Supporting Online Material for

Time-Dependent Central Compensatory Mechanisms of Finger Dexterity After Spinal Cord Injury

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Subjects

Five monkeys were used in the present study. Three monkeys [two Macaca mulatta (Monkey T: male 8.1 kg, Monkey K: male 6.5 kg) and a Macaca fuscata (Monkey H: female 6.7 kg)] were used for the PET study. Two monkeys [a Macaca mulatta (Monkey S: female 5.2 kg) and a Macaca fuscata (Monkey H: female 4.9 kg)] were used for the inactivation study. The experiments were subjected to prior reviews by the ethical committee of the National Institute for Natural Sciences and were performed in accordance with the NIH Guideline for the Care and Use of Laboratory Animals and the Guidelines of the Central Research Laboratory, Hamamatsu Photonics.

Behavioral test

In order to assess the capacity of dexterous finger movements before and after transection of the l-CST, the monkeys were trained to be seated on a monkey chair and reach, grasp and retrieve a small piece of sweet potato or carrot (about 7 mm cubic) through a narrow vertical slit using both the index finger and thumb. The food piece was positioned in the center of vertical slit located at the height of the monkey's shoulder and at a sagittal distance of 15 cm. Each experiment (for about 30 min, every third days) consisted of ~100 trials. In each trial, the animal started reaching for the food piece from a fixed position. A high-shutter-speed digital video camera (33 frames/s taken at a shutter speed of $\frac{1}{1000}$ of a second) was used to record the reach-retrieval sequences from a lateral viewpoint (as in Fig.1A).

The CST lesion

Establishment of the l-CST lesion was performed as previously described (1). First, the animals were anesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and xylazine (1 mg/kg, i.m.), and then maintained with sodium pentobarbital (20 mg/kg, i.v.). 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 CST lesion was made under a surgical microscope, in three steps: 1) A small opening in the pia mater was made at the lateral convexity of the spinal cord. A horizontal strip in a mediolateral direction relative to the lateral funiculus was made by inserting a minute L-shape hook that could not be inserted >5 mm deep, which corresponds to the distance from the lateral convexity of the spinal cord to the midline. 2) The dorsal part of the lateral funiculus was transected, with watchmaker's forceps, from the dorsal root entry zone ventrally to the level of the horizontal strip lesioned in step 1. 3) The lesion was
extended ventrally, with watchmaker's forceps, at the most lateral part of the lateral funiculus. The opening of the dura mater was closed. The skin and back muscles were sutured with nylon or silk.

Between the postoperative Day 1 to Day 14, the monkey was intensively trained every day, sometimes with manual assistance of movements. Usually the intact hand was restricted not to be used. The dairy session continued for 1 hour, until the monkey stopped eating the food pieces. Between the postoperative Day 15 until the day of the first PET scan session (at about 1 month postoperative), the animal was trained every day without the weekend. The dairy session continued for about 1 hour and contained 200–300 reaching and precision grip movements. After the first PET scan, the training was performed every third day and the dairy session continued for about 1 hour and contained 200–300 movements.

**PET experiments**

**Subjects**

Monkey T and K performed with the left hand and received the l-CST lesion on left side. Monkey H performed with the right hand and received the l-CST lesion on the right side. During the test, the other hand was restricted. After taking an anatomical image of the brain using MRI, an acrylic head holder was attached to the skull. The head holder was used for painless fixation of the monkeys’ head during the PET scanning (2).

**Behavioral tasks in PET experiment**

As described above, the monkeys were intensively trained to be seated on a monkey chair and trained to reach from a particular starting position, grasp and retrieve a small piece of sweet potato or carrot through a narrow vertical slit using both index finger and thumb with a constant pace (once per 5s)(Fig.1A) also in the scanner. In addition, they were trained on the control task, in which the food piece was stuck on the tip of the rod and give to their mouth through a long tube with the same frequency and trials as the precision grip task with both arms restricted.

