Effects of Motor Training on the Recovery of Manual Dexterity After Primary Motor Cortex Lesion in Macaque Monkeys

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INTRODUCTION

Lesioning of the motor cortex is a well-established experimental model for studying recovery of brain function, with >100 yr of research documenting postlesion behavior in non-human primates (Liu and Rouiller 1999; Ogden and Franz 1917; Passingham et al. 1983; Travis and Woolsey 1956; Vilensky and Gilman 2002). Although motor recovery has also been investigated in human patients (Lang and Schieber 2003, 2004; Wade et al. 1983; Wenzelburger et al. 2005), experimental animal studies are important to understand the recovery process after induction of irreversible lesions in specific brain regions.

Among brain functions, the recovery of dexterous hand function, a characteristic of some primate species such as humans, apes, and Old World monkeys (Courtine et al. 2007; Lemon and Griffiths 2005), has been investigated in detail because it is important for human quality of life, as in the making and using of tools. For example, it has been reported that macaque monkeys with motor cortex lesions retain whole-hand grasping ability, but that the precision grip, with finger-to-thumb opposition, did not recover when damage included the hand area of the primary motor cortex (M1) (Passingham et al. 1983). The results of lesion studies are consistent with the suggestion that precision grip is mediated by a monosynaptic projection from M1 to hand motoneurons in the spinal cord (Galea and Darian-Smith 1995; Lawrence and Hopkins 1976; Lemon 1999) and, as such, it is generally accepted that precision grip does not recover, at least without intensive training, after damage to the M1 hand area.

On the other hand, it has become clear that training after a brain lesion can induce plastic changes in neural circuits and subsequent recovery of function. Several studies using rat models showed that motor training or an enriched environment after a brain lesion enhanced motor performance, and that reorganization in dendritic morphology and movement representation in the motor cortex accompanied training-induced recovery (Biermaszkie and Corbett 2001; Johansson and Ohlsson 1996; Ohlsson and Johansson 1995; Ramanathan et al. 2006).

In the present study, behavioral analyses were conducted in a macaque monkey model of brain lesion, as the first step in investigating the mechanism of training-induced recovery of hand function. We made an irreversible lesion in the digit area of M1 and then compared the motor recovery of monkeys that received intensive motor training with those that received no training after the lesion.

METHODS

Prelesion training and testing

Six adult macaque monkeys (Japanese monkeys, Macaca fuscata; 5.7–7.1 kg) were used in this study (Table 1). Before prelesion training, we assigned the monkeys to two groups, trained (n = 3, monkeys S, R, and N) and untrained (n = 3, monkeys O, M, and Q), keeping the average weight similar between the two groups. Both the trained and untrained groups of monkeys received...
training before the M1 lesion, but only the trained group received training after the lesion was established (Fig. 1A). Adequate measures were taken to minimize pain or discomfort, in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 86–23, revised 1987) and the Guide for the Care and Use of Laboratory Primates established by the Primate Research Institute, Kyoto University. All experimental protocols were approved by the National Institute of Advanced Industrial Science and Technology Animal Care and Use Committee.

The monkeys were tested for hand preference using a Klüver board containing five cylindrical wells (13, 12, 11, 10.5, and 10 mm in diameter, 7.5 mm in depth; Fig. 1, B and C), an apparatus similar to that used in previous studies for testing manual dexterity and hand preference in monkeys (Lawrence and Hopkins 1976; Lawrence and Kuypers 1968; Nudo et al. 1992, 1996). A small spherical food pellet (3 mm in diameter) was placed randomly into one of the wells and the monkey was allowed to retrieve it. We recorded which hand was used for the initial reach to the well and determined the preferred hand as that used in >80 of 100 daily trials over 2 consecutive days. The preferred hand for all except one monkey (monkey O) was the left (Table 1). The monkeys were then trained to retrieve the small spherical food pellets from the wells (Klüver board task) using the preferred hand. Two sets of tests and training sessions were conducted each day, one in the morning and one in the afternoon. Food was restricted for 16 h prior to the morning session. In a single test session, 25 pellets were placed pseudorandomly into the wells, 5 in each of the five different sized wells. Within the daily training sessions (30 min × 2 sets), the size of the training well was fixed. On the first day of training, pellets were placed only in the largest well (13 mm in diameter, 7.5 mm in depth). When the total number of pellets retrieved on a given day exceeded 1,000, all the pellets were placed into the next smaller well on the following day. Training was completed when the number of pellets retrieved from the smallest well exceeded 1,000 on 2 consecutive days. The prelesion training was completed in 10–15 days to complete (Table 1).

With the exception of monkey S, manual skill in all monkeys was also evaluated by the “vertical slot task,” in which they retrieved a small food morsel inserted into a vertical slot (Fig. 1D). This type of task has also frequently been used to evaluate manual dexterity in studies using monkeys (Darian-Smith and Ciferri 2005; Galea and Darian-Smith 1997; Sasaki et al. 2004). We used food morsels of two different sizes: the smaller was a raisin (a cylinder ~7 mm in diameter and ~15 mm in length), whereas the larger was a piece of sweet potato cut into a rectangular parallelepiped (7 × 7 × 20 mm in size). Before the test session, we stabbed the food morsel with a toothpick and put it into the vertical slot through a 7.5-mm-square opening located in the center of the slot. The monkey retrieved the food from the opposite side of the slot, using a precision grip without special force. We reloaded the food morsels within 1 s after the monkey retrieved them. Behavioral scores were obtained by counting the number of food morsels retrieved during 1 min. The vertical slot task

