Behavioral Findings during Recovery after Experimental Stroke in Monkeys —Assessment with Modified Hand Performance Test—

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Abstract. It is still unclear how rehabilitative intervention assists recovery from strokes. In order to address this question, we made an animal model to examine the recovery process after experimental strokes. The forelimb movements in 2 monkeys retrieving food from a well were analyzed with a videotape recording. A small infarction by coagulation of blood vessels was made in the forelimb areas after mapping of the primary motor areas. The recovery process was evaluated by measuring the time required for taking food. We used the modified Klüver board with the small diameter in order to restrict the monkeys' compensatory movements such as pronation or supination. After the infarction, the monkeys could not use their impaired hands for the first 2 days. Failure to retrieve food and compensatory movements such as involvement of other joints were often observed during the first week. However, the forelimb function of both monkeys showed no significant impairments on the 6th week, compared to the results before the infarction. Throughout the experiments, the monkeys showed no supination or pronation of the forearm. These results suggest that this animal model with the modified Klüver board may be useful for the evaluation of motor deficit.

Key words: Animal model, Cerebral infarction, Recovery process

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INTRODUCTION

Although various rehabilitative therapeutic exercises are reported to improve the function of patients after strokes from various aspects^{1–3)}, how they assist recovery from dysfunction has not been elucidated. It has been reported that rehabilitative therapeutic exercises have a certain effect on the reorganization of the nervous system of the cerebral cortex and cerebellum^{4, 5)}. Nudo et al.^{6, 7)} showed the effects of exercises on somatotopic reorganization by means of microstimulation in the

primary motor cortex of the monkeys that had received experimental procedures of cerebral infarction. Xerri et al.⁸⁾ reported the changes of somatotopy in the areas of 3a and 3b after the experimental infarction in squirrel monkeys. Both studies showed changes in the areas surrounding the infarction that compensated for the function of the areas with infarction. These studies indicate that rehabilitative therapeutic exercises are involved in the recovery of motor function in stroke patients, suggesting that the regions surrounding the infarction may compensate for the impaired

function of the infarcted areas.

Barbay et al. indicated that delayed training resulted in an increase of co-contractive movements evoked with microstimulation⁹⁾. This suggests motor skill training in the chronic stages following stroke may modulate peri-infarct cortical plasticity in a different way. They also observed increase of multi-joint movements in both wrist, forearm and other proximal areas of the upper extremities evoked with microstimulation. This indicates that the reorganization of the brain is different from that induced by the training started earlier. Moreover, these multi-joint responses in the forearms⁹⁾ showed a negative correlation with the behavioral improvement in clinical evaluation¹⁰⁾.

Friel et al. examined precise abnormal multi-joint movements during behavioral recovery after experimental cortical injury in non-human primates¹¹⁾. They reported various kinds of compensatory movement patterns such as pronation and supination of the forearm, in addition to flexion and extension of the wrist and digits to take food pellets. Although precise observation of behavioral recovery may be important for future studies, it may be difficult to evaluate the results quantitatively, if the animals show various kinds of compensatory movement patterns. Moreover, compensatory movements as abnormal patterns in acute hemiparesis should be suppressed for recovery, according to Bobath's methods¹²⁾.

The purpose of the present study is to evaluate the effects of therapeutic exercises on the recovery of forelimbs after the experimental lesion in the primary motor cortex, and to investigate whether the compensatory movements such as pronation or supination can be suppressed using the modified Klüver board with small diameters and different depths. We also attempted to compare the recovery process of two monkeys that received experimental lesions in either the digit or shoulder area.

MATERIALS AND METHODS

General procedures

Two adult male monkeys (Monkey A: Rhesus Monkey 6.4 kg, Monkey B: Japanese Monkey 7.1 kg) were used in this study. All experiments were performed in strict compliance with the Guide for the Care and Use of Laboratory Animals (NRC 1997). The procedures described below were evaluated and approved by the Animal Care and

Use Committee of Hokkaido University School of Medicine. All procedures except for surgery were performed in the cages where the monkeys were reared. After examining the hand preference, control data before experimental strokes were taken. Then, under anesthesia, electrophysiological lesions were made in the areas of forelimbs in the primary motor cortex after mapping. After surgery, the monkeys started therapeutic exercises and continued them every day. Except for during the Hand Performance Test, both Monkeys were allowed free access to water and food, without any restriction.

