Effects of early versus late rehabilitative training on manual dexterity after corticospinal tract lesion in macaque monkeys

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1Human Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Ibaraki, Japan; 2Graduate School of Comprehensive Human Science, University of Tsukuba, Ibaraki, Japan; 3Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama, Japan; 4Precursory Research for Embryonic Science and Technology (PRESTO), Japan Science and Technology Agency, Saitama, Japan; 5Department of Developmental Physiology, National Institute for Physiological Sciences, Aichi, Japan; and 6Systems Neuroscience Section, Primate Research Institute, Kyoto University, Aichi, Japan

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Sugiyama Y, Higo N, Yoshino-Saito K, Murata Y, Nishimura Y, Oishi T, Isa T. Effects of early versus late rehabilitative training on manual dexterity after corticospinal tract lesion in macaque monkeys. J Neurophysiol 109: 2853–2865, 2013. First published March 20, 2013; doi:10.1152/jn.00814.2012.—Dexterous hand movements can be restored with motor rehabilitative training after a lesion of the lateral corticospinal tract (l-CST) in macaque monkeys. To maximize effectiveness, the optimal time to commence such rehabilitative training must be determined. We conducted behavioral analyses and compared the recovery of dexterous hand movements between monkeys in which hand motor training was initiated immediately after the l-CST lesion (early-trained monkeys) and those in which training was initiated 1 mo after the lesion (late-trained monkeys). The performance of dexterous hand movements was evaluated by food retrieval tasks. In early-trained monkeys, performance evaluated by the success rate in a vertical slit task (retrieval of a small piece of food through a narrow vertical slit) recovered to the level of intact monkeys during the first 1–2 mo after the lesion. In late-trained monkeys, the task success rate averaged 30% even after 3 mo of rehabilitative training. We also evaluated hand performance with the Klüver board task, in which monkeys retrieved small spherical food pellets from cylindrical wells. Although the success rate of the Klüver board task did not differ between early- and late-trained monkeys, kinematic movement analysis showed that there was a difference between the groups: late-trained monkeys with an improved success rate frequently used alternate movement strategies that were different from those used before the lesion. These results suggest that early rehabilitative training after a spinal cord lesion positively influences subsequent functional recovery.

functional recovery; hand movement; primate model; rehabilitation; spinal cord lesion

RECOVERY OF HAND FUNCTION is a major issue after central nervous system injuries because it is essential for the quality of human life. To maximize the functional recovery of hand movements, determining the ideal timing for the onset of rehabilitative training after the injury is crucial. Experimental studies in animals are useful in this regard because they allow investigators to test the effects of different onset schedules under defined conditions. For example, rats subjected to rehabilitative training of forelimb reach to grasp movements initiated 5 days after a brain lesion showed better improvement in skilled forelimb ability than did those in which training was initiated 2 wk or 1 mo after the lesion (Biernaskie et al. 2004). In this case, the results suggest that the brain is more sensitive to rehabilitative training in the early postlesion period. However, in other studies, delayed rehabilitative training was shown to be as effective as early training in promoting recovery of forelimb movements after spinal cord injury in rats, and the recovery of reduced cAMP signaling in the affected motor cortex was even better in those with delayed rehabilitative training (Girgis et al. 2007; Krajacic et al. 2009). Thus, time-dependent effects of rehabilitative training may vary depending on the affected regions and pathways in the central nervous system. Moreover, early training has been suggested to exacerbate brain injury (Humm et al. 1998). Therefore, the ideal timing for the onset of rehabilitative training after central nervous system injury is still debated, and further evidence is needed to establish a standard strategy for rehabilitative training in patients with such injuries.

Nonhuman primate models of central nervous system injury are important because the brain and body structure of primates are similar to those of humans (Courtine et al. 2007). In some primate species, such as humans, apes, and Old World monkeys, dexterous hand function is highly developed, and the recovery of hand movements has been investigated in many previous studies, including those of our group (Lang and Schieber 2003; Murata et al. 2008; Passingham et al. 1983; Vilensky and Gilman 2002; Wenzelburger et al. 2005). In the present study, we investigated how the timing of the initiation of postlesion training influences the recovery of dexterous hand function in macaque monkeys with a lesion of the lateral corticospinal tract (l-CST), which is involved in dexterous hand movements (Kuypers 1982; Lemon 2008). A spontaneous recovery of voluntary movement occurs after a lesion of corticospinal tracts by pyramidalotomy and spinal hemisection in macaque monkey, while a long-lasting impairment in dexterous hand movements frequently remains, such as disability in precision grip, loss of independent finger movements, and slowing of the fingergrip (Courtine et al. 2005, 2007; Galea and Darian-Smith 1997; Hepp et al. 1974; Lawrence and Kuypers 1968; Nout et al. 2012a, 2012b; Schmidlin et al. 2004, 2011; Zaaijen et al. 2012). Previously, we reported that macaques...
with a l-CST lesion at the midcervical spinal cord, in which daily training to retrieve a food morsel from a slit was initiated immediately after the lesion, regained dexterity in hand movements within 1–2 mo after the lesion (Isa et al. 2007; Nishimura and Isa 2012; Nishimura et al. 2007; Sasaki et al. 2004). Brain imaging, reversible inactivation, and gene expression analyses suggested that plastic changes in several motor-related areas in the cerebral cortex are involved in the functional compensation during recovery of dexterous hand movements (Higo et al. 2009; Nishimura et al. 2007). We hypothesized that early training may induce the plastic changes in the motor cortex, because transient increases in the expression of plasticity-related molecules have been reported in the motor cortex after a lesion in the central nervous system (Carmichael et al. 2005), and somatotopically organized movement representations (motor maps) in the motor cortex were shown to be reorganized with early but not delayed rehabilitative training (Barbay et al. 2006; Nudo et al. 1996).

