THE DOCTORAL THESIS

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

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Contents

Abstract

The primary motor (M1) and primary somatosensory (S1) cortices show a somatotopic representation, which is a one-to-one correspondence of each body part to a small region of these cortices. The M1 is located in the pre-central gyrus including the anterior bank of the central sulcus (CS), while the S1 is located in the post-central gyrus. The hindlimb, body trunk, forelimb, and orofacial regions are represented in the M1 and S1 mediolaterally along the CS. The supplementary motor area (SMA), which is located in the medial wall of the hemisphere, is also somatotopically organized with the orofacial, forelimb, and hindlimb representations in the rostro-caudal direction. The aim of this study is to investigate how such somatotopy is affected when the subjects lose their body parts.

There are several reports on reorganization of the M1 of monkeys after accidental amputation of their upper limbs. A previous physiological study showed a reorganization of the somatotopic arrangement of the M1 in an adult macaque long after the accidental amputation of the upper limb below the humeral head. An acute experiment was done under general anesthesia. The thresholds of electrical intra-cortical micro-stimulation (ICMS) to evoke movements of the stump and shoulder in the affected cortical side were higher than those to evoke movements of forelimb digits in the healthy cortical side. They concluded that the shoulder region seemed to be expanded a little. Another experiment was done chronically under light anesthesia. ICMS thresholds to evoke movements in the M1 in the affected side were higher than those in normal monkeys. They mentioned that the remaining arm region in the affected side was smaller than the intact arm region in the healthy side, and that these high intensity currents could activate large cortical regions representing proximal body parts and lead to a vague somatotopic map. Anesthesia obscures somatosensory responses and increases the thresholds of ICMS. There are no reports on the reorganization of the SMA. Therefore, I decided to perform physiological mappings of M1, S1 and SMA using chronic set-ups in the awake state without any anesthesia.

I trained two female Japanese monkeys (*Macaca fuscata*), who lost their left distal forelimbs below the elbows accidentally in their childhood, to sit quietly in a monkey chair. After they were accustomed, under general anesthesia, they underwent surgery to fix two PEEK tubes on the skull for head fixation. Craniotomy was done over the M1/S1 on both sides and SMA, and 3 rectangle plastic chambers were fixed onto the skull with acrylic resin. ICMS mappings were started after a few days of a recovery period. Evoked body part movements by ICMS, threshold currents, and somatosensory responses of body parts were recorded in wide areas of the M1, S1, and SMA including the hindlimb, forelimb, and orofacial regions in the awake state.

This precise ICMS electrophysiological mapping revealed that there was shrinkage of the distal forelimb region in the M1 on the affected side where less than 10 μA ICMS could evoke movements. In the SMA, the stump region was lost or shrunk on the affected side. The mean threshold to evoke distal forelimb movements on the healthy side and that to evoke stump movements on 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 representations, was preserved in the M1, S1, and SMA.

In this study, chronic recording in the awake monkeys enabled us to obtain precise somatotopic mappings with lower thresholds than previous studies. The stump regions previously representing the distal forelimb shrank in the M1 and SMA, while that in the S1 was rather preserved. The reorganization of the M1 and SMA may occur when the monkeys lost their body parts to control, while the S1 may remain to be reorganized because somatosensory inputs from stumps still exist.

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 (Tanji, 2001; Ebbesen and Brecht, 2017). Among these, the primary motor cortex (M1) is the most explored cortical area regarding the precise somatotopic layout in many species including humans and nonhuman primates (Penfield and Rasmussen, 1950; Woolsey et al., 1952; Wise and Tanji, 1981a, b; Sessle and Wiesendanger, 1982). 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 (Penfield and Rasmussen, 1950) or simiusculus (Woolsey et al, 1952).