After the l-CST lesion, the recovery time course of the 3 animals was not uniform, although we intended the lesions to be as similar in size as possible (Fig. S2). In the three monkeys (Monkey T, H and K), the success rate of the precision grip recovered to nearly 100 % before 3 weeks postoperative. We performed the PET scans at as similar a performance level as possible in each monkey; during an early stage of recovery when recovery was incomplete and during full recovery stage when the performance level was fairly stabilized (cf. SOM text, PET scan).
PET scan

Thirty-one slices with a center-to-center distance of 3.6 mm were collected simultaneously by PET scanner in a 3D mode. Transaxial resolution of the PET scanner was 2.6 mm at full-width-half-maximum (FWHM). Before the PET scan, the monkey was seated in a primate chair and was transferred to the PET scanner without anesthesia. A 30 min transmission scan with a rotating $^{68}$Ge-$^{68}$Ga pin source was obtained to evaluate the relative attenuation factor for image reconstruction. PET scans were performed in an upright sitting position in a scanner that was tilted at 80° from the vertical position to expand the view of the monkey. After the delivery of a bolus of $[^{15}$O]H$_2$O (~300 MBq in 1.5 ml followed by 1.0 ml of saline) via a cannula placed into the sural vein, the scan was initiated automatically when the radioactivity in the brain was greater than 30 kcps. The monkey was allowed to begin the behavioral task (20 trials) ~10 s before the start of the PET scan. On the scan, the monkeys performed a series of reach-grip-retrieve-eat movements 20 trials by every 5 s. In addition, the brain activation during the control task was also recorded in other sessions randomly mixed with the sessions of the precision grip task. PET data were collected for 80 s (one 40 s frame and followed by four 10 s frames). During the scanning session, video tape was always taken and if it was found that the monkey did not start from the fixed starting point, or did not start reaching immediately (within less than 15 flames = 500 ms) after presentation of the food or did not move the hand directly to the food piece, the data from the session was excluded from analysis.

Approximately 20 PET scans were performed per day with an interscan interval of ~15 min. PET experiments were conducted twice a week.

A series of PET scans was conducted during the preoperative, early recovery and late recovery stages. The timing of PET scans at each stage was determined by the recovery level in the behavioral test. PET scans during the early recovery stage were initiated when the success rate for retrieval reached 80%. PET scans during the late recovery stage were initiated when the success rate for retrieval consistently reached 100% after 3 months postoperative. In Monkey H, PET scans during the early recovery stage were conducted between postoperative Day 15 to 29, and those during the late recovery stage were conducted between postoperative Day 90 to 112. In Monkey K, PET scans during the early stage were conducted between postoperative day 22 to 41, and those during the late stage were conducted between postoperative Day 92 to 115. In monkey T, PET scans during the early stage were conducted between postoperative Day 36 to 64, and those during the late stage were conducted between postoperative Day 106 to 122. The PET scans of the precision grip task and the control
task were conducted on the same day. Twenty scans were conducted in Monkey H and K in both tasks for every stage, and 24 scans were conducted in Monkey T.

High-resolution whole-brain MRI images were obtained using a conventional T1-weighted, fast-spin echo sequence (in-plane resolution, 0.80 × 0.80 mm; slice thickness, 0.81 mm) with a 3.0 tesla MR imager. In addition, the cortical borderline and spatial coordination of $^{15}$O$\text{H}_2$O PET images was determined by the $^{18}$F2-fluoro-2-deoxy-D-glucose ($^{18}$FFDG) PET scan in each subject once by injecting a bolus of $^{18}$FDG (40 MBq/kg).

**Data analysis for PET**

PET images obtained from the scan sessions that satisfied the behavioral criteria were summated for their first 60 s epochs, and were used for statistical analysis. The reconstruction was performed on projection data, after which images were corrected for attenuation using a transmission scan with a 4.0 mm Hanning filter. Reconstructed brain images (voxel size, 1.2 x 1.2 x 3.6 mm), which were scalped and smoothed with a 4.0 mm FWHM isotropic kernel, were processed using statistical analysis of parametric mapping (SPM99) software. The significant foci of individual and inter-subject data were assessed using the analysis of covariance (ANCOVA) with global normalization. For the inter-subject analysis, brain shapes of individual monkeys were morphologically normalized to a pseudo-brain template MRI, which was constructed by averaging 8 brain MRI images from young adult male macaque monkeys using a $^{18}$FDG-PET image of each subject. In order to localize activity that reflected functional recovery, we defined the contrast as (precision grip task at postoperative stage - control task at postoperative stage) - (precision grip task at preoperative stage - control task at preoperative stage). This definition examines the main effect of the functional recovery of finger dexterity. We also examined the contrasts (precision grip task - control task) in each stage. The statistical threshold was set at $P < 0.01$, uncorrected ($Z > 2.34$). Any region that consisted of less than ten-clustered voxels was not considered a significant signal because of the limitation of spatial resolution. To determine the anatomical localization of activated foci, the SPM$\{Z\}$ PET images were precisely co-registered with the matching individual MRI image or the pseudo-brain template MRI using a three-dimensional alignment program.