As the first step toward understanding the time-dependent effects of rehabilitative training on functional compensation after a l-CST lesion, we conducted behavioral analyses and compared the recovery of dexterous hand movements between monkeys in which hand motor training was initiated immediately after the l-CST lesion and those in which training was initiated 1 mo after the lesion.

MATERIALS AND METHODS

The subjects of the present study were eight young adult rhesus monkeys (Macaca mulatta) purchased from a local provider (Table 1). The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of the National Institutes of Natural Sciences of Japan. Veterinary care was provided throughout the experiment. Adequate measures were taken to minimize animal pain or discomfort, in accordance with the Policies on the Use of Animals and Humans in Neuroscience Research revised and approved by the Society for Neuroscience in January 1995.

Monkeys were trained on dexterous hand movements before the l-CST lesion was made (Fig. 1A). Three monkeys began the same training on the day after the l-CST lesion (early-trained group, monkeys Early1–Early3; Table 1), and five monkeys started training 1 mo after the lesion (late-trained group, monkeys Late1–Late5; Table 1). The training and testing procedures of dexterous hand function were almost identical to those used in our previous study (Murata et al. 2008) (Fig. 1B). Training and testing were conducted by a worker blinded to both the experimental condition of the monkeys and the purposes of the study.

Lesion of l-CST. The l-CST lesion was made at the C4/C5 segment of the animal’s left spinal cord, as previously described (Nishimura et al. 2007; Sasaki et al. 2004). Briefly, under deep anesthesia (20 mg/kg iv pentobarbital sodium), a skin incision was made along the back of the neck, and a muscle incision was made until the cervical spine was exposed. The border between the C4 and C5 segments was exposed by laminectomy of the C3 and C4 vertebrae, and a transverse opening was made in the dura. The lesion was made under a surgical microscope as follows; first, in a small opening in the pia mater was made at the lateral convexity of the spinal cord. A horizontal strip oriented in a mediolateral direction relative to the lateral funiculus was made by inserting a minute L-shaped hook with a maximum possible insertion of 5 mm, which corresponded to the distance from the lateral convexity of the spinal cord to the midline. Second, using a pair of fine forceps, the dorsal part of the lateral funiculus was transected from the dorsal root entry zone ventrally to the level of the horizontal strip lesioned as described above. Finally, the lesion was extended ventrally to the most lateral part of the lateral funiculus by using forceps. All monkeys exhibited limb paralysis after the l-CST was lesioned.

Training and testing. Before the l-CST was lesioned, monkeys were trained on manual dexterity with a Klüver board, which contains cylindrical wells of five different sizes (10, 10.5, 11, 12, and 13 mm in diameter, 7 mm in depth; Fig. 1C). In the Klüver board task, the board was placed on the floor in front of the cage, and monkeys retrieved small spherical food pellets (3 mm in diameter) from the wells. Monkeys were trained to retrieve the food pellets from one of the five wells of different sizes for 30 min/day and 5 days/week. On the first day of training, pellets were placed only in the largest well. When the total pellets retrieved on a given day exceeded 500, the next smaller well was used on the following day. Training was completed when the number of pellets retrieved from the smallest well exceeded 500 on 2 consecutive days. The Klüver board task was also used in the test session (Fig. 1B), when the daily changes in manual dexterity were evaluated. The test session consisted of 50 trials; in each trial, a pellet was placed pseudorandomly into a well such that 10 pellets were placed in each of the 5 differently sized wells over the course of the session. Manual dexterity was also evaluated by a task in which monkeys grasped and retrieved a small piece of food through a narrow vertical slit by using both the index finger and thumb (Fig. 1D). In this vertical slit task, the food piece (7 × 7 × 7 mm in size) was positioned in the center of a slit (30 mm in height and 10 mm in width) located at shoulder height and at a sagittal distance of 15 cm from the cage. This is a similar but slightly more difficult task than that used in our previous studies (Nishimura et al. 2007; Sasaki et al. 2004), which was performed with the monkey sitting on a monkey chair. Monkeys reached their hand out between the cage bars in the present vertical slit task, and thus a higher level of coordination between the hand and arm was required. The test sessions with the Klüver board task and vertical slit task were conducted before and after the training session, respectively (Fig. 1B). Food was restricted for 14–18 h before the test/training sessions with water available ad libitum. Supplemental feedings were given if adequate feeding was not obtained from the test/training sessions. Monkeys were housed in adjoining individual