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 (Wise and Tanji, 1981b; Sessle and Wiesendanger, 1982; Sato and Tanji, 1989; Tokuno and Tanji, 1993; Tokuno et al., 1997). 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 (Makin and Flor, 2020; Manger and Jones, 1996; Valyear et al, 2020; Weiss et al, 2000). A clinical study with transcranial magnetic stimulation (TMS) in humans also suggests that the face region may have expanded to the lost hand region in the M1 (Pascual-Leone et al, 1996; Vargas et al, 2009). However, this expansion of the face region could not be observed in the ICMS experiments on nonhuman primates (Schieber and Deuel, 1997; Wu and Kaas, 1999). 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 (Wu and Kaas, 1999; Qi et al, 2000). 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 (Qi et al., 2000). Their investigation setups were acute and long (10-20 hours) experiments under anesthesia with ketamine and xylazine or urethane. 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 (Tokuno and Tanji, 1993). Therefore, I would like to try to confirm precisely the somatotopic changes after amputation using the alert chronic ICMS method.

On the other hand, the supplemental motor area (SMA) is located on the medial wall anterior to the M1 leg region (Hatanaka et al, 2001), and its somatotopy from face to leg is arranged antero-posteriorly (Shima and Tanji, 2000; Tanji, 2001). The distal-proximal segregation in the SMA was not clear. There are several individual differences (Wise and Tanji, 1981b; Mitz and Wise, 1987; Tokuno and Inase, 1994). However, there are no reports about effects of the loss of body parts on the SMA somatotopy using electrophysiological approach. To address this question, I also investigated somatotopic changes after loss of the forelimb in the SMA as well as in the M1.

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 (Penfield and Rasmussen, 1950; Kaas et al., 1979) and sensorimotor integration (Lee et al., 2013). Further, recent physiological studies revealed that the S1 received efference copy from the M1 just before sensory feedbacks from the spino-thalamic system (Delhaye et al, 2018; Umeda et al, 2019). The S1 also showed a reorganization of the somatotopy; the face representation expanded into the former region of the amputated forelimb (Florence and Kaas, 1995; Jain et al, 1998). An experiment with unilateral column lesions at the cervical spinal cord level in macaque monkeys revealed that after transient sensory loss, the S1 showed partial reactivation by sensory stimulation of the face (Qi et al, 2014). They thought that the reactivity of the S1 after injury of the spinal cord might be caused by thalamo-cortical connectivity. Therefore, it is necessary to investigate more carefully the somatotopic reorganization of the S1 in amputated monkeys.

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 (Figure 1 A).

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. Both monkeys accidentally lost their left distal forelimbs, including their wrists and hands, at the level of the distal radial bone in 4-month-old (Figure 1 B, C) 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 (10 mg/kg body weight, i.m.), xylazine hydrochloride (1-2 mg/kg, i.m.), and propofol (6-9 µg/mL blood concentration, i.v.; using target-controlled infusion pump, TE-371, Terumo). 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 (Unifast II; GC Corporation). Two PEEK tubes were mounted for head fixation in parallel over the frontal and occipital lobes and fixed on the monkey's head. Additionally, broad depilation of the face forelimb, trunk, and hindlimb was carried out for easy detection of body part movements.

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 (10 mg/kg, i.m.) and xylazine hydrochloride (1-2 mg/kg, i.m.), 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. A glass-insulated Elgiloy-alloy microelectrode (0.6 MΩ at 1 kHz;