**Inactivation study**

**Subjects**

Two monkeys (Monkey S and C) were used for the inactivation study. Both monkeys performed with the left hand and received the l-CST lesion on the left side.
**Surgery for electrophysiological mapping**

In both monkeys, surgery was performed to gain easy access for electrophysiological mapping and muscimol injections to the M1 and PMv. Under general anesthesia initiated by ketamine hydrochloride (10 mg/kg, i.m.) plus xylazine (1 mg/kg, i.m.) and maintained with sodium pentobarbital (20 mg/kg, i.v.), the monkeys were fixed in a stereotaxic apparatus. The skull over the bilateral frontal cortices was widely exposed by skin incision. After partial removal of the skull, the cortex around the precentral gyrus was exposed bilaterally, and a pair of delrin chambers were attached to cover each opening. Small titanium-steel screws were attached to the skull as anchors. The skull and screws were completely covered with acrylic resin. Two stainless-steel tubes were mounted in parallel over the frontal and occipital lobes for head fixation. The chambers and stainless-steel tubes were fixed to the screws by the acrylic resin. In addition, body parts such as the face, forelimb, and trunk were broadly depilated for easy detection of movements.

**Electrophysiological mapping**

After recovery from the surgery, the monkeys were sedated with ketamine (10 mg/kg, i.m.) and seated quietly in a primate chair with their head fixed in a stereotaxic frame attached to the chair. A glass-coated Elgiloy-alloy microelectrode (0.9-1.4 MΩ at 1 kHz) was inserted perpendicularly to the cortical surface using a hydraulic micromanipulator. Regions in the precentral gyrus were mapped with intracortical microstimulation (ICMS). Each track was separated by more than 2 mm. In each penetration, extracellular unit activities were recorded initially, followed by examination of neuronal responses to somatosensory (by passive joint movement or light touch of the skin) and visual stimuli. Subsequently, the monkeys underwent ICMS at the same site. Each pulse had a negative phase followed by a positive phase, with each phase having a duration of 0.2 ms. Stimulus trains (currents of less than 50 µA at 333 Hz) were delivered through a constant-current stimulator. The number of pulses per train was 15, 25 or 35. Evoked movements of various body parts were carefully observed. The evoked movements detected by visual inspection were further monitored by direct muscle palpation.

**Microinjection of muscimol**

The somatotopic maps constructed during the preoperative stage were used to determine the injection sites. The unit recordings and ICMS were performed with a microelectrode at the site chosen for the injection, in order to confirm the depth in the gray matter. The microelectrode was then withdrawn and replaced by a stainless steel microinjection cannula connected to a 10 µl Hamilton microsyringe. The cannula was
mounted on the same micromanipulator used for recording, so that the needle was inserted into the same track as the microelectrode. The cannula was lowered to 500 µm below the depth of the site chosen for the injection and subsequently raised again to the correct depth. Muscimol, a GABA<sub>A</sub> receptor agonist (5 µg/µl, dissolved in 0.1 M phosphate buffer at pH 7.4), was slowly injected by pressure at a rate of 0.2 µl/1 min. The depth chosen for muscimol injection was 2 and 4 mm for the PMv and 3 and 6 mm for M1 (anterior bank of the central sulcus).

In the experiments of muscimol injection at the postoperative stages, we always tried cell recording and electrical stimulation (up to 40 µA) at the intended injection site in advance. We applied systematic ICMS mapping in monkey S after finishing inactivation study (over postoperative month 4). Because of the thickening of the dura mater, we gave up systematic mapping in monkey C. In both monkeys, because the major descending tract was transected, the threshold for inducing the body movements was higher; we did not observe motor responses of fingers at the early recovery stage by stimulation of the co-M1. Accordingly, we decided to inject muscimol to the center of the digit area at the preoperative stage using the same coordinate system. Stimulation at most sites of the preoperative digit area could evoke finger and wrist movements after the late recovery stage. We could observe finger and wrist movements (in monkey S; 20-30 µA with 15 pulses, and in monkey C, 40 µA with 25 pulses). Thus, we determined the injection site at the tracks where finger and wrist movements were evoked.