Hand preference testing

To determine the hand preference, we placed a food pellet on a flat table in front of each monkey's cage. Monkeys could easily reach the food through the window of the cage. We determined hand preference according to which hand was used more often. After determining the dominant hand, a board with wells was placed at the left side of the cage in front of the monkeys, so that the monkeys could use only their dominant hands.

Pre-infarct training procedure

To obtain the data before and after surgery, and the training of digits, we performed the Hand Performance Test using a modified Klüver board¹³⁾ (Fig. 1). The modified Klüver board had 5 wells with the same diameters (30 mm) but with different depths (10, 20, 30, 40 and 50 mm), based on the width and length of the monkeys' hands. The reason why we used the wells with the same small diameters is that monkeys are unable to pronate or supinate their forearms in the wells. The food for the test in the present study was a piece of apple cut to 1 cubic cm. To retrieve food, the monkeys had to stretch their dominant forelimbs from a 10 × 10 cm window located at the left side of the cage in front of them. At first, food was placed on a flat table. Then they were trained to take it from the shallowest well of 10 mm depth. After they learned to take food from it consistently, they took it from the well with the depth of 20, 30, 40 and 50 mm, one after another.

The baseline of the time required for retrieval was determined by the averaged time for taking it from each well for 3 days before the surgery. In 8 of the 63 sessions, recordings were made with a videotape system (Digital Video Cassette Recorder, DCR-

Fig. 1. Hand performance test. A. Plexiglas board (modified Klüver board) containing five wells was mounted on the left side in front of the animal's cage. The depth of each well was 10 mm (a), 20 mm (b), 30 mm (c), 40 mm (d), and 50 mm (e). The diameter of each well was 30 mm. B. A single small piece of food was placed in a well, from which the monkeys retrieved it.

TRV900 Sony) with a sampling rate of 30 frames/sec to examine the joint movements of the forelimbs. In the other sessions, an experimenter measured the time required for retrieving food, using a stopwatch. Although the usual training sessions consisted of 30 minutes, the training was discontinued when the monkeys did not take food for more than 5 minutes.

Electrophysiological mapping and the lesion for experimental stroke

Before making experimental lesions, we applied intracortical electrical stimulation to determine the hand area of the primary motor cortex. After we confirmed that both monkeys were able to retrieve food from all of the wells, the monkeys were sedated by ketamine (6–7 mg/kg). They were anesthetized by halothane/nitrous oxide and an intraperitoneal injection of pentobarbital sodium (2.0 mg/kg). Under aseptic conditions, from a small opening, a tungsten electrode with an impedance of 0.8–1.0 M Ω was inserted into the monkeys' hand areas of the right primary motor cortex (M1; A12 L18). At each site, current was delivered through the electrode whose tip was positioned 1,500–1,900 μ m below the cortical surface. The strength of the current was set 50 μ A (0.2 ms, 300 Hz, 11 cathodal pulse), at first. However, because of the effects of anesthesia, we increased the current to 200 μ A until we could get responses. We determined the stimulated point as “no response”, when we could not get any responses despite applying more than 200 μ A.

Acute ischemic lesions were made by a microcoagulator to pinch the blood vessels at the digit areas in M1 in Monkey B, and the shoulder area in Monkey A.

Assessment of motor performance on the Klüver board

Motor performances were analyzed with the video recording, to examine the following points: a) how long it took to retrieve food (the beginning was determined as the time when a part of the hand entered the well, and the end was the time for leaving the whole part of the hand from the well); b) how many times each joint (e.g. digit, wrist, elbow and shoulder) was moving to take food; c) failure, defined as when the monkeys could not take food despite putting their hands into the well. The failure percentage was calculated as the number of failures divided by the number of the trials to take food ($\times 100$).

Statistical analysis

The required time for each well was compared with one-way and two-way ANOVA. Fisher's PLSD was also used for testing the significance level ($p < 0.05$).

RESULTS

Hand performance in normal monkeys

The movement pattern of successfully taking food was relatively constant. During the hand preference test, Monkey A used only the left hand.

