Table 1 shows averages for the accuracy of movement in the three prism conditions after prism adaptation. Compared with the intertarget distance (60 mm), the average horizontal reaching error was relatively small, regardless of whether the prisms were present or absent. The difference in the accuracy of the movement of monkey 1 under the three prism conditions was not statistically significant (ANOVA, P > 0.05), although monkey 2 showed statistically significant differences in the accuracy of movement between prism conditions (ANOVA, P < 0.05).

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Right 10° Prism, mm</th>
<th>No Prism, mm</th>
<th>Left 10° Prism, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.25 ± 0.17</td>
<td>7.55 ± 0.19</td>
<td>8.97 ± 0.14</td>
</tr>
<tr>
<td>2</td>
<td>18.83 ± 0.11</td>
<td>13.88 ± 0.08</td>
<td>10.65 ± 0.11</td>
</tr>
</tbody>
</table>

Histology

After collecting the single-unit data and studying the effect of muscimol microinjections, electrolytic marking lesions were produced by passing 20 μA of cathodal DC through the microelectrodes for 15 s. Ten days later, the monkeys were anesthetized deeply with pentobarbital (50 mg/kg im) and perfused through the heart with saline followed by a fixative containing 3.7% formaldehyde in 0.1 M phosphate buffer at pH 7.4, and then 10 and 20% sucrose solution in the same buffer.

After marking the location of the recording chamber with five pins at known electrode coordinates, the brain was removed from the skull and photographed. Then it was stored in fixative solution at 4°C for 10 days. After the fixation period, it was sectioned at 50-μm intervals in the frontal plane on a freezing microtome for histological reconstruction of the neuronal recording and drug injection sites. Histological examination revealed no serious damage in the cerebral cortex other than electrode and tubing tracks.

The premotor cortex (PM) was defined as the area within the agranular frontal cortex where ICMS failed to evoke muscle activity at an intensity <50 μA. The border between the PM and MI, as defined by ICMS, corresponded to the cytoarchitectonic border between areas 6 and 4 in the present study and in previous reports (Kurata 1989, 1993; Kurata and Hoffman 1994; Kurata and Tanji 1986; Weinrich and Wise 1982). In the present study, a boundary was drawn at the mediolateral level of the spur of the arcuate sulcus to divide the PMd and PMv, a division based on cytoarchitectonic and histochemical studies (Barbas and Pandya 1987; Matelli and Luppino 1996; Matelli et al. 1985).
Neuronal activity and selection of muscimol injection sites

Figure 4 shows representative movement-related neuronal activity recorded in the PMv. Immediately before the movement during RT, the activity of the neuron changed from the level seen before the target presentation. The mean discharge rate between the onset of neuronal activity and the onset of movement was higher before movement toward the targets on the left (1, 4, and 7) than those on the right (3, 6, and 9). The greatest neuronal activity was before movement toward target 7. The directional selectivity was confirmed statistically by ANOVA (P < 0.05). For two of the monkeys, the number of neurons with directionally selective movement-related activity was 94 (40 and 54 from monkeys 1 and 2), 57 (11 and 46), and 56 (31 and 25) in the PMv, PMd, and MI, respectively.

Figure 5 shows cortical maps of the two monkeys, to indicate the sites where neurons with movement-related neuronal activity were recorded in the PMv, PMd, and MI (top). The injection sites were determined by the distribution of the movement-related neurons. The movement-related neurons were located in two foci in the PM. One of these was located in the PMv in a region immediately caudal to the genu of the arcuate sulcus and lateral to the spur of the sulcus. A majority of the movement-related neurons were located close to the border between the PM and MI and near the arcuate spur, but only a few neurons were recorded in the caudal bank of the arcuate sulcus. The other focus was located within the PMd, between the superior precentral sulcus and the arcuate spur. In the MI, movement-related neurons were recorded in close proximity to the border between the MI and PM, as well as in a region near the central sulcus in the proximal forelimb representation area, as identified by ICMS.

Figure 5, bottom, shows the muscimol injection sites in the PMv and PMd of the two monkeys. Muscimol was injected at 9 sites in the PMv and 7 sites in the PMd of monkey 1, and at 11 sites in the PMv and 4 sites in the PMd of monkey 2. In both the PMv and PMd, the injection sites were locations where movement-related neurons were recorded frequently. At each injection site, 1.0 µl of muscimol was injected at a depth of 1.5–2.0 mm from the cortical surface. Muscimol was not injected in the deep caudal bank of the arcuate sulcus or the deep ventral bank of the arcuate spur for two reasons. First, movement-related neurons the activity of which preceded the onset of a reaching movement were recorded in those areas infrequently. Second, it is very likely that deep insertion of the injection tubing might damage the PMv. Muscimol was not injected in the MI.