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Table 1. Characteristics of the lateral corticospinal tract-lesioned monkeys studied

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Sex</th>
<th>Initiation of Postlesion Training, days after lesion</th>
<th>Completion of Behavioral Analysis, days after lesion</th>
<th>Lesion Extent, %</th>
<th>Signal Intensity of CaMKII-α, %background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early1</td>
<td>Female</td>
<td>1</td>
<td>96</td>
<td>69.3</td>
<td>−0.54</td>
</tr>
<tr>
<td>Early2</td>
<td>Male</td>
<td>1</td>
<td>91</td>
<td>67.2</td>
<td>−2.12</td>
</tr>
<tr>
<td>Early3</td>
<td>Female</td>
<td>1</td>
<td>89</td>
<td>80.6</td>
<td>3.08</td>
</tr>
<tr>
<td>Late1</td>
<td>Male</td>
<td>29</td>
<td>121</td>
<td>72.7</td>
<td>6.72</td>
</tr>
<tr>
<td>Late2</td>
<td>Female</td>
<td>28</td>
<td>120</td>
<td>83.6</td>
<td>3.27</td>
</tr>
<tr>
<td>Late3</td>
<td>Male</td>
<td>28</td>
<td>123</td>
<td>64.7</td>
<td>4.62</td>
</tr>
<tr>
<td>Late4</td>
<td>Female</td>
<td>30</td>
<td>119</td>
<td>71.1</td>
<td>3.29</td>
</tr>
<tr>
<td>Late5</td>
<td>Male</td>
<td>30</td>
<td>119</td>
<td>77.1</td>
<td>2.79</td>
</tr>
</tbody>
</table>

CaMKII-α, α-subunit of Ca^2+ /calmodulin-dependent protein kinase II.
primate cages (600 × 750 mm in width and 900 mm in height), and four cages were placed in a room (2.1 × 4.1 m in width and 2.1 m in height), allowing social interactions. Monkeys were monitored daily by the animal care staff to check conditions of health and welfare.

There was complete paralysis of the left hand after the l-CST was lesioned in the left dorsolateral funiculus, and monkeys were carefully observed and treated, especially during the first several days after the lesion. Their deficit was limited to the movements of the limbs, and there was no obvious impairment in cognitive functions and motivation. The three monkeys in the early-trained group started postlesion training on the first day after the lesion (Fig. 1A). For several days after the lesion, the training procedure was different from that used in the prelesion period because the affected hand was paralyzed and monkeys could not perform the same training as in the prelesion period. Training was stopped immediately if the animal showed fatigue or if motivation toward the reward was low. First, we assisted the monkeys in using the affected hand to reach the well. In the next stage, when the trained monkeys could hold a large piece of food (rectangular parallelepiped of 20 × 20 × 10 mm) by themselves, they were trained to retrieve a raisin placed on a flat surface. When they could perform this task, monkeys began postlesion training on the same procedure as in prelesion training. After the completion of postlesion training on the Klüver board task, which occurred when the number of pellets retrieved from the smallest well exceeded 500 on 2 consecutive days, monkeys underwent test sessions to evaluate hand skills twice a week with both the Klüver board and vertical slit tasks.

### Table: Experimental Procedure

<table>
<thead>
<tr>
<th>Lesion of l-CST at C4/C5</th>
<th>Pre-lesion training and testing</th>
<th>confirmation of lesion extent</th>
<th>Late training Post-lesion training and testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early training</td>
<td></td>
<td>1 day</td>
<td>1 month</td>
</tr>
<tr>
<td>Late training</td>
<td></td>
<td>3 months</td>
<td>4 months after lesion</td>
</tr>
</tbody>
</table>

#### A: Experimental Design of the Present Study

- Early- and late-trained monkeys began training 1 day and 1 mo after lesion of the lateral corticospinal tract (l-CST), respectively. After 3 mo of postlesion training and testing, the extent of the lesion in the spinal cord was confirmed by histological analyses.

#### B: Experimental Procedure for Behavioral Training and Testing

- Each shaded box represents the outline of the behavioral experiment in a single day. Monkeys were trained for manual dexterity using a Klüver board task, in which the monkeys retrieved small spherical food pellets from the wells on the board. Monkeys were trained to retrieve the food pellets from one of five different-sized wells for 30 min/day and 5 days/wk. In the test session, 50 pellets were pseudorandomly placed into the wells, 10 pellets in each of the 5 wells of different sizes. The manual dexterity of monkeys was also evaluated by the vertical slit task, in which the ability of monkeys to grasp and retrieve a small piece of food through a narrow vertical slit was tested. The test sessions using the Klüver board task and vertical slit task were conducted before and after the training session, respectively.

#### C: Photograph and Schematic Drawing of the Klüver Board

- The Klüver board, with cylindrical wells of five different sizes.