### TABLE 1. Monkeys used in the present study

<table>
<thead>
<tr>
<th>Weight, kg</th>
<th>Preferred Hand*</th>
<th>Lesion Volume, mm³**</th>
<th>Prelesion Training Period, Days</th>
<th>Postlesion Training Period, Days</th>
<th>Postlesion Survival Period, Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey S</td>
<td>7.1 Left (89.0%)</td>
<td>64.8 (53.4%)</td>
<td>10</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Monkey R</td>
<td>5.8 Left (92.0%)</td>
<td>101.0 (89.2%)</td>
<td>13</td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>Monkey N</td>
<td>6.8 Left (94.5%)</td>
<td>125.5 (95.2%)</td>
<td>13</td>
<td>60</td>
<td>123</td>
</tr>
<tr>
<td>Average</td>
<td>6.6 —</td>
<td>97.1 (79.3%)</td>
<td>12</td>
<td>44.7</td>
<td>66.3</td>
</tr>
</tbody>
</table>

*The percentage of usage of the preferred hand is shown in parentheses. **The percentage relative to the whole digit area is shown in parentheses.
was easier than the Klüver board task because intact monkeys could retrieve food morsels of both sizes with no training. Before the lesion was made, 4–5 days of tests using the vertical slot task were conducted.

**Intracortical microstimulation and cortical lesion**

A map of the hand representation area in M1 was constructed using the intracortical microstimulation (ICMS) technique (Fig. 2). Under sterile conditions and using pentobarbital anesthesia (25 mg/kg), a limited craniotomy (20 × 30 mm) was made over M1. A stainless steel chamber and head holders were then affixed to the skull with dental acrylic. ICMS was conducted under ketamine anesthesia and the rate of ketamine infusion was adjusted to maintain relatively stable anesthesia. A flexible tungsten microelectrode (MicroProbe, Carlsbad, CA) was advanced perpendicular to the cortical surface to a depth of 5–15 mm at intervals of 500 μm using a hydraulic microdrive. Electrode penetrations were spaced at 2-mm intervals and a conventional intracortical microstimulation technique (1–50 μA, 200 μs in duration, monophasic cathodal pulses at 333 Hz) was used to evoke movement at each electrode penetration site. The sensory response was also investigated to determine the border between the primary motor and sensory areas, using light tactile stimuli to the face, digit, wrist, and forearm of the monkey with a small handheld brush. We defined the digit region of M1 as the 2-mm square of cortical surface centered on the penetration site that satisfied at least one of the following criteria: 1) digit movements were obtained at a current <10 μA or 2) digit movements were obtained with the lowest threshold current and the current was <20 μA. We continued mapping until the digit region was differentiated from the proximal forelimb and face sites. Ibotenic acid (15 μg/μL in 0.1 M phosphate-buffered saline, pH 7.4; 3–9 μL) was then injected intracortically to destroy the digit region of the M1 contralateral to the preferred hand. Multiple injections were used to spread ibotenic acid throughout the digit region. The penetration sites on the cortical surface were separated by 2–3 mm (Fig. 2) and two or three injections separated by 3 mm in depth were made in each track. The depth of injection from the cortical surface was determined by the threshold of ICMS. At each injection site, 0.8–1.0 μL of ibotenic acid was injected at a rate of 0.2 μL/min.

**Postlesion training**

The three monkeys in the trained group began postlesion training on the day after the lesion. Because of hand paralysis, the monkeys could not perform the same training exercises as in the prelesion period for the first several days after the lesion. Therefore we introduced a training procedure different from that used in the prelesion period. At first, we assisted the monkeys in using their affected hand to hold a large food pellet (a rectangular parallelepiped of 20 × 20 × 10 mm; Laboratory Animal Diet, Oriental Yeast, Tokyo, Japan). At 3–6 days after the lesion, when the trained monkeys could hold a large food pellet by themselves, they were trained to retrieve a raisin placed on the plane 200 mm away from the cage. At 6–10 days after the lesion, the trained monkeys began postlesion training using the same procedure as for prelesion training (i.e., two sets of test sessions and 30-min training sessions were conducted each day). At the beginning of postlesion training, the monkeys had difficulty retrieving the food pellets from even the largest well used in prelesion training (13 mm in diameter, 7.5 mm in depth); therefore we introduced an additional shallower well (13 mm in diameter, 5 mm in depth). When the total number of pellets retrieved from the shallower well on a given day exceeded 1,000, all the pellets were placed into the largest well used in prelesion training (13 mm in diameter, 7.5 mm in depth) on the following day. These sets of test and training sessions were conducted 5 days/week. During the experimental period, the monkeys wore a jacket (Alice King Chatham Medical Arts, Hawthorne, CA, or Lomir Biomedical, Notre-Dame-de-l’Île-Perrot, Quebec, Canada) with a dead-end sleeve covering the unaffected hand, forcing them to use the affected hand. Postlesion training was completed when the number of pellets retrieved from the smallest well exceeded 1,000 on 2 consecutive days (Table 1). Two of the three trained monkeys (monkeys S and R) were killed on the day after completion of postlesion training and histological analysis was performed to confirm the location of the lesion (see following text). One trained monkey (monkey N) was killed 2 mo after completion of postlesion training to evaluate whether the recovered manual dexterity was maintained without training. During this survival period, the monkey underwent tests to evaluate manual skill only once/week, using both the Klüver board and vertical slot tasks.

