THE OUTLINE OF DOCTORAL THESIS

Somatotopic reorganization of the sensorimotor cortex in Japanese macaques after accidental arm amputation

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The Graduate University for Advanced Studies, SOKENDAI

School of Life Science

Department of Physiological Sciences

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Nondhalee, Pimpimon

Introduction

Somatotopy is the point-for-point correspondence of a part of the body to a specific region of the brain. In the cerebral cortex, there are several sensori-motor cortices and they have their own somatotopic maps. Among these, the primary motor cortex (M1) is the most explored cortical area in many species. In the primate M1, representation of each body part has been arranged in the precentral gyrus from leg to face mediolaterally, as defined by a homunculus or simiusculus.

A strong attention has been paid to the somatotopic arrangement of the upper limb in the primate M1 because it was a clue of neural network analysis to achieve precise manipulation of body parts. By using the intracortical microstimulation (ICMS) method, the fine somatotopic arrangement of the upper limb has been revealed. The ICMS maps obtained showed nest-like distal-proximal organization that digit representations were surrounded by more proximal representations in the anterior bank of the central sulcus (CS) and the precentral gyrus.

Several lines of physiological evidence have demonstrated plasticity of the sensori-motor cortices. Kaas and his colleagues surveyed on certain somatotopic arrangement differences between the healthy and the affected side after loss of the forelimb (including a finger loss case) and hindlimb in the monkey M1. These reports showed that neuronal activity coding the lost body part was responsible for the stump, a remaining part of the limb.

The threshold of stimulation currents inducing muscle movements in macaque monkeys was not different significantly between the amputated and the healthy side or the normal monkey data. Their investigation setups were under anesthesia. They mentioned that they did not have enough time to map entire pre- and post- central gyrus completely. Moreover, it is especially important that the threshold to evoked muscle movements is increased by application of general anesthesia, such as ketamine and xylazine.

On the other hand, the supplemental motor area (SMA) is located on the medial wall anterior to the M1 leg region, and its somatotopy from face to leg is arranged antero-posteriorly. The distal-proximal segregation in the SMA was not clear. However, there are no reports about effects of the loss of body parts on the SMA somatotopy using electrophysiological approach.

The somatosensory cortex (S1) located in the postcentral gyrus, and the posterior bank of the central sulcus. It is well known that this cortical area plays a role in the somatosensory processing and sensorimotor integration. The S1 also showed a reorganization of the somatotopy; the face representation expanded into the former region of the amputated forelimb.

In this study, I tried to investigate differences of somatotopic arrangement in terms of both of evoked movement and of somatosensory inputs using the chronic ICMS method in the M1, SMA and S1 in alert macaque monkeys with hemi-amputation of the distal forelimb.

Materials and Methods

Animals

Two female Japanese monkeys (*Macaca fuscata; Monkey N*, 7.5 kg body weight, 9 years old; *Monkey H*, 7.3 kg, 14 years old) were used in this study. They accidentally lost their left distal forelimbs, including their wrists and hands, at the level of the distal radial bone in 4-month-old in the breeding colony of National BioResource Project "Japanese monkey" in Japan. The experimental protocols were approved by the Institutional Animal Care and Use Committee, and all experiments were performed in accordance with the guidelines of the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Each monkey was housed in an individual primate cage under a 12-h light-dark cycle with *ad libitum* food. They were trained to sit quietly in a primate chair.

Surgery

After chair training, each monkey received surgical operations to fix its head painlessly in a stereotaxic frame. Each monkey was anesthetized with ketamine hydrochloride, xylazine hydrochloride, and propofol. The monkey's head was fixed in a stereotaxic apparatus. The skull was widely exposed under aseptic conditions. Small screws made of polyether ether ketone (PEEK) were implanted in the skull as anchors. The exposed skull and screws were covered with transparent acrylic resin. Two PEEK tubes were mounted for head fixation in parallel over the frontal and occipital lobes.