#### D: Photograph of a Monkey’s Hand Retrieving a Piece of Food through the Vertical Slit

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Fig. 1. A: experimental design of the present study. Early- and late-trained monkeys began training 1 day and 1 mo after lesion of the lateral corticospinal tract (l-CST), respectively. After 3 mo of postlesion training and testing, the extent of the lesion in the spinal cord was confirmed by histological analyses. B: experimental procedure for behavioral training and testing. Each shaded box represents the outline of the behavioral experiment in a single day. Monkeys were trained for manual dexterity using a Klüver board task, in which the monkeys retrieved small spherical food pellets from the wells on the board. Monkeys were trained to retrieve the food pellets from one of five different-sized wells for 30 min/day and 5 days/wk. In the test session, 50 pellets were pseudorandomly placed into the wells, 10 pellets in each of the 5 wells of different sizes. The manual dexterity of monkeys was also evaluated by the vertical slit task, in which the ability of monkeys to grasp and retrieve a small piece of food through a narrow vertical slit was tested. The test sessions using the Klüver board task and vertical slit task were conducted before and after the training session, respectively. C: photograph and schematic drawing of the Klüver board, with cylindrical wells of five different sizes. D: photograph of a monkey’s hand retrieving a piece of food through the vertical slit.
and then perfused through the ascending aorta with 0.5 liters of ice-cold saline containing sodium heparin (1,000 units/ml) and 1–2 liters of ice-cold saline followed by ice-cold fixative consisting of 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer (pH 7.4). The volume of fixative perfused was 1 l/kg body wt. After perfusion, spinal cords were immediately removed and immersed in a postfixative solution containing 2% paraformaldehyde and 5% sucrose in phosphate buffer for several hours and then successively immersed in 10%, 20%, and 30% sucrose in phosphate buffer. Transverse frozen sections (50 μm thick) were prepared from C3–C7 spinal cord segments.

Klüver-Barrera staining was performed to visualize and evaluate the extent of the lesion in the C4/C5 segment (Fig. 2A). In all monkeys, the lesion was located around the dorsolateral funiculus where most of the corticospinal tract fibers descend (Fig. 2C). To quantify the extent of the lesion in each monkey, we measured the area of the intact part of the lateral and ventral funiculi on the lesioned side (α; Fig. 2A) and the entire area of the lateral and ventral funiculi on the intact side (β; Fig. 2A). We then calculated the lesion extent (lesion extent; Table 1) as a percentage: 100 × (1 – α × β). Thus, this value indicates the extent of the lesion in the white matter and does not take into account the lesion in the gray matter. We also performed immunofluorescence staining for the α-subunit of Ca2+/calmodulin-dependent protein kinase II (CaMKII-α), a marker of the corticospinal tract (Terashima et al. 1994), to further evaluate the loss of corticospinal axons in the segment caudal to the lesion (Fig. 2B).

After pretreatment for 3 h in 0.05 M PBS with 2% normal goat serum (NGS), 0.5% fish gelatin, 0.5% carrageenan, 0.02% Na3VO4, and 0.1% Triton X-100, sections were rinsed in 0.05 M PBS with 0.05% Triton X-100 and 2% NGS for 10 min. Sections were then incubated for 1 h with primary antibody (anti-CaMKII-α monoclonal antibody, 6G9, Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:100 in PBS containing 2% NGS. After washes with 0.05 M PBS, sections were incubated for 2 h at room temperature with Alexa Fluor 594-conjugated goat anti-mouse IgG secondary antibody (Molecular Probes, Eugene, OR) diluted 1:100 in 0.05 M PBS containing 2% NGS, 0.5% fish gelatin, 0.5% carrageenan, and 0.02%NaN3. After additional washes with 0.05 M PBS, sections were coveredslipped using Fluoromount (Diagnostic BioSystems, Pleasanton, CA). The optical density (OD) of immunofluorescence signals in the dorsolateral funiculus (ODDLF), through which the l-CST passes, was normalized by subtracting that of the background signals in the ventral part of both the lateral and ventral funiculi (OD background; Fig. 2B). To compare values in the lesioned (left) and intact (right) hemicords, we calculated the signal intensity (SI) of CaMKII-α according to the following formula:

\[
\text{SI of CaMKII-α} = \frac{(\text{OD}_{\text{DLF}} - \text{OD}_{\text{background}} \text{in the left hemicord})}{(\text{OD}_{\text{DLF}} - \text{OD}_{\text{background}} \text{in the right hemicord})} \times 100
\]

The result is 0 when the immunofluorescence signals of CaMKIIα in the left dorsolateral funiculus are completely lost and 100 when the l-CST is completely intact.

**RESULTS**

**Extent of l-CST lesions.** We used two different measurements to evaluate the extent of l-CST lesions. The lesion extent was calculated on sections stained with the Klüver-Barrera method, and the SI of CaMKII-α was calculated from sections that had undergone immunofluorescent staining for CaMKII-α (see MATERIALS AND METHODS for details). The lesion extent exceeded 60% in all of the monkeys (Table 1 and Fig. 2C), consistent with the findings of our previous study in which an anatomic tracer experiment confirmed that most of the l-CST fibers were interrupted (Nishimura et al. 2007). The SI of CaMKII-α was 6.72 even at the highest value (monkey Late1 in Table 1), indicating that immunofluorescence signals of
CaMKII-α in the left dorsolateral funiculus are almost completely lost. There were no significant differences in the lesion extent or SI of CaMKII-α between early- and late-trained monkeys (P = 0.786 for both lesion extent and SI of CaMKII-α by Mann-Whitney U-test).