exposed tip 7-15 μm) was held by a hydraulic micromanipulator (Narishige Scientific Instrument Lab) attached to the stereotaxic frame and inserted perpendicularly to the cortical surface through the dura mater with local application of lidocaine. Signals from the electrode were amplified (×10,000), filtered (300–5,000 Hz), and continuously monitored with an oscilloscope and a sound monitor. When neuronal activity was first recorded, the depth was defined as the depth 0 (zero). Then, neuronal responses to somatosensory (deep sensation, such as passive joint movements and muscle palpation for M1 and SMA mapping; and superficial sensation, such as light skin touch for S1 mapping) and visual (such as, presentation of spotlight or a piece of fruit) stimuli were examined. Following extracellular recording, ICMS was performed. The connection of the electrode was switched from the pre-amplifier to the isolator connected to the stimulator. Currents less than 50 μA were delivered with a train of 12 (for M1 mapping) or 22 (for SMA mapping, occasionally used for M1 mapping) cathodal pulses (200-μs duration at 333 Hz), and the evoked movements of various body parts, such as joint extension/flexion and muscle twitches, were examined by visual inspection and palpation. The stimulating currents were increased or decreased gradually, and the current threshold was determined as a current that evoked movements in two-thirds of trials. Then, the electrode was advanced by 0.5 mm, and somatosensory responses and ICMS evoked movements were examined. These procedures were repeated at every 0.5 mm along the penetration track from the first spike depth to the last spike depth, where no neuronal activity was recorded. The electrode was penetrated at 1.0 - 2.0 mm apart around the central sulcus in the M1 and S1 and along the longitudinal fissure in the SMA. Thus, motor, threshold, and somatosensory maps were drawn in the M1 and SMA, and somatosensory maps were drawn in the S1.

These mappings were performed for 3-4 hours in five days a week for 11 months in *Monkey N* and for 12 months in *Monkey H*.

Mapping of normal monkeys

Somatotopic arrangements in the M1 of two normal Japanese monkeys with intact both forelimbs were drawn from the database of M1 mapping in Division of System Neurophysiology, National Institute for Physiological Sciences. The purpose of these M1 mappings is to localize only the distal and proximal forelimb regions for implantation of stimulating electrodes, and the mappings were not intensively done. However, enough data are available to compare with the results obtained in this study.

Data presentation

The penetration in the M1 near the CS went through the anterior bank of the CS, and these data were plotted in the anterior bank of the CS. The penetration in the M1 far from the CS (≥ 2mm) went through the surface part of M1, and the body part movement with the lowest threshold and the major somatosensory inputs in each track were plotted in the surface part. On the other hand, the posterior bank of the CS is thin, and S1 mapping was performed mainly in the surface region of the postcentral gyrus. The major somatosensory inputs in each track were plotted in the surface part of the postcentral gyrus. The penetration along longitudinal fissure went through the SMA, and these data were plotted in the mesial wall.

The core region in the M1 was defined as the region where weak ICMS (< 10 μA) evoked distal forelimb or stump movements, and the distal forelimb region in the M1 was defined as the region where ICMS (< 50 μA) evoked distal forelimb or stump movements. The distal forelimb in the S1 was defined as the region where somatosensory responses were recorded in response to distal forelimb stimulation, such as light skin touch. Therefore, the following regions can be identified: the core region in the M1, and distal/stump and total forelimb regions in the M and SMA examined by ICMS-evoked motor responses; and the distal and total forelimb regions in the M1, SMA, and S1 examined by somatosensory inputs. The border between the regions was drawn by MATLAB (R2018b) software. A two-dimension convex hull was drawn to make a border that contains stimulation/recording sites of each body part in the bank by connecting stimulation/recording sites from superficial layer to the end of tracks (until disappearance of neural spikes). Stimulation/recording sites on the surface were connected together to draw boarders of each body part. Quickhull algorithm (Barber et al., 1996) was used to calculate the areas within the border.

Results

Somatotopic changes in the M1

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 (Figures 2A, C, D, left; 3A, C, D, left) seems to be the same as that in normal monkeys (Figure 4). 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).

Somatotopy of the M1 was similarly represented in the both cortical sides of normal monkeys (Figure 4A, B). On the other hand, in the affected cortical side of *Monkeys N and H* (Figures 2A, C, D, right; 3A, C, D, right), the cortical region that was supposed to represent the distal forelimb was lost, and instead represented the stump: Neurons in this region responded to the palpation of the stump, and ICMS in this region induced movements of the stump at threshold < 10 μA (core region). Therefore, the distal forelimb region in the M1 is considered to be substituted by the stump region. Other general somatotopic map of the M1, such as orofacial, proximal forelimb, trunk, and hindlimb regions, seems to be the same as that in the healthy sides (Figures 2A, C, D; 3A, C, D).