The untrained monkeys wore a jacket with a dead-end sleeve that covered the affected hand to restrain use of the affected hand in daily activities.
life in the cage. The sleeve was made of leather or canvas such that the monkeys could grasp and climb the lattice of the cage but could not grasp small objects with the restrained hand. These unrestrained monkeys underwent a single session of tests to evaluate manual skill in the affected hand once or twice/wk, using both the Klu¨ver board and vertical slot tasks. The animals were killed 1.5–6 mo after the lesion (Table 1) and histological analyses were performed to confirm the location of the lesion.

Confirmation of lesion location

After completing the behavioral experiments, brain tissue was obtained to determine lesion locations. Animals were perfused through the ascending aorta with 0.5 L of ice-cold saline containing 2 mL (2,000 units) of heparin sodium followed by 5–7 L of ice-cold fixative, containing 2% paraformaldehyde and 0.1% glutaraldehyde. Brain segments, including M1, were sectioned coronally at a thickness of 16 mm on a cryostat (Cryocut 3000; Leica, Nussloch, Germany) and then Nissl-stained with cresyl fast violet. Images of the Nissl-stained sections were photographed under an Olympus BX60 microscope, using a 3CCD color video camera (DXC-950; Sony, Tokyo, Japan) and digitized with an image analysis system (MCID; Imaging Research, St. Catherines, Ontario, Canada). We defined the lesioned area as the area of gliosis, as shown by Nissl staining (Fig. 3, B and C). Based on the Cavalieri principle (Mayhew 1992), unbiased volumes of the lesioned region and the M1 digit region were calculated using the following equation

\[ \text{Volume} = T \times a(p) \times \sum P_i \]

where \( T \) is the distance between section planes, \( a(p) \) is the area associated with each grid point, and \( P_i \) is the number of grid points on each section.

Data analysis

We performed five types of behavioral analysis to evaluate changes in manual dexterity over time and to evaluate the differences in dexterity between the trained and untrained groups. First, we calculated the percentage of successful retrieval from the five wells in the Klu¨ver board task (Figs. 4A, 5, and 6, A and B). A successful retrieval occurred only when the monkey retrieved a pellet from the well and brought it into the cage without dropping it. Next, the number of index finger flexions per retrieval was counted in the daily test session trials of the Klu¨ver board task to evaluate the extent of trial and error in a single retrieval (Figs. 4B and 6C). Then, the number of food morsels retrieved during 1 min in the vertical slot task was counted (Figs. 4C and 6D). These behavioral indexes of manual dexterity were estimated in real time during test sessions by the same persons who had trained the monkeys and thus there may have been a risk of bias in estimating them. However, hand movements during the task were recorded using a digital video camera (66-Hz frame rate, noninterlaced; DCR-PC101K, Sony) attached to both the Klu¨ver board and the vertical slot; a subset of the videos of behavior was reanalyzed by a person blinded to the treatment of the monkeys. Results of this reanalysis were consistent with those of the real-time evaluations. For these three behavioral analyses, we used the Mann–Whitney \( U \) test to assess the statistical significance of differences between prelesion measures and the measures in each postlesion period, and between the trained and untrained groups, treating each day as an independent data point. Next, we analyzed digit movements during a single trial of both the Klu¨ver board and vertical slot tasks (Figs. 7C and 9, C–F). The location of each joint in the thumb and index finger was measured in each frame of the recorded video, using video analysis software (Dipp-Motion XD; Ditect, Tokyo, Japan) on an Endeavor MT7000 (Epson Direct, Tokyo, Japan). In this study, two-dimensional images of hand movements were obtained using a single video camera. The visual axis of the camera was perpendicular to the axis between the opening of the cage and the wells on the Klu¨ver board and also to the axis between the opening of the cage and the vertical slot. The movement of digits was restricted within the plane perpendicular to the visual axis of the camera (z-axis) in the vertical slot task, whereas the location of the digits could be displaced in the z-axis by supination/pronation of the wrist and flexion of the thumb in the Klu¨ver board task. Thus we measured the \( y \)-coordinate of the tip of the thumb in the Klu¨ver board task, placing the origin of the coordinate axis at the aperture of the well, as an independent value from the displacement of the thumb toward the z-axis. The circular aperture of the well appeared as an ellipse in the video frames because of the perspective and thus we placed the origin on the long axis of the ellipse (Fig. 7C). We viewed the recorded movie frame by frame and identified the moment of contact between the tip of the index finger and the aperture of the well, contact between the tip of the index finger and the bottom of the well, the greatest flexion of the index finger, and grasping of the food pellet (a, b, c, and d in Fig. 7A, respectively). The \( y \)-coordinate of the tip of the thumb at the moment of grasping the food pellets was then measured and average values in both the trained and untrained monkeys were calculated (Fig. 7D). When the thumb interphalangeal (IP) joint flexed away from the camera and the tip of the thumb was hidden behind the IP joint, we used the \( y \)-coordinate of the IP joint because the distal phalanx of the thumb was almost parallel to the visual axis of the camera. The Mann–Whitney \( U \) test was used to assess the statistical significance of differences between prelesion measures and the measures in the postlesion period, and between the trained and untrained groups, treating each day as an independent data point. These kinematic analyses were conducted by a person blinded to the treatment of the monkeys. Moreover, temporal changes in the distance between the tips of the thumb and index finger and the distance between the tip of the thumb and the food morsel were calculated in the vertical slot task using the same software (Fig. 9, C–F). Finally, we quantified the temporal changes in grasping in the Klu¨ver board task through the pre- and postlesion periods. We classified types (forms) of grasping into three groups according to the location of the food pellet on the thumb and counted the number of times each type of grasp was used in both the pre- and postlesion periods (Fig. 8).
RESULTS