Vertical slit task. Before the lesion, all eight monkeys smoothly retrieved the piece of food from the vertical slit, and the average success rates of retrieval were 97.8 ± 1.0% and 97.7 ± 2.7% for the early- and late-trained groups, respectively (Fig. 3A). They used a precision grip in most trials, and the average precision grip success rate was 95.2 ± 2.7% and 79.37 ± 41.9% for the early- and late-trained groups, respectively (Fig. 3B). Movement in the affected hand was absent immediately after the l-CST was lesioned.

Although the success rate of retrieval on the first day of postlesion training was zero in all three early-trained monkeys, the average value in early-trained monkeys increased sharply during the first month after the lesion, gradually increased during the subsequent 2 mo, and finally reached 89.1 ± 7.6% (Fig. 3A). The precision grip success rate (Fig. 3B) also increased during the postlesion training period and returned to 39.9 ± 48.0% after 3 mo of postlesion training. There were no significant differences in the success rate of retrieval after postlesion training compared with rates before the lesion (P > 0.05 by Mann-Whitney U-test). Sequences of photographs of hand and digit movements as well as digit kinematic analysis of early-trained monkeys also showed that the tip of the thumb approached the index finger smoothly during retrieval and that the food pellet was held between the pads of the index finger and thumb (Fig. 4, B and D), similar to movements observed before the lesion (Fig. 4, A and D; see Supplemental Video S1 in the Supplemental Material). Thus, task performance comparable with that observed before the lesion was recovered when postlesion training was initiated on the day after the l-CST was lesioned.

The success rate of retrieval on the first day of postlesion training, i.e., 1 mo after the l-CST was lesioned, was 0% in all late-trained monkeys except for monkey Late1, in which the rate was 26.7% on the first day. The average success rate of retrieval in late-trained monkeys increased gradually during the first 3 wk of the postlesion training period, with a slower rate of subsequent increase (Fig. 3A). The average success rate of retrieval after 3 mo of postlesion training was 34.4 ± 34.0%, which was significantly lower than that of early-trained monkeys (P < 0.001 by Mann-Whitney U-test). In these late-trained monkeys, a precision grip was not observed even after the completion of postlesion training; the final average precision grip success rate was 0% (Fig. 3B). Both retrieval and precision grip success rates after postlesion training were significantly lower than those before the lesion (P < 0.005 by Mann-Whitney U-test). Sequences of photographs and digit kinematic analysis showed that the animals could approach the food pellet with their index fingers but could not then bring the thumb close to the index finger (Fig. 4, C and D; see Supplementary Video S1). In trials with successful retrieval, monkeys frequently retrieved the food pellet by raking it out of the slit by using the index finger and then holding it between this finger and the proximal joint of the thumb. Thus, deficits in thumb movement or in coordinated movement between the
index finger and thumb, or both, remained in late-trained monkeys.

Scattergram analyses indicated that there were individual differences in both retrieval and precision grip success rates after postlesion training regardless of the lesion extent among the three early-trained monkeys (Fig. 3, C and D). Individual differences were also observed among the five late-trained monkeys. At present, it is not clear why such individual differences exist. Nevertheless, both retrieval and precision grip success rates after postlesion training in all of the early-trained monkeys were higher than those in all of the late-trained monkeys. The results of two early-trained monkeys, monkeys Early1 and Early2, and those of three late-trained monkeys, monkeys Late1, Late2, and Late4, were similar in that they frequently retrieved the food piece without using the precision grip. However, kinematic analysis showed that there was a difference between them; the distance between the tips of the thumb and index finger at the moment of contact of the index finger tip with the food morsel (see Fig. 4) in two early-trained monkeys (21.9 ± 4.6 mm) was significantly shorter than that in three late-trained monkeys (40.3 ± 4.5 mm, P < 0.0001 by Mann-Whitney U-test), indicating that the digit movements in two early-trained monkeys after postlesion training were more similar to those observed before lesion. The average number of postlesion training and test days was larger in late-trained monkeys (58.8 ± 13.5 days) than in early-trained monkeys (47.0 ± 2.6 days), although the difference was not significant (P > 0.05). Therefore, the present results indicate that the hand performance of late-trained monkeys showed less recovery than that of early-trained monkeys, even

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**Fig. 3.** A: temporal changes in success rates of retrieval in the vertical slit task. The average values of early-trained monkeys (n = 3) and late-trained monkeys (n = 5) are shown with SDs. The day of postlesion training in early-trained monkeys corresponds to the day after lesion, whereas there was a gap of 1 mo in late-trained monkeys (Table 1). Thus, the day of postlesion training in late-trained monkeys is indicated in the top horizontal bar. All monkeys smoothly retrieved the piece of food from the vertical slit before lesion, while impairment of hand function was observed immediately after l-CST lesion. The average success rate of retrieval in early-trained monkeys increased gradually after the lesion and reached the prelesion level during the postlesion training period. The average success rate of retrieval in late-trained monkeys after 3 mo of postlesion training was significantly lower than that in early-trained monkeys (*P < 0.001 by Mann-Whitney U-test). B: temporal changes in the success rates of precision grip in the vertical slit task. A certain degree of recovery of precision grip was observed in early-trained monkeys, whereas almost no recovery was observed in late-trained monkeys. C and D: correlations between the lesion extent and average success rate of retrieval (C) and precision grip (D) for the last 3 days of the postlesion training period. Both values in all of the early-trained monkeys were higher than those in the late-trained monkeys.
though the former had received a longer period of training and 1 more month had passed since the lesion in the former than in the latter.