The core region in the affected side was smaller than that in the healthy side. The difference was quantitatively examined in Figure 5. The core region examined by motor responses evoked by ICMS in the affected side is smaller than that in the healthy side (*Monkey N*, 63%; *Monkey H*, 6%; the percent ratio of the affected side to the healthy side). The distal forelimb region examined by motor responses evoked by ICMS in the affected side is also smaller than that in healthy side (*Monkey A*, 54%; *Monkey G*, 38%). The distal forelimb/stump region examined by somatosensory responses shows similar tendency (*Monkey N*, 42%; *Monkey H*, 25%). On the other hand, the total forelimb region in the affected side did not show consistent difference: smaller in *Monkey N* (motor, 70%; somatosensory, 81%) and larger in *Monkey H* (motor, 121%; somatosensory, 108%) in comparison to the healthy side.

Threshold currents were compared in the affected and healthy sides (Figure 6). In the healthy side, threshold currents to evoke distal forelimb movements were low (*Monkey N*, 21 ± 1.2 μA; *Monkey H*, 24 ± 1.2 μA) and those to evoke proximal forelimb movements were slightly higher (*Monkey N*, 24 ± 1.3 μA; *Monkey H*, 32 ± 3.0 μA). Similar thresholds were observed in normal monkeys (Figure 4C). In the affected side, threshold currents to evoke distal forelimb (*Monkey N*, 16 ± 1.4 μA; *Monkey H*, 23± 13.1 μA) and proximal forelimb (*Monkey N*, 23 ± 1.2 μA; *Monkey H*, 30 ± 12.8 μA) movements were in the same range as in the healthy side with similar tendency.

Somatotopic changes in the S1

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) (Figure 2B, left; 3B, left). The most medial area represented the hindlimb. The more lateral area next to the hindlimb region represented the trunk. The forelimb region was located next to the trunk region. The orofacial region was located at the lateral end. In the forelimb region, the distal forelimb region (core region) occupied its lateral part (lateral large portion in *Monkey N* and lateral half in *Monkey H*). In the healthy S1, ICMS with 22 pulses at 50 μA usually rarely evoked movements (*Monkey N*, 0/49 tracks; *Monkey H*, 2/56).

Somatotopy of the S1 was similarly represented in the both sides of normal monkeys (Figure 4A). On the other hand, in the affected side (Figures 2B, right; 3B, right), the stump region occupied some region in the forelimb region (lateral large portion in *Monkey N* and anterior and posterior mid part in the forelimb region in *Monkey H*; Figures 2B, left; 3B, left). Therefore, the area that had been dedicated to the distal forelimb region was considered to be partly dedicated to the stump. Other body parts, such as orofacial, trunk, and hindlimb, are similarly represented as in the healthy side. There was no sites in the affected side that showed evoked movements by ICMS with 22 pulses at < 50 μA (*Monkey N*, 0/43; *Monkey H*, 0/60). The areas of the distal forelimb and total forelimb regions were compared between the affected and healthy sides (Figure 5). The distal forelimb region in the affected side was smaller than that in the healthy side (*Monkey N*, 74%; *Monkey H*, 94%), while the total forelimb regions showed inconsistent changes (*Monkey N*, 74%; *Monkey* H, 125%).

Somatotopic changes in the SMA

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 N* and *H* (Figures 7, left; 8, left). 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 (Figures 7, left; 8, left).

On the other hand, in the affected side, motor and somatosensory examination identified the forelimb regions, and most of the forelimb region represented the proximal forelimb, not the stump (Figures 7, right; 8, right); only small stump region in *Monkey N* and no stump region in *Monkey H*. The distal/stump and total forelimb regions in the SMA were compared between the affected and healthy sides (Figure 9). The stump region in the affected side was smaller than the distal forelimb region in the healthy side in both *Monkey N* (motor map 16%, somatosensory map 39%) and *Monkey H* (motor map 0%; somatosensory map 0%), while the total forelimb regions were similar between the affected and healthy sides in *Monkey N* (motor map 120%, somatosensory map 127%) and *Monkey H* (motor map 115%, somatosensory map 53%).