After a few days of recovery period, the monkey was positioned in a stereotaxic apparatus with its head restrained painlessly using the pipes under anesthesia with ketamine hydrochloride and xylazine hydrochloride, and the skull over the M1 and S1 of left and right hemispheres and SMA was removed for electrophysiological mapping. Three rectangle plastic chambers covering exposed cortical areas were fixed on the skull with acrylic resin.

Cortical mapping

Cortical mappings were started a few days after fixation of the plastic chambers. The monkey was positioned in a stereotaxic apparatus with its head restrained painlessly using the pipes under awake state. Evoked body part movements by ICMS, threshold currents, and somatosensory responses to body parts were recorded in wide areas of the M1, S1, and SMA including hindlimb, forelimb, and orofacial regions. Motor, threshold, and somatosensory maps were drawn in the M1 and SMA, and somatosensory maps were drawn in the S1.

Results

Somatotopic changes in the M1, SI, and SMA

The M1 occupies the surface area of the precentral gyrus and the anterior bank of the central sulcus. In the healthy cortical side of *Monkeys N and H,* the somatotopic map of the M1 seems to be the same as that in normal monkeys. Based on the motor and somatosensory maps, the most medial parts of both the surface and bank M1 were the hindlimb region, including hip, knee, ankle, and hindlimb digits. The more lateral parts next to the hindlimb region represented the trunk. The forelimb region was located laterally to the trunk region in both the surface and bank M1 and represented shoulder, elbow, wrist, and digits. Distal forelimb, such as digits and wrist was represented in the more lateral area in the forelimb region. Week currents induced distal forelimb movements, core region, in both the surface and bank M1 of *Monkey H*, while only in the surface M1 of *Monkey N*. The most lateral area represented the orofacial body part. The area anterior to the M1 required stronger current (> 40 µA) with 22 pulses and is considered to be the premotor cortex (PM).

The S1 is located posteriorly to the CS. In the healthy side, the somatotopic arrangement of the S1 of *Monkeys N* and *H* was similar to that reported previously (Jones, 2000). In the forelimb region, the distal forelimb region (core region) occupied its lateral part. In the healthy S1, ICMS with 22 pulses at 50 µA usually rarely evoked movements.

In the SMA, it is known that the orofacial, forelimb, trunk, and hindlimb regions are presented rostro-caudal direction in the mesial wall. In the healthy side, both motor and somatosensory examination identified the forelimb region between the orofacial and hindlimb/trunk regions in both monkeys. In the forelimb region, the distal forelimb region representing the digit, wrist, and palm occupied the rostral part. Motor and somatosensory maps usually matched each other with some exceptions.

This precise ICMS electrophysiological mapping revealed that there was shrinkage of the distal forelimb region in the M1 in the affected side that required less than 10 µA. In the SMA, the stump region was lost or shrunk in the affected side. The mean threshold to evoke distal forelimb movements in the healthy side and that to evoke stump movements in the affected side were comparable in the M1 and SMA. On the other hand, only a little shrinkage of the S1 distal forelimb region was detected. General arrangement of somatotopy, such as hindlimb, trunk, forelimb and orofacial, was preserved in the M1, S1, and SMA.

Discussion

In this study, I investigated somatotopic changes in the sensorimotor areas of two adult macaque monkeys who lost their distal forelimbs including the wrists in their childhood. I mapped the M1, S1 and SMA under awake state. The stump region was identified in the M1 and SMA: ICMS induced movements in the stump with low threshold as in the distal forelimb of the healthy side, and palpation of the stump induced neuronal activity. The stump regions of the M1 and S1 in the affected side were smaller than the distal forelimb regions in the healthy side. On the other hand, the stump region of the S1 in the affected side examined by somatosensory inputs was rather preserved in comparison to the distal forelimb region in the healthy side.