Klüver board task. In addition to the vertical slit task, we also evaluated the hand performance of monkeys with the Klüver board task, which has been used in several previous studies for testing manual dexterity (Lawrence and Hopkins 1976; Lawrence and Kuyper 1968; Nudo et al. 1996). Although wrist extension may be affected in late-trained monkeys during the vertical slit task (Fig. 4C), the influence of deficits in wrist extension should be small in the Klüver board task because the monkeys approach the board with their hand directed downward to the well on the board.

Similar to their performance on the vertical slit task, all monkeys smoothly retrieved the food pellets from the board before the l-CST was lesioned; the average success rates of retrieval were 95.6 ± 2.7% and 96.8 ± 1.5%, and the average precision grip success rate was 94.4 ± 2.0% and 96.5 ± 1.6% for the early- and late-trained groups, respectively. After the l-CST was lesioned, both success rates decreased markedly because of hand paralysis. The average success rate of retrieval for early-trained monkeys gradually rose during the postlesion training period and finally returned to 85.9 ± 9.9%. The precision grip success rate also gradually increased to 73.7 ± 24.6% (Fig. 5B).

The success rate of retrieval on the first day of postlesion training was 0% in all late-trained monkeys except for monkey Late1, in which the rate was 78.0% on the first day. Both their retrieval and precision grip success rates also gradually increased during the postlesion training period, reaching 75.7 ± 30.8% and 68.5 ± 41.2%, respectively, at the end of postlesion training (Fig. 5, A and B). Unlike the results in the vertical slit task, the average success rates of both retrieval and precision grip on the Klüver board task for the last 3 days did not differ significantly between the early- and late-trained groups (P > 0.05 by Mann-Whitney U-test). Scattergrams showing data for individual monkeys indicated that all animals in the early- and late-trained groups, with the exception of one late-trained monkey (monkey Late5), showed substantial levels of recovery in both retrieval and precision grip (Fig. 5, C and D).

Although the behavioral scores did not differ between early- and late-trained monkeys, kinematic analysis showed that there was a difference between the groups. Before the l-CST was lesioned, monkeys first inserted the index finger into the well and then smoothly brought the tip of the thumb close to the aperture of the well in coordination with index finger flexion and finally held the food pellet between the tips of the thumb and index finger (Fig. 6A; see Supplementary Video S2), as observed in our previous study (Murata et al. 2008). The hand movements in early-trained monkeys after postlesion training
were similar to those observed before the lesion (Fig. 6 B). In contrast, the hand movements in late-trained monkeys were different from those observed before the lesion (Fig. 6, C and D). Monkey Late1 did not bring the tip of the thumb close to the aperture of the well, presumably because of a deficit in coordinated movements between the index finger and thumb (Fig. 6 C). Instead, the monkey flicked the food piece with the index finger toward the thumb pad while retrieving the food pellet; consequently, the thumb was placed farther from the aperture of the well when the monkey held the food pellet.

To evaluate the differences in digit kinematics during precision grip performance in the Klüver board task among the groups, another measurement was carried out in trials with a successful precision grip. The distance between the tip of the thumb and the nearest aperture of the well during grasping (thumb distance) was evaluated in each monkey before the l-CST was lesioned and after postlesion training (the distance between the double arrowhead and the arrowhead in Fig. 6, right; see MATERIALS AND METHODS for details). The measurements were not carried out in one late-trained monkey (monkey Late5) because a precision grip was not observed in this animal even on the last day of behavioral analysis (Fig. 5 D). The increase in distance after postlesion training was substantial in late-trained monkeys, and this was also observed in an early-trained monkey, monkey Early2 (Fig. 7 A). The average thumb distance in early-trained monkeys was slightly, but not significantly, larger after postlesion training than before the lesion (Fig. 7 A). In contrast, the average thumb distance of late-trained monkeys after postlesion training was significantly greater than that in prelesion monkeys (P < 0.05 by Mann-
Whitney U-test; Fig. 7A). Therefore, late-trained monkeys tended to use a strategy to perform a precision grip different from that used before the lesion, i.e., flicking the piece of food with the index finger toward the thumb pad during retrieval to compensate for the lack of coordinated movements between the index finger and thumb.

An exception in late-trained monkeys was monkey Late2, in which the thumb distance after postlesion training was almost the same as that before the lesion (Fig. 7A). The sequence of photographs shows that monkey Late2 slowly put its palm down and pressed the thumb against the board (Fig. 6D, middle right) and then flexed its thumb and index finger synchronously during grasping (Fig. 6D, right), presumably to compensate for the lack of independent movement between the thumb and index finger. Thus, monkey Late2 was thought to use a retrieval strategy different from that of other late-trained monkeys, bringing the thumb close to the aperture of the well at the cost of taking more time to accomplish the task. The results of a population analysis of the contact time (the time interval between insertion of the index finger into the well and contact of the thumb with the food pellet) indicated that this value in monkeys Late2 and Late4 was higher after postlesion training than before the l-CST was lesioned (Fig. 7B).