Before lesion, all six monkeys smoothly retrieved food pellets from the smallest well of the Klüver board using a precision grip following 10–15 days of prelesion training. The behavioral indexes used to evaluate manual dexterity were not different between the two groups (“Before lesion” in Fig. 4, A–C). Ibotenic acid was then injected intracortically to destroy the digit area of M1 (Figs. 2 and 3, B–D). Histological analysis after completion of the behavioral analysis confirmed that the ibotenic acid lesion was mostly confined to M1 and very little lesioning was observed in neighboring cortical areas, such as the premotor and the primary sensory areas (Figs. 2 and 3). The average lesion volume was similar between the trained and untrained groups (Table 1). Impairment of function in the contralateral hand was observed immediately in all monkeys examined. The behavior was identical across all animals in that there was complete paralysis of the affected hand. During the first few days after lesioning, monkeys were unable to grip even large objects on the ground with the affected hand (Fig. 1F). Hand motor function then recovered gradually. All six monkeys became able to grip large objects about 1 wk following lesion induction. They were also able to grasp the lattice of the cage and support some weight with the affected hand at this time, but no independent finger movement was observed in any monkey.

Effects of postlesion training on behavioral scores for manual tasks

The percentage of successful retrievals from the five wells was significantly higher in the trained than that in the untrained group in all postlesion periods: 1–2, 3–5, and 6–10 wk after the lesion (Fig. 4A). We also calculated the average percentage of successful retrievals during the last 3 days of the experimental period. Although the average survival period after the lesion was much longer in the untrained (128.7 days) than that in the trained group (66.3 days; Table 1), the percentage of successful retrievals was significantly higher in the latter than that in the former (Fig. 4A). In the trained group, the average percentages of successful retrievals during the period from 6 to 10 wk after the lesion and the last 3 days of the experimental period were not significantly different from that in the prelesion period. In the untrained group, the percentages of successful retrievals were significantly lower in all postlesion periods than that in the prelesion period. In particular, those from the smallest well remained low even during the last 3 days of the experiment (triangles in Fig. 4A). We also evaluated the difference in manual dexterity between the trained and the untrained groups using another criterion: “average number of index finger flexions per retrieval,” which reflected the extent of trial and error in a single retrieval in the Klüver board task (Fig. 4B). Before the lesion, this value was close to 1 in both groups, indicating that the monkeys smoothly retrieved the food pellets without trial and error. The average number of index finger flexions per retrieval was higher in the untrained than that in the trained group in all postlesion periods, indicating that untrained monkeys needed more trial and error to retrieve a single food pellet. The average percentage of successful retrievals in the trained group during the last 3 days of the experimental period was not significantly different from that in the prelesion period, whereas in the untrained group those in all postlesion periods were significantly lower than that in the prelesion period (Fig. 4B). Similarly, the number of food morsels retrieved during 1 min in the vertical slot task was higher in the trained group than that in the untrained group in all postlesion periods. In the untrained group, this number remained low, especially for the smaller food morsels, even during the last 3 days of the experiment (diamonds in Fig. 4C).

Time course of changes in test scores in the trained monkeys

In the trained monkeys, the sets of test and training sessions were conducted 5 days/wk and daily changes in the test scores evaluating manual dexterity were recorded (Fig. 5). This daily analysis showed that manual skill, evaluated by the percentage of successful retrievals from the five wells of the Klüver board task, recovered progressively during the postlesion training period. All monkeys showed periods of transient improvement in success rate (yellow ovals in Fig. 5), followed by a relapse to poor performance. This relapse period was then followed by another period of improvement. The behavioral criteria for the completion of postlesion training were reached after 1 to 2 mo (Table 2; see METHODS for details of the criteria). At this stage, the success rate for all monkeys was restored to the same level as that before lesion (98.7 ± 1.1, 85.3 ± 6.1, and 83.0 ± 27.0% for monkeys S, R, and N, respectively) and smooth finger movements were observed. The timing of the recovery period was positively correlated with lesion size (i.e., the size of the lesion was largest in monkey N, the second largest in monkey R, and the smallest in monkey S) and the length of the recovery period was in the same order. Moreover, the monkey with the smallest lesion (monkey S) showed a transient increase followed by a decrease in test scores only once during the recovery period, whereas the other two monkeys (monkeys R and N) with more severe lesions showed this pattern of change (increase, then decrease in success rate) twice during recovery. The success rate of one monkey (monkey N) was reevaluated 1–2 mo after completion of postlesion training. This analysis confirmed that manual dexterity was maintained even after training had ceased: the average success rate 102–122 days after lesion induction (41–61 days after completion of training) was 81.0 ± 7.6%.

The transient increase and subsequent decrease in the percentage of successful retrievals was observed in the retrievals from both large and small wells during the postlesion recovery period (Fig. 6B), but not during the prelesion training period (Fig. 6A). The transient increase and subsequent decrease in manual dexterity was also evident in the average number of index finger flexions per retrieval; during the days when the success rate transiently decreased (days 23–25 for monkey R), the number of flexions per retrieval increased (Fig. 6C). This measure, when taken with the success rates, suggested transient unskilled digit movements in the Klüver board task during the middle stage of the recovery period. In contrast, no such apparent fluctuation in the test score was observed in the vertical slot task (Fig. 6D).