Changes in the M1 and SMA

Studies with nonhuman primates with amputation have been limited because such subjects were obtained by a result of injury and therapeutic treatment. Qi and collegues examined four adult (from 5 to 17 years old) macaque monkeys long after the injury, who lost their forelimbs below/above the elbow or at mid-upper arm at different ages (from 4 month to 7 years). Threshold current levels for stump movements were comparable to those for normal arm movements. They found that extensive regions of the M1 formerly devoted to the missing hand evoked movements of the stump and the adjoining shoulder. Few or no sites in the estimated former territory of the hand evoked face movements. The stump size seems to be preserved, and reorganization across the somatotopy is less probable. Wu and Kaas examined two adult squirrel monkeys and one adult galago long after amputation (from 4 to 12 years). Stimulation of the deprived portion of the M1 elicited movements of remaining muscles just proximal to the amputation. There was no expansion of face representation into the deefferented forelimb representation. The minimal levels of current needed to evoke these movements ranged from normal to higher than normal.

In contrast to previous studies, the present results showed that the stump region in the affected side was shrank by both ICMS and somatosensory mappings. This study has been done in awake monkeys with lower threshold current (1-50 μA), which are commonly used for ICMS mappings. These procedures enabled us the precise mapping of the M1. Previous studies were conducted under anesthesia: an acute and long (10-20 hours) experiment under deep anesthesia, and a chronic recoding by using recording chambers (for 15 days) under light anesthesia of ketamine. Anesthesia increased ICMS threshold, and they needed stronger stimulation to evoke movements than the present study: higher currents or longer stimulation trains. Stronger ICMS might excite other neuronal axons and somata that were not adjacent to the electrode tip, leading to proximal body parts movements and vague somatotopy as discussed by Mitz and Wise.

The stump regions in the M1 shrank probably because the M1 lost the body part to control. Limitation or immobilization of the distal forelimb by use of a soft case in adult squirrel monkeys decreased digit representation and increased wrist/forearm representations with the same ICMS threshold.

To my best knowledge, this is the first report on the somatotopic changes in the SMA of animals with limb amputation. The forelimb region of the SMA shrunk after the forelimb amputation in this study. This may be reasonable because the SMA also lost body parts to control as in the case of the M1.