Effects of muscimol injection into the PMv and PMd: qualitative observations

Figure 6 shows the most typical behavioral deficits in task performance following muscimol injection into the PMv of monkey 1. The effects of muscimol injection on task performance also were observed in the other monkey. In this figure, data from the first 10 trials after prism application were excluded from the analyses, because approximately that many trials were needed for the monkeys to adapt to the prisms (Fig. 3). Before muscimol injection into the PMv, there were some horizontal and vertical reaching errors, possibly due to the experimental design: the monkeys were required to initiate and execute the reaching movement in relatively short time of periods but were allowed to move their hand toward the targets after contacting the screen (see METHODS). Even after muscimol injection into the PMv in the no prism condition (Fig. 6, top), the distribution of the points of first contact was similar to that without the injection, and the points were close to the targets. Thus in the no prism condition, the monkey performed the task accurately without any apparent behavioral deficits even after muscimol was injected. In contrast, the same monkey showed severe deficits when looking through the left 10° prisms (Fig. 6, middle). Although the monkey could initiate and execute reaching movements, the horizontal reaching errors were frequently larger in the post- than in the preinjection periods, especially in the case of targets 4, 6, 7, 8, and 9. The contact points for targets 8 and 9 were closer to where they appeared.
to be when seen through the prisms (the visual coordinates; Figs. 1 and 2). The errors for the remaining targets (1, 2, 3, and 5) were similar in the pre- and postinjection periods. It is important to note that, when the monkey reached for targets in the same motor coordinates without prisms (see METHODS), no such errors were observed. In contrast, when right 10° prisms were applied, these deficits were not observed. The contact points for each of the targets were distributed similarly before and after muscimol injection (Fig. 6, bottom). We never observed such deficits when muscimol was injected into the PMd.

Table 2 summarizes the changes in the horizontal reaching errors and other behavioral changes we observed for each injection site in the PMv and PMd of the two monkeys. The specific behavioral deficits in task performance were frequently observed when muscimol was injected into the PMv (5 of 9 sites in monkey 1 and 4 of 12 sites in monkey 2) but only when the left 10° prisms were introduced. In addition to these specific deficits observed after muscimol injections into the PMv, the monkeys frequently stopped performing the task after injection into the PMv. After PMd injections, such behavioral changes were not observed.

Quantitative analysis of behavioral deficits after muscimol injection into the PMv

The specific deficits observed after PMv injections were analyzed quantitatively. Figure 7 shows the horizontal reaching error ($z$ axis) for the data shown in Fig. 6 plotted on the visual coordinates ($xy$ axes) of the screen. Again, data for the first 10 trials, those required for prism adaptation, were excluded from the analyses. In the left 10° prism condition, the horizontal reaching errors were larger around $xy$ coordinates in the lower left corner of the screen, especially around targets 8 and 9. The difference in the horizontal reaching errors between the pre- and postinjection periods was statistically significant (ANOVA, $P < 0.05$). In contrast, the plotted data for the pre- and postinjection periods and the smoothed distance-weighted least squares were similar in the no-prism and right 10° prism conditions. The differences in the horizontal reaching errors between the pre- and postinjection periods were not statistically significant (ANOVA, $P > 0.05$). Because the monkeys also showed vertical reaching errors, the difference in the vertical horizontal reaching errors between the pre- and postinjection periods was examined by ANOVA. However, the difference was not statistically significant in any prism condition ($P > 0.05$).