The scattergram shown in Fig. 7C demonstrates the results of kinematic analyses showing the thumb distance and contact time along each axis. This graph indicates that at least one of the two measures after postlesion training in late-trained monkeys was substantially higher than before the lesion. In early-trained monkeys, neither measure after postlesion training was substantially different from its value before the lesion. An exception was that the thumb distance of monkey Early2 increased after the lesion, although the value was still lower than in three late-trained monkeys, monkeys Late1, Late3, and Late4. Therefore, although the success rate of the precision grip recovered even in late-trained monkeys, except for monkey Late5 (Fig. 5D), the monkeys frequently used alternative strategies to achieve a precision grip.

DISCUSSION

Effects of early rehabilitative training after spinal cord injury. In the present study, we showed that postlesion training after a l-CST lesion was more effective in restoring dexterous
EFFECTS OF EARLY REHABILITATIVE TRAINING ON MANUAL DEXTERITY

Contact time (s)

and the nearest aperture of the well during grasping (thumb distance; Fig. 7.

2862

2.862

EFFECTS OF EARLY REHABILITATIVE TRAINING ON MANUAL DEXTERITY

Contact time (s)

2.862

Great effects of early rehabilitative training on manual dexterity

2.862

on the day after the lesion that after 1 mo after the lesion. The significant difference in the success rate of both retrieval and precision grip was observed between early- and late-trained monkeys in the vertical slit task but not in the Klüver board task, indicating that the success rate of the vertical slit task is more sensitive than that of the Klüver board task to detect a difference between early versus late training. Kinematic analysis in the Klüver board task also showed a difference among the groups: late-trained monkeys with the improved success rate frequently used alternate movement strategies that were different from those used before the lesion. Compensatory movement patterns that differ from those used before lesion induction have also been reported in other studies that have used monkey motor cortex lesion models (Friel and Nudo 1998; Murata et al. 2008). Interestingly, at least two different compensatory movements were used by each of the late-trained monkeys in the present study: flicking the food piece with the index finger toward the thumb pad and pressing the thumb against the board. Although these compensatory movements were likely established by trial and error, they could also be induced by a rehabilitative intervention such as assisting digit movements. There was an interindividual variability of lesion extent among the three early-trained monkeys and among the five late-trained monkeys (Fig. 2C and Table 1), and the recovery of dexterous hand movements in all of the early-trained monkeys was consistently better than those in the late-trained monkeys.

The results in early-trained monkeys were consistent with our previous studies, in which daily training to retrieve a food morsel from a slit was initiated immediately after the l-CST lesion and the precision grip recovered within 1–2 mo after the lesion (Nishimura et al. 2007; Sasaki et al. 2004). However, some of the early-trained monkeys in the present study showed lower success rates after postlesion training than those in our previous studies, possibly because the present study used a more difficult task than that used in the previous studies, as described in MATERIALS AND METHODS. A case study of four spinal cord injury patients suggested that rehabilitative training is more effective in recovering motor performance when applied within a few days after the lesion (Winchester et al. 2005). Other clinical studies have shown that the recovery of motor performance was comparable between spinal cord injury patients with early and late rehabilitative training, although the former group showed higher scores in daily living skills than the latter (Scivoletto et al. 2005; Sumida et al. 2001). The present study, which tested the effects of varying the timing of rehabilitative training onset under defined conditions, supports the findings of these human patient studies and provides an experimental model for studying the neural basis of functional recovery induced by early rehabilitative training.

In the present study, monkeys were forced to use the affected hand at all times during the postlesion training period by using a jacket with a dead-end sleeve covering the unaffected hand. The experimental conditions resembled the methods used in constraint-induced movement therapy (CI therapy), which has been widely adopted in the rehabilitation of stroke patients (Taub et al. 2002; Wolf et al. 2005). The results of the present study suggest that CI therapy immediately after a spinal cord lesion is beneficial for promoting recovery of hand movement. Upregulation of plasticity-related genes may be associated with CI therapy-induced functional recovery, as shown in