A total of 5 µl of muscimol solution was injected into the ipsilesional M1 or PMv in both monkeys. For the contralesional M1, 0.8 or 1.5 µl of muscimol solution was injected in Monkey S and C, respectively. For the contralesional PMv, 1.5 µl of muscimol solution was injected in both monkeys. The same volume of muscimol was injected in the same sites before and after the l-CST lesion. Furthermore, in both monkeys the multiple injections were spaced at 2 mm distances from each other in the contralesional PMv and ipsilesional M1 during the early and late recovery stages.

**Tracer injections**

In Monkey T, biotinylated dextran amine (BDA) was injected into the co-M1 at 563 days after the l-CST lesion and 114 days before termination of the experiments to confirm the extent of the lesion. Under anesthesia with sodium pentobarbital (20 mg/kg, i.v.), based on the somatotopic map constructed by ICMS, eight tracks were selected for injection of BDA in the M1 digit area. BDA was injected at 2 or 3 locations of different depth (2 mm steps) in the precentral gyrus. In total, BDA was injected into
20 positions. 10% BDA (10,000 MW) dissolved in 0.01 M phosphate buffer (pH 7.3) was injected through a 10-μl Hamilton microsyringe. BDA was slowly injected by pressure at a rate of 0.2 μl/1 min. A total of 0.5 μl of the BDA solution was injected at each position. The tracers were slowly deposited over 10 minutes and the injection needle or micropipette was kept in place for an additional 10 minutes.

**Electrophysiological confirmation of lesion**

In Monkeys H, T and S, the terminal acute electrophysiological experiments were conducted to confirm the extent of the l-CST lesion. The methods of electrophysiological experiments have been described previously (1, 3, 4). The animals were first anesthetized with ketamine (0.1 ml/kg) and xylazine (0.05 ml/kg), and, after the tracheotomy, isoflurane was used throughout the surgery. After surgery, the anesthesia was changed to α-chloralose (75–100 mg/kg, i.v.). Blood pressure was maintained above 80 mmHg and pCO₂ at 3.3-4.2%. Drips of ringer-glucose were given continuously during the entire experiment, and the urinary bladder was emptied regularly. Atropine (0.5 mg), dexamethasone (4 mg), and gentamycin (1 ml) were given for premedication. Atropin was given at intervals of 4–5 h. The animals were paralyzed with pancuronium bromide (1 ml, 0.2 mg/ml) given at 30-min intervals and artificially ventilated. Pneumothorax was made just prior to electrophysiological recording.

A craniotomy was performed that exposed the posterior part of cerebellum and the caudal brain stem to place the stimulating electrode to the medullary pyramid. The appropriate location of the electrode was determined when the threshold for eliciting the descending pyramidal volley recorded at C2 became the lowest (~5 μA) for both sides. The location was ~2.5 mm rostral and 1.2 - 2.2 mm lateral to the obex and at the depth of ~6.0 mm from the bottom of IVth ventricle. Probably due to shrinkage of the CST axons on the lesion side, the best locations for stimulation of the medullary pyramid were asymmetric across the midline. Monopolar cathodal pulses (0.1-ms duration) were applied using tungsten electrodes with an impedance 50-100 kΩ and a tip diameter of 10 μm. A laminectomy was performed at C2–Th1 and at Th6–Th10 segments. The deep radial (DR) nerve was dissected and mounted in a cuff with bipolar silver electrodes. Other forelimb nerves were stimulated with needle electrodes inserted through the skin. Recordings of extracellular synaptic field potentials were made from lateral motor nuclei of the C6–Th1 segments identified antidromically. Glass capillary electrodes (tip diameter: ~1.0 μm, impedance: ~3–5 MΩ) filled with 2 M potassium citrate were used. The cord dorsum potential (CDP) was monitored with a silver ball
electrode. The extent of the lesion was assessed by measuring either the negative peak of the CDP and the amplitude of the monosynaptic excitatory field potential induced by the stimulation of the medullary pyramid. As shown in Fig. S3, extracellular recordings of field potentials in the lateral motor nuclei in C6 (a train of 3 stimuli was applied to the contralateral medullary pyramid) and the CDP at the same segment on the intact and lesion sides in Monkey T. After a delay of 0.3 ms from the onset of the direct corticospinal volley (onset; solid line, peak; open arrow in the lower record of Fig. S3A), a negative field potential (filled arrow) was recorded on the intact side (the upper record of Fig. S3A). This potential results from monosynaptic CM excitation of hand motoneurons. In contrast, on the lesion side (the upper record in Fig. S3B), this potential was absent, although a small negative volley could be observed in the CDP (asterisk in the lower record of Fig. S3B). The extent of the lesion estimated by the amplitude of the negative volley in CDP was 96.4% in Monkey T, 100% in Monkey H, and 99.6% in Monkey S (relative to the intact side).