The horizontal reaching errors for each target in the pre- and postinjection periods were compared statistically when the left or right prisms were introduced after the no-prism condition (Figs. 8 and 9, respectively) and were removed (data not shown). The data were taken from the effective injection sites in monkey 1 (sites C, E, I, K, and M, see also Fig. 5 and Table 2) and combined. The results were similar in monkey 2. Before muscimol injection (interrupted lines in Fig. 8), the difference in horizontal reaching errors between the two prism conditions was not statistically significant for any target location (Scheffé, $P > 0.05$). In the no-prism condition (trials $-5$ to $-1$ on the abscissa in Fig. 8), the difference in errors between the pre- and postinjection periods was not statistically significant (Scheffé,
P > 0.05). It should be noted that, in Figs. 8 and 9, horizontal reaching errors to each of the nine targets (panels 1–9) were plotted along its approximately five appearances contained in each recording block (trial –5 to –1 or 1–5). Because a target was selected randomly from nine possible locations in a trial (see METHODS) and the monkeys adapted to the prisms in –10 trials (Fig. 3), only 1 or 2 trials to one of the targets during prism adaptation were contained in each recording block. Consequently, adaptation curves as shown in Fig. 3 were not seen in Figs. 8 and 9 when the left 10° prisms were applied during the preinjection periods. By contrast, the errors in the left 10° prism condition (trials 1–5 in Fig. 8) during the postinjection periods were significantly larger for targets 1, 4, 5, 6, 7, 8, and 9 (Scheffé, P < 0.05) than those in the no-prism condition during the postinjection periods (trials –5 to –1) and those in the left 10° prism condition during the preinjection periods (trials 1–5 of the interrupted lines). In the analysis, horizontal reaching errors were calculated for the period throughout the adaptation period in recording blocks (see METHODS). Importantly, when targets in the same motor coordinates (Fig. 1B) were presented to the monkeys without prisms, the differences in error between the pre- and postinjection periods were not statistically significant (ANOVA, P > 0.05). Thus after muscimol injection into the PMv, the monkeys showed deficits in reaching the targets in the left 10° prism condition but could reach the same targets in the no-prism condition. When the left 10° prisms were removed, the difference in horizontal errors between the pre- and postinjection periods were not statistically significant for any target location (Scheffé, P > 0.05). In the left 10° prism condition, adaptation curves taken from data of the first 10 trials (regardless of target location) after prism application between the pre- and postinjection periods were compared by ANOVA with repeated measures, and it was judged that the difference in the adaptation curves was statistically significant (see METHODS). When postadaptation baselines taken from data of the last 10 trials in successive two recording blocks (see METHODS) were compared, the difference between the two prism conditions was not statistically significant.

![Graph showing horizontal reaching errors before and after muscimol injections into the PMv and PMd in the left 10° prism conditions.](http://jn.physiology.org/)

**TABLE 2.** Horizontal reaching errors before and after muscimol injections into the PMv and PMd in the left 10° prism conditions

<table>
<thead>
<tr>
<th>Injection</th>
<th>Monkey 1</th>
<th>Monkey 2</th>
</tr>
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<tbody>
<tr>
<td>Site</td>
<td>Preinjection</td>
<td>Postinjection</td>
</tr>
<tr>
<td>PMv</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td>6.7 ± 5.5</td>
<td>6.1 ± 5.3*</td>
</tr>
<tr>
<td>C</td>
<td>7.1 ± 5.8</td>
<td>14.7 ± 6.5†</td>
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<td>E</td>
<td>6.3 ± 6.0</td>
<td>9.2 ± 4.8†</td>
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<td>G</td>
<td>7.3 ± 6.4</td>
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<td>H</td>
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<td>—</td>
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<td>I</td>
<td>6.6 ± 5.7</td>
<td>10.0 ± 6.3†</td>
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<tr>
<td>J</td>
<td>—</td>
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<tr>
<td>K</td>
<td>10.0 ± 5.3</td>
<td>14.0 ± 6.2†</td>
</tr>
<tr>
<td>L</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>M</td>
<td>11.9 ± 4.6</td>
<td>15.3 ± 5.4†</td>
</tr>
<tr>
<td>N</td>
<td>5.5 ± 5.1</td>
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<td>O</td>
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<tr>
<td>H</td>
<td>8.1 ± 5.5</td>
<td>9.1 ± 5.6</td>
</tr>
<tr>
<td>L</td>
<td>8.2 ± 5.7</td>
<td>9.9 ± 6.3</td>
</tr>
<tr>
<td>N</td>
<td>9.3 ± 6.5</td>
<td>10.4 ± 5.3</td>
</tr>
</tbody>
</table>

Overall means ± SD (in millimeters) of the horizontal reaching errors toward the nine targets. Capital letters (A–Q) indicate location sites shown in Fig. 5. PMv and PMd, ventral and dorsal premotor cortex areas. * Frequent cessation of task performance was observed after muscimol injection. † Difference in horizontal reaching errors between pre- and postinjection periods was statistically significant (ANOVA, P < 0.05, see METHODS).
No Prism