ICMS threshold currents were compared between the affected and healthy sides (Figure 10). Threshold currents to evoke movements in the distal and total forelimb regions were similar between the affected (distal forelimb, *Monkey N*, 40 ± 4.1 μA, *Monkey H*, no movements were evoked; total forelimb, *Monkey N*, 40 ± 9.3 μA, *Monkey H*, 44 ± 8.9 μA) and healthy sides (distal forelimb, *Monkey N*, 42 ± 9.2 μA, *Monkey H*, 38 ± 9.6 μA; total forelimb, *Monkey N*, 40 ± 9.6 μA, *Monkey H*, 39 ± 9.6 μA).

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 (Figure 11). 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 et al. (2000) 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. Schieber and Deuel (1997) examined a single macaque monkey 15 years after arm amputation at about 2 years old. They found that movements of the stump of the amputated limb could be evoked throughout the normal territory of missing distal limb in the M1. The expansion of face representation into the deefferented forelimb representation was little. The threshold current levels for evoking movements of the stump were about the same or higher than those for the upper arm in the M1 contralateral to the normal arm. Wu and Kaas (1999) 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 (Qi et al., 2000), and a chronic recoding by using recording chambers (for 15 days) under light anesthesia of ketamine (Schieber and Deuel, 1997). Anesthesia increased ICMS threshold, and they needed stronger stimulation to evoke movements than the present study: higher currents or longer stimulation trains (10-80 μA, Qi et al., 2000; 250-ms trains of 333 Hz at 80 or 100 μA, Schieber and Deuel, 1997). 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 (1987).

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 (Milliken et al, 2013). Forelimb immobilization in adult rats (Viaro et al, 2014) showed similar results: decreased distal forelimb representation and increased proximal representation with increased threshold currents.

Studies using other subjects reported large somatotopic changes in the M1 after limb amputation. The pioneering works by Donoghue and Sanes (1988) and Sanes et al (1988, 1990) reported large reorganization of the M1 of rats followed by the loss of a forelimb or the section of a motor nerve to the movable vibrissae of the face. ICMS at locations normally evoked movements of the forelimb or facial vibrissae evoked movements of remaining movable body parts instead. Non-invasive human studies using TMS suggested that face and stump representations expanded into the former territory of the amputated forelimb (Chen et al, 1998; Dettmers et al, 1999).

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. A human study reported that the SMA was activated during imaginary movements of phantom toes (Maruno et al, 2000). In the present study, many sites in the SMA represented forelimb digits in the healthy side (especially *Monkey N*, Figure 7A, C, left), while, in normal monkeys, only a limited area represent forelimb digits (Shima and Tanji, 2000). This might be caused by compensatory mechanism to use digits of a healthy forelimb.

Changes in the S1

Florence and Kaas (1995) 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 (Florence et al., 1998) and similar reorganization in the ventroposterior (VP) nucleus of the thalamus as well as area 3b (Florence et al, 2000). On the other hand, spinal cord injuries induced large scale reorganization in the S1: Macaque monkeys with unilateral dorsal column lesion after recovery periods of 22-23 months were examined, and the intact face inputs expanded into the deafferented hand region of areas 3b and VP nucleus (Jain et al, 1998; Jain et al, 2008; Qi et al, 2014).

Clinical significance

The M1 could be divided into the old M1 and new M1 (Rathelot and Strick, 2009). 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. It will also be needed to investigate what the neuronal activity codes in the stump region. It was reported that monkeys with chronic amputation learned to control prosthetic hands by cortical activity to perform a reaching and grasp task (Vaidya et al, 2018).

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. Many hypotheses have been proposed to explain the mechanism of phantom limb, but they are still under debate (Collins et al, 2018). 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.