Digit kinematics in the Klüver board task

Digit movement during the test sessions was recorded on video and subsequent analysis of the recorded images revealed
sequential changes in digit kinematics during the recovery period. During the first several weeks following the lesion, index finger movement was observed first, followed by thumb movement. In the thumb, movement of the carpometacarpal (CM) and metacarpophalangeal (MP) joints preceded movement of the IP joint. The sequences of changes in digit movement were similar among the three trained monkeys.

Figure 7, A and B shows sequential photographs during a single trial in the Klüver board task before lesion and in two stages of the recovery process in one each of the trained and untrained monkeys.

![Graphs and diagrams showing the changes in retrieval, flexions per retrieval, and retrievals per minute over time.](http://jn.physiology.org/).
In the prelesion period, the movements of the thumb differed between the trained and untrained groups, we focused on thumb kinematics. In the prelesion period, the y-coordinate of the tip of the thumb in the recorded images decreased continuously, in accordance with the approach of the index finger to the well (Fig. 7, A, leftmost panels and C). The monkey grasped the food pellets around the aperture of the well. At the point in recovery when both the thumb and index finger began to move again and the success rate increased transiently, the y-coordinate of the tip of the thumb did not decrease, indicating that the tip of the thumb did not move close to the aperture of the well (day 21 in Fig. 7, A and C). This was due to the thumb IP joint flexing toward the palm in accordance with index finger flexion because independent digit movements had not recovered at this time. When postlesion training was successfully completed, the y-coordinate of the tip of the thumb once again decreased during grasping (day 44 in Fig. 7, A and C). Although digit movements during the grasping process differed from those in the prelesion period, i.e., the index finger MP joint remained hyperextended (open double arrowheads in Fig. 7A) at the moment of contact between the tip of the index finger and the bottom of the well, digit posture when grasping objects was almost identical in the last phase of the postlesion period (closed arrowhead on day 44 in Fig. 7A).

In the untrained monkeys, index finger movement was also observed first, followed by movement of the thumb CM joint, and then that of the thumb IP joint. Similar to the trained monkeys, the thumb IP joint of the untrained monkeys flexed toward the palm in accordance with index finger flexion while holding the food pellet at the point of recovery when both the thumb and index finger began to move again (day 32 in Fig. 7B). Unlike the trained monkeys, the synchronized movements of the thumb and the index finger remained in the untrained monkeys and the y-coordinate of the tip of the thumb did not decrease during grasping, even on the last day of the behavioral experiment (day 45 in Fig. 7B). The average y-coordinate of the tip of the thumb at the moment of grasping the food pellets in the untrained group (6.43 ± 3.72 mm) was significantly higher than that in the trained group (−0.50 ± 0.83 mm) during the period from 6 to 10 wk after induction of the lesion (Fig. 7D).

Changes in grip strategies in the Klüver board task

Combined with the test scores during the recovery period, our analysis of digit movement indicated that a transient improvement in test scores in the middle of the recovery period occurred in the trained monkeys, despite incomplete digit movement (e.g., monkey R on day 21, in Figs. 5, 6B, and 7, A and C). Analysis of the recorded video images indicated that monkeys retrieved food pellets using various alternate grip strategies during the middle of the recovery period because of a lack of independent digit movement. Although the tip of the index finger was always used to hold food pellets, all monkeys used several forms of grasping not involving the tip of the thumb (see photographs on day 21 in Fig. 7A). To quantify changes in the form of grasping over time, we classified the grasping observed into three groups, according to the location of the food pellet on the thumb, and counted the number of each grasp type in the pre- and postlesion periods (Fig. 8, A and B). This quantitative analysis of grip types revealed changes in grip strategies over the postlesion period. In both trained and untrained monkeys, use of the precision grip involving the tips of both the index finger and the thumb was observed in almost all trials before lesion induction. In the trained monkeys, many alternate grips, such as holding the food pellet between the tip of the index finger and around the proximal joint of the thumb, were observed during postlesion sessions in which transient

**FIG. 4.** Changes in behavioral indexes used to evaluate manual dexterity. A: the percentage of successful retrievals in the Klüver board task during the last 4 days of the prelesion period; 1–2, 3–5, and 6–10 wk; and the last 3 days of the behavioral experiment after lesion induction. Open circles indicate data for all of the 5 different well sizes. Squares and triangles indicate data for the largest (diameter 13 mm) and smallest wells (diameter 10 mm), respectively. The data are expressed as the mean value for an individual monkey on multiple days. The dark gray and light gray bars indicate the mean values of the trained and untrained monkeys, respectively. B: the number of index finger flexions per retrieval in the Klüver board task, which evaluates the extent of trial and error in a single retrieval. #, no data, because the untrained monkeys could not retrieve the food pellet at all during the period from 1 to 2 wk after lesion induction. The symbols are the same as in A. C: the number of food morsels retrieved during 1 min in the vertical slot task. Stars and diamonds indicate data for the larger (sweet potato) and smaller food morsels (raisin), respectively. The other symbols are the same as in A. All of the behavioral indexes (A, B, C) indicated that the recovery of dexterous digit movement occurred earlier in the trained than in the untrained monkeys. Although the indexes recovered to the same level as seen in the prelesion period in the trained monkeys, the indexes in the untrained monkeys remained below those before the lesion, even in the last behavioral experiment. The Mann–Whitney U test was used to assess the statistical significance of differences between prelesion measures and those in each postlesion period, and between the trained and untrained groups, treating each day as an independent data point (*P < 0.005, **P < 0.001).
increases in the success rate occurred (e.g., days 21 and 31 in Fig. 8A). The location of the food pellet on the thumb changed from more proximal regions (around the MP joint) to more distal regions (around the IP joint). The number of observed precision grips gradually increased during the subsequent period, which involved a transient decrease and subsequent increase in the success rate. At the point when the success rate decreased transiently, the monkeys often seemed to attempt to retrieve food pellets using a precision grip, but most attempts failed due to incomplete coordination between the index finger and thumb (day 34 in Fig. 8A). At 1–2 mo after lesion induction, when behavioral criteria were met, the precision grip was used as often as during the presleion period.