**Histological assessment of lesion completeness**

At the end of the experiments, the monkeys were deeply anesthetized with an overdose of sodium pentobarbital (50-75 mg/kg, i.v.) and perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.3), followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.3) and, finally, the same fresh PB containing 10%, 20% and then 30% sucrose. The spinal cords were removed from the bone immediately, saturated with 30% sucrose in 0.1 M PB (pH 7.3), then cut serially into 50-µm-thick coronal sections on the freezing microtome. In Monkey T, which was injected with BDA into co-M1, the sections were divided into three groups. The first group of sections was processed for BDA histochemistry. The second group, which were adjacent sections (50 µm apart), were processed for Klüver-Barrera staining. The remaining sections were Nissl-stained with 0.1% Cresyl Violet. For visualization of injected and transported BDA, the sections were incubated in 0.05 M PBS (pH 7.3) and incubated in methanol containing 0.6% H₂O₂ for 30 min, followed by the same fresh PB containing avidin-biotin-peroxidase complex for 2 hours. Subsequently, the sections were reacted for 10-20 minutes in 0.05 M Tris-HCl buffer saline (pH 7.6) containing 0.04% diaminobenzidine, 0.04% NiCl₂, and 0.003% H₂O₂, and washed in 0.1M PB. The sections were mounted on gelatin-coated slide glasses.

On the lesioned side of the spinal cord, BDA-labeled axons at the C5 level (caudal to the lesion) were counted and compared with the number of BDA-labeled axons at the C3 level in Monkey T. The number of labeled axons was quantified at 100x
magnification using a 10 x 10 counting grid mounted in the microscope ocular. The identity of each object was verified by focusing through the plane of section. All BDA-labeled objects were identified as axons. The extent of the lesion was assessed by the ratio of caudal versus rostral BDA-labeled axons. In Monkeys C, K, H and S, the sections were processed for either Klüver-Barrera or Nissl-staining with 0.1% Cresyl Violet.
Fig. S1

The extent of the l-CST lesion. (A) The CST lesion at the caudal C4 in Monkey T (Nissl staining). Dotted line indicates the border between the lesioned and intact tissue. (B) BDA-labeled CST axons at C3 (rostral to lesion). (C) a few BDA-labeled CST axons labeled at the rostral C5 (box) (caudal to the lesion).
Fig. S2

Drawings of the C4/C5 segments showing the extent of the l-CST lesion (painted in black) in the 5 monkeys used in this study.
Fig. S3

Recordings of extracellular field potentials (Field; *upper*) in the motor nuclei and the cord dorsum potentials (CDP; *lower*) evoked by electrical stimulation of the contralateral pyramid, recorded at C6 on the intact side (A) and on the lesioned side (B). Solid vertical line, the onset of the direct CST volley (very small on the lesioned side). Open arrow, peak of the direct CST volley on the intact side (very small on the lesioned side: asterisk). Filled arrow in the top, the monosynaptic pyramidal field in the motor nuclei on the intact side (lacking on the lesioned side). Asterisk, a small remaining CST volley. Open circles, the disynaptic field potential in the motor nuclei on either side.
**Table S1**

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Acb; accumbens, Cb; cerebellar cortex, contra; contralesional hemisphere, ipsi; ipsilesional hemisphere, Ins; insula, M1; PM; premotor area, primary motor area, PMv: ventral premotor area, S1; primary sensory area, S1; secondary sensory area, V1: visual area 1, V2: visual area 2

**Table S1**

Statistical analysis of the rCBF increase related to functional recovery during the early (upper) and late (lower) recovery stages compared to that during the preoperative stage. Z- and P-values at the center of individual masses of activation (the locations are indicated with the positions along the x-, y- and Z-axis) are indicated.
References