Left 10° Prism

Right 10° Prism

**DISCUSSION**

The main finding of this study is that after muscimol injection into the left PMv (but not the PMd), the monkeys showed significant horizontal errors when reaching selective targets with their right arm. This occurred only when the left 10° prisms were introduced. Under this condition, the motor coordinates of the targets were shifted contralaterally (rightward) relative to the injected, left hemisphere, but the visual coordinates of the targets remained in the same positions regardless of the prism condition. Before muscimol injection into the PMv, the monkeys reached the targets correctly in the left prism condition. After muscimol injection into the PMv, however, the reaching points were located systematically between the visual and motor coordinates of selective targets, thus relatively closer to the visual coordinates than before muscimol injection. The results would be expected if a small amount of muscimol inactivated only a small fraction of the PMv that is involved in prism adaptation specifically for the targets but sparing other functionally distinct parts of the PMv. But it is possible that deficits in prism adaptation were occurred more generally. The most typical deficits were observed at targets 8 and 9 (Fig. 6) located in a lower right part of the screen. Because the monkeys used their right hand, the monkeys were required to approach to the screen from lower right and reach the two target virtually without visual feedback. In reaching other targets, however, they could see their hand after it appeared in the visual field.

**RT and MT**

The RT and MT were compared before and after muscimol injections into the PMv and PMd. For statistical comparison, we combined the behavioral data from each monkey at the sites in PMv where muscimol induced the specific deficits (sites C, E, I, K, and M). Figure 10 shows the RT and MT before and after muscimol was injected in all the effective sites within the PMv of *monkey 1*. The differences in the RT and MT between the pre- and postinjection periods were not significant in any of the prism conditions (ANOVA, *P* > 0.05). Similarly, there were no statistically significant differences when RT and MT were compared in the data for PMd injections in *monkeys 1 and 2*, or in the data for PMv injections in *monkey 1* (ANOVA, *P* > 0.05).
matching the screen during reaching. Then they might have corrected reaching trajectories, masking deficits in prism adaptation to the targets.

Muscimol injection only had effects in the left 10° prism condition, while the monkeys did not make reaching errors in either the no-prism or right 10° prism conditions. In addition,
the RT and MT did not change after muscimol injections in any prism conditions. These results suggest that the monkeys’ ability to reach with their arms was unhindered and that their visual recognition of the targets in space was intact. Our observations show that the effect of PMv injections is different from the reported effect of parietal lobe lesions. When the parietal lobe is damaged, reaching errors are observed in humans (Chieffi et al. 1993) and in monkeys (LaMotte and Acuna 1978; LaMotte and Mountcastle 1979) in virtually all conditions, including the equivalent of the ‘‘no-prism’’ condition of the present study. Therefore we conclude that the specific behavioral effects after PMv injection were due to reacquisition deficits of prism adaptation when required to recalibrate the motor coordinates in a direction contralateral to the injected hemisphere.

**Comparison with previous lesion studies**

Our results shown in Fig. 8 (panels marked by *) are comparable with the deficits seen in patients with inferior olive hypertrophy (reported by Martin et al. 1996). In their report, patients with focal lesion in the inferior olive, which sends climbing fibers to the cerebellum, lost their ability to adapt while throwing darts at a target when looking through wedge prisms. The anatomic connections between the PMv and cerebellum may explain this similarity. The PMv is intercon-nectected with area X of the thalamus, which receives inputs from the cerebellum (Kurata 1994; Matelli et al. 1989; Orioli and Strick 1989; Schell and Strick 1984). The PMv sends signals to the pontine nuclei (Brodal 1978; Glickstein et al. 1985; Leichnetz et al. 1984; Wiesendanger et al. 1979) and the parvocellular, but not the magnocellular, part of the red nucleus (Hartmann von Monakow et al. 1979; Humphrey et al. 1984; Kennedy et al. 1986; Kuypers and Lawrence 1967). The pontine nuclei connect with mossy fibers that are the major afferents to the cerebellum, whereas the parvocellular red nucleus projects to the inferior olivary nucleus, which sends signals to the cerebellum via climbing fibers (see Ito 1984 for review).