In the untrained monkeys, there were many trials using alternate grips, such as holding the food pellet between the tip of the index finger and around the MP and IP joints of the thumb, during the recovery period (Fig. 8B). However, unlike the trained monkeys, these alternate grips were not replaced by the precision grip in the untrained monkeys up to the last behavioral experiment. Although the untrained monkeys became skilled in using these alternate grips and took shorter times to retrieve pellets by the end of the experimental period (Fig. 7B), they still took a longer time than did the trained monkeys using a precision grip (day 44 in Fig. 7A; day 45 in Fig. 7B).

Digit kinematics in the vertical slot task

We also investigated sequential changes in digit movement while retrieving food morsels in the vertical slot task. Before lesion induction, the monkeys opened their index finger and thumb and formed an appropriate configuration for the target object to be grasped (preshaping; time point b in Fig. 9, A and C) before holding the food morsel. The monkey’s index finger and thumb simultaneously touched the food morsels and the morsels were between the tips of the index finger and thumb.

In the trained monkeys, the number of food morsels retrieved increased gradually over several weeks after lesion induction (Fig. 4C). The monkeys could actively move their index finger, but moved their thumb poorly during the middle stage of recovery (e.g., 24 days after lesion induction in Fig. 9, A and D). The monkey frequently touched the food morsel with the index finger twice or more in a single trial because it failed to hold it between the index finger and thumb with the first touch of the index finger. Thus a single retrieval took much longer than before the lesion. As recovery progressed, the thumb became more easily opposed to the index finger and came closer to the food morsels (61 days after lesion induction). However, the digit posture when grasping objects changed little during the recovery period. Thus in contrast to the Klüver board task, we did not observe an apparent change or shift in the grip strategy in the vertical slot task. When monkeys approached the food morsels after recovery, the index finger MP joint was more extended, resulting in a wider aperture between the index finger and thumb than before lesion induction (open arrowhead in Fig. 9A) and apparent preshaping was not observed. Moreover, in contrast to the prelesion period, the index finger touched the food morsels first followed by the thumb (time points c and c’ in Fig. 9, A, D, and E).

Active movements of the index finger were also observed in the untrained monkeys, whereas movements of the thumb were poor and difficulty in achieving opposition to the index finger remained even several months after lesion induction (Fig. 9, B and F). Moreover, dissociative movement of each finger was inadequate such that both the middle finger and the index finger were frequently inserted into the slot. The flexion force of the index finger seemed to recover completely and the monkeys frequently raked food morsels out of the slot using the index finger.

FIG. 6. Time course of changes in behavioral indexes in a trained monkey (monkey R). A and B: the percentage of successful retrievals in the Klüver board task increased almost continuously during the presleion training period (A), whereas a transient increase and subsequent decrease in the percentage of successful retrievals were observed for both the large and small wells during the postlesion recovery period (B). The dotted and solid lines indicate the data for the largest and the smallest wells, respectively. C: the transient increase and subsequent decrease in manual dexterity were evident using another criterion in the Klüver board task: average number of index finger flexions per retrieval. The number of flexions per retrieval increased during the period when the success rate decreased transiently (days 23–25). The Mann–Whitney U test was used to assess the statistical significance of the differences (**P < 0.05, ***P < 0.01). Data are expressed as means ± SD. D: daily changes in the number of food morsels retrieved during 1 min in the vertical slot task. The dotted and solid lines indicate the data for the larger (sweet potato) and smaller food morsels (raisin), respectively. In contrast, no apparent fluctuation in the test score was observed in the vertical slot task during the recovery period.
FIG. 7. A: sequence of photographs showing hand and digit movements of a trained monkey (monkey R) while performing the Klixer board task. The arrows in the top left show the locations of the carpometacarpal (CM), metacarpophalangeal (MP), and interphalangeal (IP) joints of the thumb. The joints and tips of the thumb and index finger are linked by solid and dotted lines, respectively. Rows a, b, c, and d show the moment of contact between the tip of the index finger and the aperture of the well, contact between the tip of the index finger and the bottom of the well, the greatest flexion of the index finger, and grasping of the food pellets, respectively. The closed arrowhead indicates opposition of the tips of the thumb and index finger. The open arrowhead indicates that the thumb and the index finger were not in opposition while holding the pellet. The double arrowhead indicates hyperextension of the index finger MP joint in the trained monkey. B: sequence of photographs showing hand and digit movements of an untrained monkey (monkey M) while performing the Klixer board task. C: sequential changes in the y-coordinate of the tip of the thumb, placing the origin of the coordinate axis at the long axis of the eclipse of the well, during a single trial before, and 21, 44, trained monkey, the same trials as shown in A), and 45 days after lesion induction (untrained monkey, the same trial as shown in B). Arrowheads indicate the moment of grasping the food pellets. D: population data for the y-coordinate of the tip of the thumb at the moment of grasping the food pellets. The averages across the monkeys are shown with the SD. The Mann–Whitney U test was used to assess the statistical significance of differences between prelesion measures and those 6–10 wk after lesion induction, and between the trained and untrained groups, treating each day as an independent data point (*P < 0.005, **P < 0.001).
ence the recovery of independent digit movement. The trained monkeys were forced to use the affected hand at all times because a jacket with a covered sleeve prevented use of the unaffected hand. These experimental conditions may prevent “learned nonuse,” in which monkeys learn not to use the impaired hand (Taub 1999; Taub et al. 2002; Wolf et al. 1989). Thus it is reasonable that both intensive daily training and restraint of the unaffected hand together contributed to functional recovery.