Changes in the S1

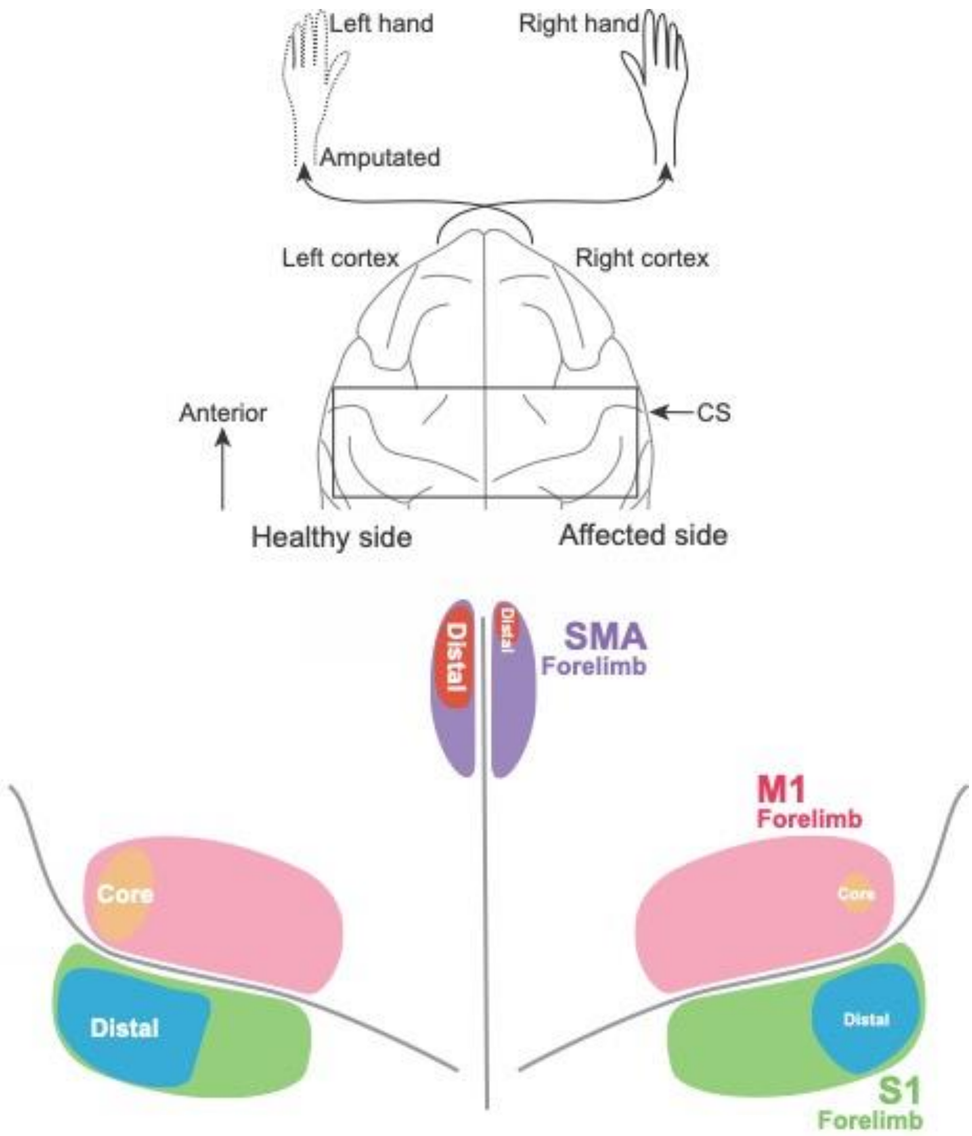
Florence and Kaas examined three owl or rhesus adult monkeys 1 - 13 years after amputation above the wrist or elbow and mapped area 3b under anesthesia. They found that the representation of the remaining forelimb skin expanded into the deprived hand representation. The overall size of the forelimb representation contralateral to the amputation was smaller than that in the normal animals. Florence et al. examined the mechanism of such reorganization of the S1 and found expanded lateral connections in areas 3b and 1 and similar reorganization in the ventroposterior (VP) nucleus of the thalamus as well as area 3b.

Clinical significance

The M1 could be divided into the old M1 and new M1. The old M1 is the rostral region, which lacks cortico-motoneuronal cells and is the standard for many mammals. The new M1 is the caudal region, which has monosynaptic connections with motoneurons innervating shoulder, elbow, and finger muscles, enables highly skilled movements and is present only in higher primates and humans. The core region with low ICMS threshold (< 10 μA) in the present study roughly corresponds to the new M1. This study showed that the areas of both core and distal forelimb regions were reduced, suggesting both the old and new M1s were affected.

The present results have a significant meaning to develop Brain Machine Interface, which is a technique that controls prosthetic hands based on the neuronal activity recorded in the motor cortex. To control prosthetic hands instead of the accidental lost hand, the best target to record neuronal activity is the M1 previously involved in the forelimb control. In this study, such the region shrunk.

Patients with amputation often experience phantom limb, sensation in their amputated limb. They can move voluntarily their amputated limb and feel its movement. Some patients feel phantom limb pain, especially when they cannot move their amputated limb. The mechanism of phantom limb are still under debate. In the present study, the forelimb region of the M1, which previously coded the forelimb movements remains after the forelimb amputation, although its size is decreased. ICMS in this region induced stump movements, and neurons in this region responded to somatosensory stimulation. Therefore, it is presumable that this region keeps motor and proprioceptive functions and gives a sensation of phantom limb. In the present study, I observed that some M1 neurons in the affected side were activated during reaching, supporting this hypothesis. On the other hand, the size of the distal forelimb in the S1 was rather preserved. The discrepancy of size changes between the M1 and S1 may cause phantom limb pain.



Summary figure The distal and total forelimb regions in the M1, S1, and SMA in the healthy and affected sides are schematically shown.