Fig. 2. The time required for retrieving the food in the Hand Performance Test with the dominant hand before experimental lesion. A. Monkey A showed significant differences for both depths and days (#). B. Monkey B showed significant differences for both depths and days (#).

Although Monkey B took food by his either left or right hand at first, he used only his left hand, after he repeated practice. From these findings, we determined that both monkeys were left-handed.

Figure 2 shows the average time required for retrieving food in hand performance tests before surgery. The average ($\pm$ standard deviation) time for the 2 monkeys was 0.67 ± 0.27 sec for the well of 10 mm depth, 1.04 ± 0.95 sec for 20 mm, 1.05 ± 0.65 sec for 30 mm, 1.25 ± 0.74 sec for 40 mm, and 2.16 ± 1.68 sec for 50 mm. Monkey A showed a significant difference for days ($F_{2, 101}=13.9$, $p<0.001$) and depth ($F_{4, 104}=22.6$, $p<0.001$).

Monkey B needed a significantly shorter time to retrieve food than Monkey A at the beginning ($F_{1, 153}=18.5$, $p<0.001$). He also showed a significant difference in the results between the 1st day and the other days ($F_{2, 68}=1.87$, $p<0.05$), although the significance level was relatively low. He showed a significant difference in the performance at different depths ($F_{4, 66}=6.33$, $p<0.001$).

Although both monkeys showed various patterns of movements at the beginning of the training, their movement pattern became rather uniform through practice. When they took food from the shallow well, they pinched the food between the thumb and digit finger. On the other hand, for deeper wells, they inserted 3 fingers (index, middle and ring) into the well at the same time to scrape the food away. They used only digit joints and rarely moved the wrist or elbow joints. After they successfully took out food, they grasped it tightly and withdrew their hands in the cage to eat it.

Mapping & cortical infarct

As the stimulation was applied medially to laterally in the hand areas of the right M1, the muscle contraction was observed in the order of left shoulder, left hand, and digits. The distal forelimb representation (digit, wrist/forearm) was bounded laterally. Movements of proximal areas (elbow and shoulder) are represented medially. Wrist movements such as pronation and supination of the left hand were elicited. From medial to lateral hand areas, flexion and extension of little, ring, middle and index fingers were observed as shown in Fig. 3. The stimulation evoked digit movements that combined several muscles, consistent with previous reports^{14, 15}. Experimental ischemic lesions were made in the shoulder area for Monkey A and the digit area for Monkey B as shown in Fig. 3.

Hand performance after experimental infarct

After surgery, the monkeys tended to use their unaffected right hands to retrieve food from the wells for a few days after the experimental ischemic lesions. On the 2nd day after surgery, the monkeys were hardly able to retrieve food from the wells as before.

Monkey A showed impairments in all the joints of the contralateral upper limbs including the shoulder. From the third day after the lesion, he started to use the affected left hand. However, he was so clumsy in holding food with his left hand that he could not successfully take it to his mouth. He sometimes showed flexion of the elbow joint and even lateral flexion of the trunk to compensate for the clumsy movements of his forelimbs. Monkey A could retrieve food with his left hand on

Fig. 3. The cortical map illustrating the sites of electrode penetration in the right M1 in the 2 monkeys. After stimulation, lesions were made in the 2 monkeys.

the 7th day after the lesion, but a longer time was needed as shown in Fig. 4A ($F_{2, 253}=32.2$, $p<0.001$). There were also significant differences among the depth of the wells ($F_{4, 251}=28.5$, $p<0.001$). However, in the test sessions from the 2nd to the 6th week, he took food only with his intact right hand and did not try to use his impaired left hand. So, we were unable to obtain data during this period. Finally, on the 42nd day after the lesion, he could take food as quickly as he had done before surgery, there being no significant difference between the results of pre-surgery and those of the 6th week ($p=0.63$). Supination or pronation of the forearm was not observed in any of the trials.

Monkey B did not show impairment in the elbow or shoulder joints after surgery, but he did show it in the wrist and digit joints. The time required for Monkey B to retrieve food was significantly increased after the lesion ($F_{4, 437}=15.0$, $p<0.001$), but decreased as the training was repeated. In the 2nd week, there was no significant difference in his results compared to that before surgery, as shown in Fig. 4B ($p=0.25$). He showed a significant difference for the depth of the wells ($F_{4, 437}=10.7$, $p<0.001$).