Figures

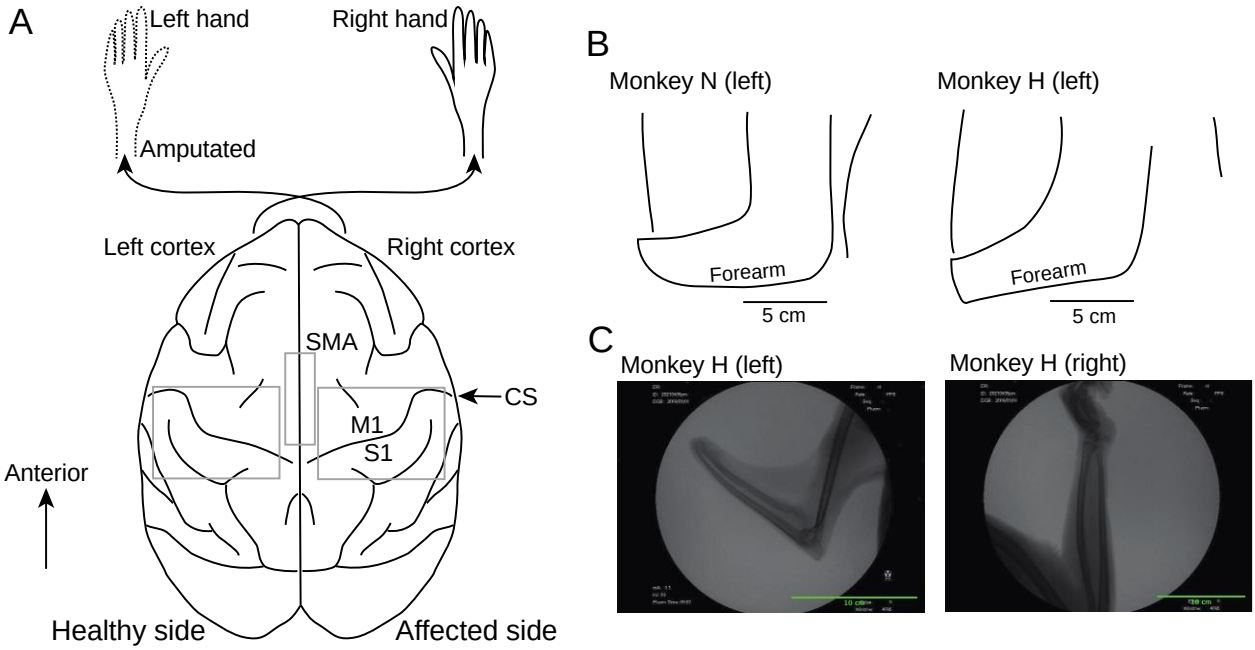

Figure 1 A, Schematic drawings of the experimental setup. The monkeys lost their left distal forelimbs in 4 month old. The M1, S1, and SMA in the healthy (left) and affected (right) cortical sides were mapped. B, Schematic drawings of the left forelimbs (amputated side) of *Monkeys N and H*. C, X-ray images of the left and right forelimbs of *Monkey H*.