However, we do not suggest that the PMv and cerebellum play identical roles in prism adaptation. It is more likely that the two structures are specialized for various aspects of motor learning. One possibility is that descending outputs from the PMv carry error signals to the cerebellum via the inferior olive. If that is the case, our inactivation effects may result from the inability of its output to assist in motor learning due to loss of the error signals from the PMv (see also Gilbert and Thach 1977; Ito 1984; Kawato and Gomi 1992; Marr 1969). Alternatively, the deficits observed in the present study may be derived from disruption of the cerebellar input to the PMv. The PMv is a major target of the cerebellar output to the cerebral cortex (Kurata 1994; Matelli et al. 1989; Orioli and Strick

**FIG. 10.** Means and standard errors of reaction times (RT) and movement times (MT) for reaching movements toward the 9 targets in the 3 prism conditions during pre- and postinjection periods. Data were taken from monkey 1.
1989; Schell and Strick 1984), and the PMv projects to the major motor centers such as the primary motor cortex and the spinal cord (Dum and Strick 1991; He et al. 1993; Leichnetz 1986; Muakkassa and Strick 1979). Therefore disruption of the input from the cerebellum may result in the deficits observed in the present study, even when the cerebellum is working normally in prism adaptation.

Our results are also similar to deficits reported in monkeys with bilateral area 6 lesions (Moll and Kuypers 1977). In their study, the monkeys were not able to "detour" the trajectories of arm movements to get a food pellet. Instead, they made direct arm movements toward the pellet, even when blocked by a transparent board. The common results imply that without the PMv reaching movements can be performed correctly if no additional calculation is needed. Perhaps, there are many brain centers that produce "hard-wired" transformation of coordinates from visual to motor space. They may mask the effect of PMv inactivation when "normal" visuomotor transformations suffice for successful behavior. However, the PMv could play the most significant role in adjusting or modifying the relationship between visual and motor coordinates when a "displaced" situation occurs. In support of this case, the monkeys showed remarkable behavioral deficits but only when adaptation was required.

Comparison with a PET study in humans

In an activation study using positron emission tomography (PET), Clower et al. (1996) recently reported that the posterior parietal cortex was activated when human subjects adapted to prisms in performing visually guided reaching tasks. Their study, however, did not reveal activation of the premotor cortex or cerebellum during the motor learning. As the authors discussed, the results do not necessarily mean that the cerebellum or premotor cortex do not play a role in that kind of motor learning. Such results depend heavily on both experimental design and the threshold to determine activated pixels.

Functional difference between the PMv and Pmd

We also injected muscimol into the Pmd, at loci where neurons with movement-related activity were concentrated densely. The results were strikingly different from PMv injections. We did not observe any remarkable or specific deficit after the injections, although it is suggested that the transformation of coordinates from visual to motor space takes place in the Pmd (Shen and Alexander 1997). This discrepancy could result from differences in experimental design. In the study by Shen and Alexander (1997), the monkeys initiated a movement in a mentally rotated direction (90° clockwise or counterclockwise) from a visually presented directional cue. Thus the task required the monkey to make a more conditional visuomotor transformation. This interpretation seems to agree with previous studies (Godschalk et al. 1985; Kurata and Hoffman 1994; Kurata and Wise 1988) in suggesting that the Pmd is involved more in conditional motor behavior than in motor control guided by spatial visual information.

Hypothesis

The horizontal reaching errors exponentially reduced after the prisms application without muscimol injection into the PMv (Fig. 3), although location of a target was selected randomly in each trial. The behavioral data suggest that the monkeys normally adapted to the prisms by generalizing visual and kinesthetic feedback signals obtained in ~10 trials immediately after the prism were applied. The specific behavioral effects of muscimol injection in the present study suggest that the PMv plays an important role in recalibration of the transformation of the coordinates from visual to motor space as required in prism adaptation. Although the monkeys were faced only with horizontal shifts in our study, similar specific deficits might be expected when vertical wedge prisms, a magnifier, or a microscope are applied. Indeed, humans can adapt to new eyeglasses quickly, and neurosurgeons adapt very quickly to a microscopic view when performing microsurgery by acquiring a new relationship between visual and motor coordinates. Common to monkeys and humans, we can adapt to changes in relative positions between eyes and limbs and in body and limb sizes during development. The results of the present study suggest that whenever such recalibration of a visuospatial frame is required, the PMv of monkeys as well as humans contributes to the rapid adaptation as part of a network that may cooperate with other motor centers such as the cerebellum.

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