Monkey B also frequently failed to hold a piece of food in the test sessions. The percentage of failure in which Monkey B could not retrieve food at his first attempt was 50.5%. On failing to hold the food, he picked it up again. The reason for the failure was lack of complete flexion and extension of the digit joints. On the 3rd day after the lesion, Monkey B moved the elbow and shoulder joints more often than before. He used flexion and

extension of the elbow and abduction of the shoulder joints and even lateral flexion of the trunk during the 1st week. However, supination or pronation of the forearm was not observed in any of the trials. Monkey B showed many failures in the first and second weeks, but from the 3rd week, he succeeded in all of the trials.

Monkey B needed 14 days to reach the same level of competence as before lesion. Because Monkey A was not using his impaired hand from the 2nd to the 6th week, we were unable to determine the accurate date of his recovery. However, he showed no significant impairment in the test sessions of the 6th week.

Movement pattern analysis

Figure 5 shows how many times each joint flexed or extended per retrieval trial in Monkey B. There were significant differences among prelesion, the 1st week and the 1st month ($F_{2, 324}=38.9$, $p<0.001$). However, the results at the 1st month did not significantly differ from those before the surgery ($p=0.69$). This indicates the number of the joint movements per retrieval increased after the lesion, but decreased as the training progressed and reached the pre-lesion level by the 1st month. Depth also showed significant differences in the results ($F_{4, 322}=12.0$, $p<0.001$), but the results of the 50-mm well did not improve significantly ($p=0.28$). There was a significant difference for joints ($F_{2, 324}=26.3$, $p<0.001$). However, the wrist joint did not show a significant recovery ($p=0.73$).

DISCUSSION

Although the number of monkeys was small, the present study not only confirmed that monkeys recovered with therapeutic exercises after the experimental stroke, but also showed the usefulness of the Modified Hand Performance Test with modified Klüver Board, because the compensatory movements were controlled.

The usefulness of the modified Klüver board in the present study

The test with the modified Klüver board showed less variability among the performances with the wells, compared to the results reported in previous studies^{6, 7)}. The conventional Klüver board was used in the previous reports^{6, 7, 13)}, and allowed the animals to use pronation and supination of the

Fig. 4. The time required for taking food with the dominant hand in the Hand Performance Test for the post-lesion monkeys. A. Monkey A showed a significant difference for both days (#) and depths (*). During the period from the 1st to the 6th week, there was no data, because he did not use his impaired hand. On the 6th week, he took food with his impaired dominant hand and the results were as fast as those of pre-lesion. The results in the wells with 50 mm depth showed a significant difference compared to the others (*). B. Monkey B showed significant differences for both days (#) and depths (*). When he succeeded in retrieving the food, the retrieval time was prolonged after the lesion but it recovered in the 2nd week. The results in the well with 50 mm depth showed a significant difference compared to the others (*).

Fig. 5. The number of joint movements to retrieve food per trial with his dominant hand for Monkey B. There was a significant difference for days, depth and joints. The movement of shoulder and elbow significantly increased after surgery for each well but recovered after 1 month (#). No significant difference was observed in wrist movements. The number of finger movements increased for each well after operation but recovered at 1 month.

forearm movements, because the sizes of the wells were 9.5, 13.5, 19.5 and 25 mm which are relatively large for squirrel monkeys. Thus, complex movements of flexion and extension including supination and pronation of the forearm may have taken place. On the contrary, in the present study, the two monkeys showed consistent patterns of successful retrieval. This enabled us to evaluate the recovery by means of observing the numbers of flexion and extension of the joints, suggesting that

the Modified Hand Performance Test of this study may be more useful for examining the hand movements involved in taking something out of the wells than the conventional one^{6, 7, 13}. It is possible that the monkeys might have wanted to take food with these compensatory movements, but the small size of wells with respect to the Monkeys' hands might inhibited them to do that. We cannot exclude the possibility that the motor commands for the compensatory movements were sent to their

extremities but the size of the wells were too small to allow them.