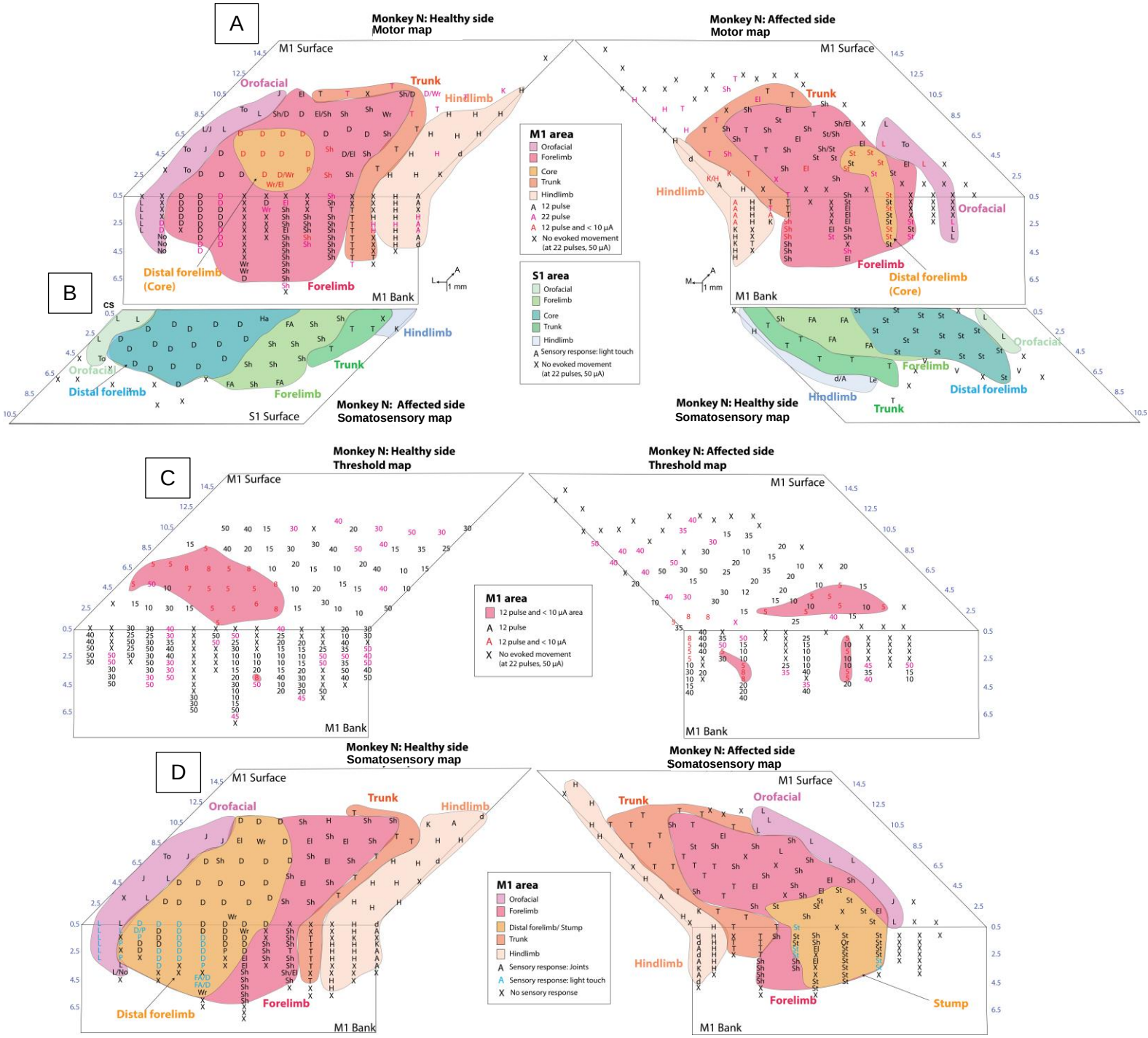

Figure 2 Somatotopic maps of the M1 and S1 in the healthy (left) and affected (right) hemispheres of *Monkey N*. **A,** M1 motor map representing body part movements evoked by ICMS. **B**, S1 somatosensory map representing body parts whose somatosensory stimulation evoked neuronal activity, **C,** M1 threshold map representing the ICMS threshold (µA). **D**, M1 somatosensory map representing body parts whose somatosensory stimulation evoked neuronal activity. Letters in A, B and D represent the following body parts; A, ankle; D, forelimb digits; d, hindlimb digits; E, ear; El, elbow; Ha, hand; L, lip; Le, leg; N, neck; Or, orofacial; P, palm Sh,

shoulder; T, trunk; To, Tongue; V, visual response Wr, wrist; X, no evoked movement or no somatosensory response.