The monkeys without postlesion training became able to flex and extend every joint of both the thumb and index finger, but rarely used a precision grip after lesion induction. During the transient improvement period, both monkeys often held the food pellets between the tip of the index finger and around the proximal joint of the thumb. In the trained monkey, the percentage of precision grips increased gradually during this transient improvement and in subsequent periods. By 44 days after lesion induction, the use of the precision grip became as frequent as that during the prelesion period. By 44 days after lesion induction, the use of the precision grip became as frequent as that during the prelesion period. By 44 days after lesion induction, the use of the precision grip became as frequent as that during the prelesion period.

Comparison with previous lesion studies in nonhuman primates

Results of the untrained monkeys were similar to those in most previous lesion studies in which monkeys came to use a
whole-hand grasp, but the precision grip with finger-to-thumb opposition did not recover when damage included the hand area of the motor cortex (Passingham et al. 1983). Thus it is possible that there was no recovery of precision grip in these previous studies because no intensive postlesion training was performed. Results of the present study suggest that although dexterous digit movements depend on M1, this function can be taken over by other brain regions when intensive postlesion training is performed after M1 damage. However, the digit movements observed after recovery were not identical to those before lesion induction. In the vertical slot task, reshaping was not observed, even after recovery of the precision grip. A recent study in macaque monkeys identified M1 neurons whose firing rates peaked during hand preshaping (Gardner et al. 2007). The present results suggest that M1 plays a key role in preshaping and that this role was not taken over by remaining brain regions, even with intensive postlesion training.

Furthermore, the lesion area in the present experiment was smaller than that in most previous studies, in which the lesions were not confined to M1, but also affected neighboring cortical areas, including premotor and primary sensory areas (Kennard 1938; Ogden and Franz 1917; Passingham et al. 1983; Travis and Woolsey 1956). In the present study, lesions were largely confined to the digit area of M1, as defined by ICMS, with much smaller lesions outside M1. This may have been due in part to differences in the technique used to induce the lesion; most previous studies used artificial infarct lesions or tissue aspiration (Vilenisky and Gilman 2002), whereas we used ibotenic acid to destroy cortical neurons. By injecting small amounts of ibotenic acid into multiple cortical regions defined by stereotactic coordinates, we were able to closely control the lesion location and size. A recent study using the transneuronal transport of rabies virus indicated that corticomotoneuronal

**FIG. 9.**  A: sequence of photographs showing hand and digit movements of a trained monkey (monkey N) while performing the vertical slot task. The joints and tips of the thumb and index finger are linked by solid and dotted lines, respectively. The asterisk indicates the location of the raisin. Letters a, b, and c in the photographs indicate the moment of contact of the tips of the index finger with the aperture of the slot, the opening between the tips of the index finger and the thumb (preshaping), and the point of contact between the tip of the index finger and the food morsel, respectively. Before lesion induction, the index finger and the thumb simultaneously touched the food morsels. After lesion induction, the index finger touched the food morsels first, followed by the thumb (c'). At 24 days after lesion induction, the monkey touched the food morsel with the index finger twice in a single trial (c1 and c2) because it failed to retrieve it with the first touch of the index finger. As recovery progressed, the thumb became more easily opposed to the index finger and came closer to the food morsels (61 days after lesion induction). The arrowheads indicate that the index finger MP joint was more extended than before lesion induction.

B: sequence of photographs showing hand and digit movements of an untrained monkey (monkey Q) while performing the vertical slot task. The untrained monkey rarely retrieved raisins successfully from the vertical slot, even several months after lesion induction. In this trial, the monkey raked out the raisin with the index finger (d). C–F: sequential changes in the distance between the tips of the thumb and the index finger (solid line), and the distance between the tip of the thumb and the raisin (broken line), indicated by the arrow lines in A, bottom left. Data from a single trial in a trained monkey (monkey R) in the prelesion period (C), 24 days after lesion induction (D), and 61 days after lesion induction (E), and an untrained monkey (monkey Q) 56 days after lesion induction. Before lesion induction, the monkeys gradually opened the index finger and thumb to form an appropriate configuration for the target object to be grasped (preshaping; time point b in C). Although the precision grip recovered in the trained monkey, the index finger MP joint was more extended while approaching the food morsels, resulting in a wider aperture between the index finger and thumb than that before lesion (time point a in E), and apparent preshaping was not observed.
cells projecting to the digit muscles exist in regions of M1 from which shoulder movements are evoked with ICMS (Rathelot and Strick 2006). Thus the remaining corticomotoneuronal projections within M1 may contribute to the recovery of precision grip in the present study. In addition, ibotenic acid is an excitotoxin that destroys only neuronal cell bodies and spares axons (Schwarz et al. 1979). Thus the method of lesioning used in the present study allowed more opportunities for functional compensation by cortical areas outside the digit region of M1.