Fig. 7. A and B: population data for the distance between the tip of the thumb and the nearest aperture of the well during grasping (thumb distance; A) and the time interval between the insertion of the index finger into the well and contact between the thumb and the food pellet (contact time; B) during precision gripping in the Klüver board task in the last 3 days of prelesion and postlesion periods. The analysis was not carried out in one late-trained monkey, monkey Late2, because this animal did not show precision grip even on the last day of behavioral analysis. The dotted line and shaded region show the average and SD of the value before lesion. In early-trained monkeys, the average thumb distance was slightly, but not significantly, larger after postlesion training than before the lesion. In contrast, the average thumb distance of late-trained monkeys after postlesion training was significantly greater than those in prelesion monkeys (P < 0.05). An exception in late-trained monkeys was monkey Late2, whose thumb distance was almost the same as that before the lesion. In contrast, the contact time in monkey Late2 was greater after postlesion training than before l-CST lesion (B). C: scattergram showing the thumb distance and contact time on each axis. The open circle and shaded region show the average and SD of the value before lesion, respectively. At least one of the values was substantially different from those before lesion in late-trained monkeys.
a rat model of corticospinal tract lesions (Maier et al. 2008). In late-trained monkeys, in contrast, we intended to prevent the active use of the affected hand for feeding and moving, which may work as spontaneous training, by wearing a jacket with a dead-end sleeve covering the affected hand during the first month after l-CST lesion. This experimental protocol, in addition to the difference in the timing to initiate the postlesion training sessions, may have accentuated the difference between the early- and late-trained groups.

Possible mechanisms underlying functional recovery induced by early rehabilitative training. The existence of a critical period for rehabilitation has also been confirmed in a rat brain lesion model: rats subjected to rehabilitative training with forelimb reach to grasp movements initiated 5 days after a brain lesion showed better improvement in skilled forelimb ability than did those in which the training was initiated 2 wk or 1 mo after the lesion (Biernaskie et al. 2004). The present results are also consistent with a previous brain lesion study that used squirrel monkeys, in which somatotopically organized movement representations (motor maps) in the motor cortex were shown to be reorganized with early but not delayed rehabilitative training; however, the study did not show a difference in recovery of hand motor skills between early- and late-trained groups (Barbay et al. 2006). Our previous studies in macaque monkeys, in which postlesion training was initiated on the day after the l-CST lesion, also demonstrated that plastic changes in several motor-related cortical areas were associated with functional compensation during recovery of dexterous hand movements (Higo et al. 2009; Nishimura et al. 2007). Anatomic changes are also known to occur in the unlesioned corticospinal projections, including those descending ipsilaterally and decussate across the midline in sublesional segments of the spinal cord (Rosenzweig et al. 2010). A recent study in a rat model of spinal cord injury showed that remodeling of the spared corticospinal tract is associated with the restoration of voluntary locomotion by rehabilitative training with a robotic postural interface (van den Brand et al. 2012). Other spared descending motor tracts, such as the rubrospinal and reticulospinal tracts, may also be involved in the recovery of hand movements after corticospinal tract lesion (Belhaj-Saif and Cheney 2000; Zaaimi et al. 2012). However, previous studies have shown that these brain stem pathways cannot fully compensate for the precision grip (Lawrence and Kuypers 1968).

Instead, our recent studies showed that precision grip did not recover after a similar lesion made at the C2 segment (Alstermark et al. 2011 and unpublished observations), which suggest that propriospinal neurons at the C3–C4 segments are involved in the recovery of precision grip. Furthermore, we have recently shown that these propriospinal neurons are involved in the control of precision grip in intact monkeys (Kinoshita et al. 2012). Early rehabilitative training after l-CST lesion may induce plastic changes in both motor cortical areas and spared descending motor axons in an activity-dependent manner.

However, a contradictory result has been reported in a rat model of spinal cord injury, in which the effectiveness of delayed rehabilitative training initiated 12 days after a lesion of the dorsolateral corticospinal tract was shown to be comparable with early training initiated 4 days after the lesion in promoting motor recovery (Girgis et al. 2007; Krajacic et al. 2009). In case the l-CST lesion was incomplete and considerable proportions of l-CST fibers remained intact in the macaque monkey, the recovery of hand motor skills with late training was comparable with that with early training (our unpublished observations). Therefore, the apparently inconsistent results may be ascribed to differences in lesion size or location, as the previous study in the rat did not examine the effect of lesion size. The inconsistency may also be due to differences in the duration of the delay: the rehabilitative training was only delayed by 8 days (4 vs. 12 days) in the rat study, whereas it was delayed by 1 mo in the present study. Moreover, we note that a direct comparison of studies in rodent and primate spinal cord lesion models is difficult because the degree of contribution of the corticospinal tract to motor behavior has been suggested to differ between rodents and primates (Courtine et al. 2007; Kuypers 1982; Lemon 2008).

Transient increases in the expression of plasticity-related molecules have been reported after a lesion in the central nervous system (Bareyre and Schwab 2003; Carmichael et al. 2005). These molecules have been suggested to constitute the underlying molecular basis of the functional recovery. Previously, we reported that expression levels of growth-associated protein 43 (GAP-43) and other plasticity-related molecules increase in motor-related cortical areas of monkeys during the recovery period after a l-CST lesion (Higo et al. 2009; Sato et al. 2009). The monkeys in these previous studies underwent postlesion rehabilitative training immediately after the lesion. Therefore, these molecules may be involved in the functional recovery induced by early rehabilitative training. However, whether the increased expression was caused by the lesion itself or induced by postlesion training remains unclear. Moreover, molecules induced only when postlesion training is performed during a particular period after the lesion could underlie the critical period of rehabilitation. Further comparisons of gene expression between early- and late-trained animals are needed to detect molecules induced by early rehabilitative training. The present study provides an experimental model in which to study such molecules.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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