One limitation of this study is that we did not perform three-dimensional recordings. Crista and Levin reported the compensatory trunk movements of stroke patients with 3-D recording¹⁶. We cannot exclude the possibility that the monkeys showed subtle compensatory movements. However, as far as we observed with VTR, there was no supination or pronation. It is also possible that the repeated training of food retrieval controlled their performances.

In the present study, the time required for the monkeys to take food from the deepest well was significantly longer than that of the other wells. It is well known that monkeys are not good at pinching things with thumb and index fingers, because of their poor abilities to oppose the thumb¹⁷. This is the reason why they scraped the food out with their index, middle and ring fingers from the deep wells.

The results of the present study also showed improvements of performances before the lesion in both monkeys A and B (Fig. 2B). Similar results were reported in the previous study¹⁸. This may be due to learning effects, suggesting that cortical reorganization may occur even in intact animals.

Recovery of retrieving movements after cortical infarcts and rehabilitation training

Although the lesion in Monkey A was located in the shoulder area, the impairment extended over the left arm and digits. A lesion made by a coagulator may cause the occlusion of the blood stream of the areas of the whole arm. Both monkeys A and B showed paresis of the contralateral forelimbs, although the severity was different. Monkey A may have had severer impairments, because of his extended lesions. He needed a longer time to show recovery after the lesion (Fig. 4 A, B). However, the reason for the difference in the recovery processes in the two monkeys may not be only the severity of the impairment. The performance of Monkey A differed from that of Monkey B even before the lesion (Fig. 2), although both monkeys showed learning effects, compared to the results at the beginning (Fig. 2 A, B). This suggests the possibility that Monkey A's dominant hand may not have been so dexterous as Monkey B's from the beginning. Therefore, many factors may be involved in the differences in the results of the two monkeys.

It has been reported that therapeutic exercise facilitated the recovery of motor performances after experimental lesions in squirrel monkeys^{6, 7, 13} and rats^{18, 19}. Kleim and Nudo¹⁹ reported the changes of somatotopy in the primary motor cortex of the intact animals after they had received training of the finger movements. These reports indicate that the reorganization of the cerebral cortex corresponded to the recovery. They also suggest the possibility that the adjacent areas may be involved in recovery of the lost function. Isa et al. reported that monkeys with cervical lesions in the corticospinal tract showed high activation in the ventral premotor area in a Positron Emission Tomography (PET) study, and that the function did not change by chemical inactivation of the primary motor cortex (M1)²⁰. This suggests that the ventral premotor area (area 6) may compensate for the impairment of the function of the corticospinal tract. In the present study, it is possible that the surrounding areas near the M1 such as the area 6 took over from the role of the impaired areas.

The animal model and the mechanisms of recovery

In recent years, understanding of motor learning, neuroplasticity and functional recovery after brain lesion has been growing. New findings in basic neuroscience have provided stimulus for research in motor rehabilitation. Above all, repetitive motor exercises have been reported as favorable for motor recovery in stroke patients²¹. Basic neurophysiological research suggests that repetitive motor activity may form the basis of motor learning and recovery²². Transcranial magnetic stimulation (TMS) in humans has revealed that even short-term repetition of execution in simple thumb movements induced changes in the cortical map²³.

The test in the present study made it possible to focus on a specific movement, and we confirmed the functional recovery after experimental stroke induction. Furthermore, the present study enabled us to evaluate the movement patterns of the forelimbs in monkeys, quantitatively.

Models in non-human primates have many advantages compared to the rodent models¹⁸, because of their resemblance to humans in brain structure and function. One might doubt whether we could successfully argue the usefulness of the present study based on two monkeys' data. However, we think it possible, even with the small sample, because of less individual difference in the

primary motor cortex in monkeys than in humans. Moreover, the lesions in the two monkeys were clear enough to evaluate, quite different from the clinical studies with patients. This animal model as those previously reported^{6, 7)} has lesions in the cerebral cortex, while clinical strokes occur more frequently in the subcortical structure, such as the internal capsules, in humans. Despite the difference of the lesions, this animal model may be useful for future studies, which will explore how the recovery process and reorganization in the brain takes place in this model.

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