In the squirrel monkey model of M1 lesion and motor recovery, postlesion training after focal lesion in the digit area of M1 induced reorganization of the undamaged motor cortex (Nudo 2003; Nudo and Milliken 1996; Nudo et al. 1996). However, behavioral analysis in the squirrel monkey model showed that even those without training returned to normal levels of motor performance and thus failed to detect any significant effect of postlesion training on motor performance (Friel et al. 2000). In contrast to macaques, the squirrel monkey does not use a precision grip to hold small objects (Costello and Fragaszy 1988). Direct corticomotoneuronal projections from M1 to hand motoneurons, absent in the squirrel monkey, are thought to be the anatomical basis of the precision grip in the macaque (Bortoff and Strick 1993; Galea and Darian-Smith 1995; Lawrence and Hopkins 1976; Lemon 1999; Maier et al. 1997; Nakajima et al. 2000). The present results suggest that the squirrel monkey model failed to detect a significant effect of postlesion training, whereas the macaque monkey model did, because intensive postlesion training particularly influenced the recovery of the precision grip.

Changes in grip strategies during the recovery process

The success rate in the Klüver board task increased transiently and then decreased during the recovery period in the trained monkeys. This fluctuation in performance was not observed during the prelesion learning process. A previous study using a rat model of motor cortex lesions also showed a transient decrease in the task success rate during motor recovery and reported that this dip may be accompanied by loss of movement representations within the peri-infarct cortex (Kleim et al. 2003). In the present study, observations of the success rate, when combined with analysis of video images recorded during task performance, indicate that the changes in grip strategies (from alternate grips to precision grip) paralleled the decrease in task success rate (Fig. 8C). We frequently observed alternate grips (e.g., holding the food pellet between the index fingertip and around the proximal joints of the thumb) during the middle stage of the recovery period. Similar compensatory movement patterns differing from those used before lesion induction have also been reported in the squirrel monkey model of M1 lesions (Friel and Nudo 1998). At the point when grip strategies changed, the monkeys frequently failed to retrieve the pellets due to inadequate coordination between digits. Thus the precision grip was used again in the trained monkeys even at the cost of a transient decrease in the success rate. It has been reported that the precision grip is the most advantageous strategy to retrieve small objects (McDonnell et al. 2005). The changes in grip strategies seem reasonable because the success rate and retrieval speed were higher during the periods when the monkeys used the precision grip than when they used alternate grips. However, the mechanism by which grip strategies were changed and precision grip recovered, even at the cost of a transient decrease in success rate, remains to be determined. A possible explanation is that a motor prototype of the precision grip (Fagg and Arbib 1998; Rizzolatti and Luppino 2001) remained intact in some cortical areas outside M1, such as the premotor cortex, and accounted for the resumption of precision grip.

In the vertical slot task, we did not observe any apparent change or shift in the grip strategy during recovery. This may have been because the monkeys had no choice but to use a precision grip in the vertical slot task because they had to insert the tips of both the index finger and the thumb into the slot to retrieve the food morsels. Trained monkeys used the precision grip to retrieve the food morsels from the vertical slot 2–3 wk after lesion induction, an earlier time point than their success in the Klüver board task. This difference in time course may have been related to differences in task difficulty. In the vertical slot task, the objects to be grasped were larger than those in the Klüver board task and thus the monkeys could hold the larger objects between the tips of the index finger and thumb, even without highly cooperative digit movements.

Plasticity of the motor cortex underlying recovery of manual dexterity

The recovery of manual dexterity after M1 lesion required ≥1 mo of intensive postlesion training. Plastic remodeling of neural circuits in the adult brain (Dancause et al. 2005; Hallett 2001) may underlie the recovery observed in the present study. The present results also indicate that there are two stages of recovery after the M1 lesion: motor recovery of the digits without independent movement, which took a shorter period, and recovery of independent digit movement, which required intensive use of the affected hand by postlesion training and took longer. Thus both use-independent and use-dependent mechanisms may contribute to motor recovery after M1 lesion.

Several studies in squirrel monkeys and human patients have shown that the adjacent intact motor cortex can compensate for the functions of the damaged area (Cramer et al. 1997, 2006; Friel et al. 2000; Liepert et al. 1998; Nudo 2006; Nudo and Milliken 1996; Teasell et al. 2005; Traversa et al. 1997). To investigate this possibility, we reexamined the functional topography of M1 after recovery of precision grip in the monkey with the largest lesion (monkey N). However, this analysis failed to detect a digit region around the lesioned area (Fig. 2A). This analysis was only conducted in the monkey with the largest lesion and, as discussed earlier, the ICMS results did not indicate a lack of corticomotoneuronal cells to the digit muscles. Nevertheless, the lack of the ICMS-defined digit region in M1 suggests that functional compensation mainly occurs in brain regions other than M1, at least when most of the digit region is damaged, as suggested by several other studies (Biernaskie and Corbett 2001; Biernaskie et al. 2005; Dancause et al. 2005, 2006; Frost et al. 2003; Johansen-Berg et al. 2002; Liu and Rouiller 1999; Schaechter et al. 2002; Serrien et al. 2004; Vandermeeren et al. 2003). Although further studies are needed to clarify the mechanism underlying the recovery of digit movement after M1 lesion, the experimental model established here will be useful for studying the mechanisms of recovery of manual dexterity following brain damage.
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REFERENCES