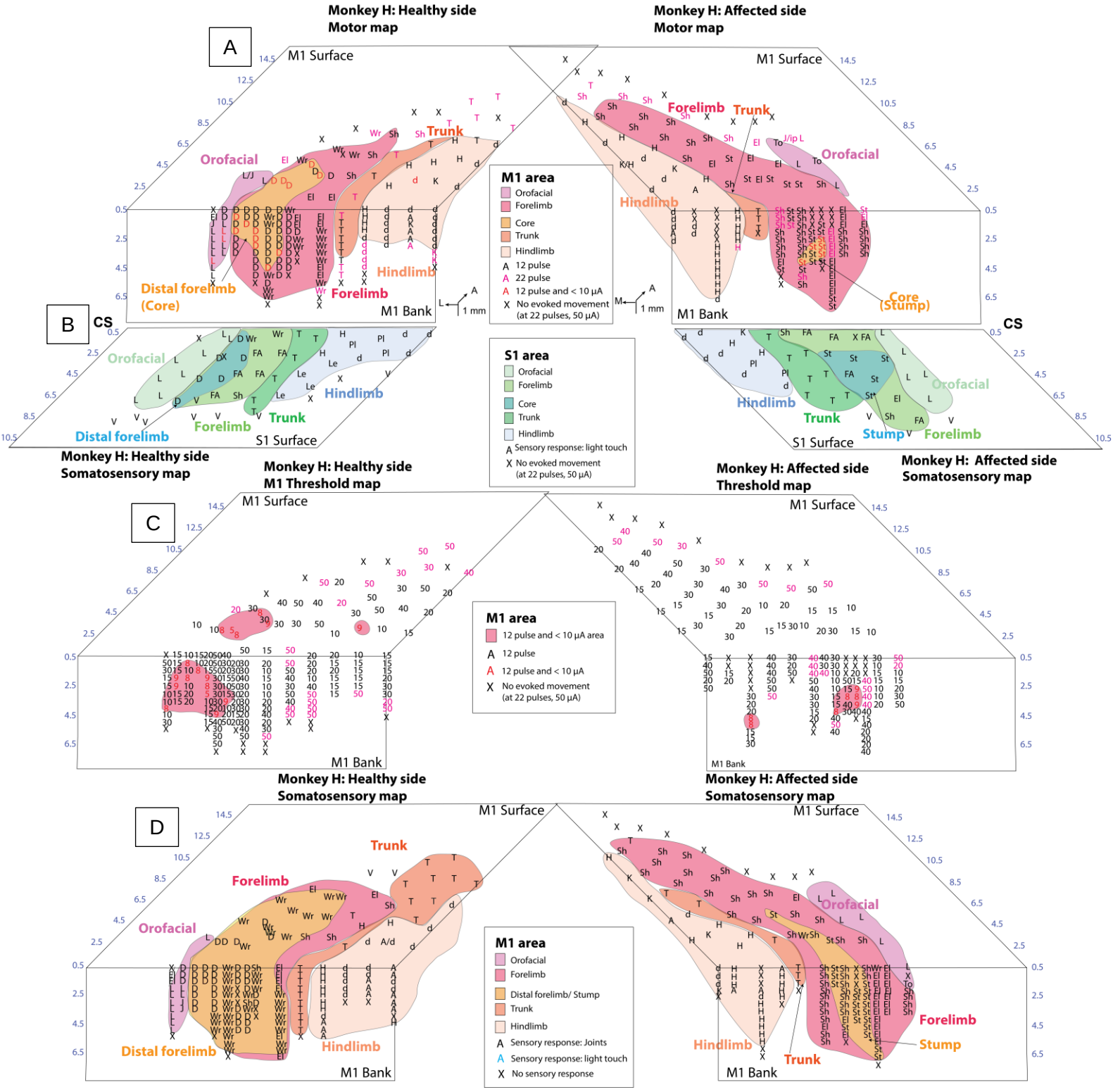

Figure 3 Somatotopic maps of the M1 and S1 in the healthy (left) and affected (right) hemispheres of *Monkey H*. All conventions are same as Figure 2.

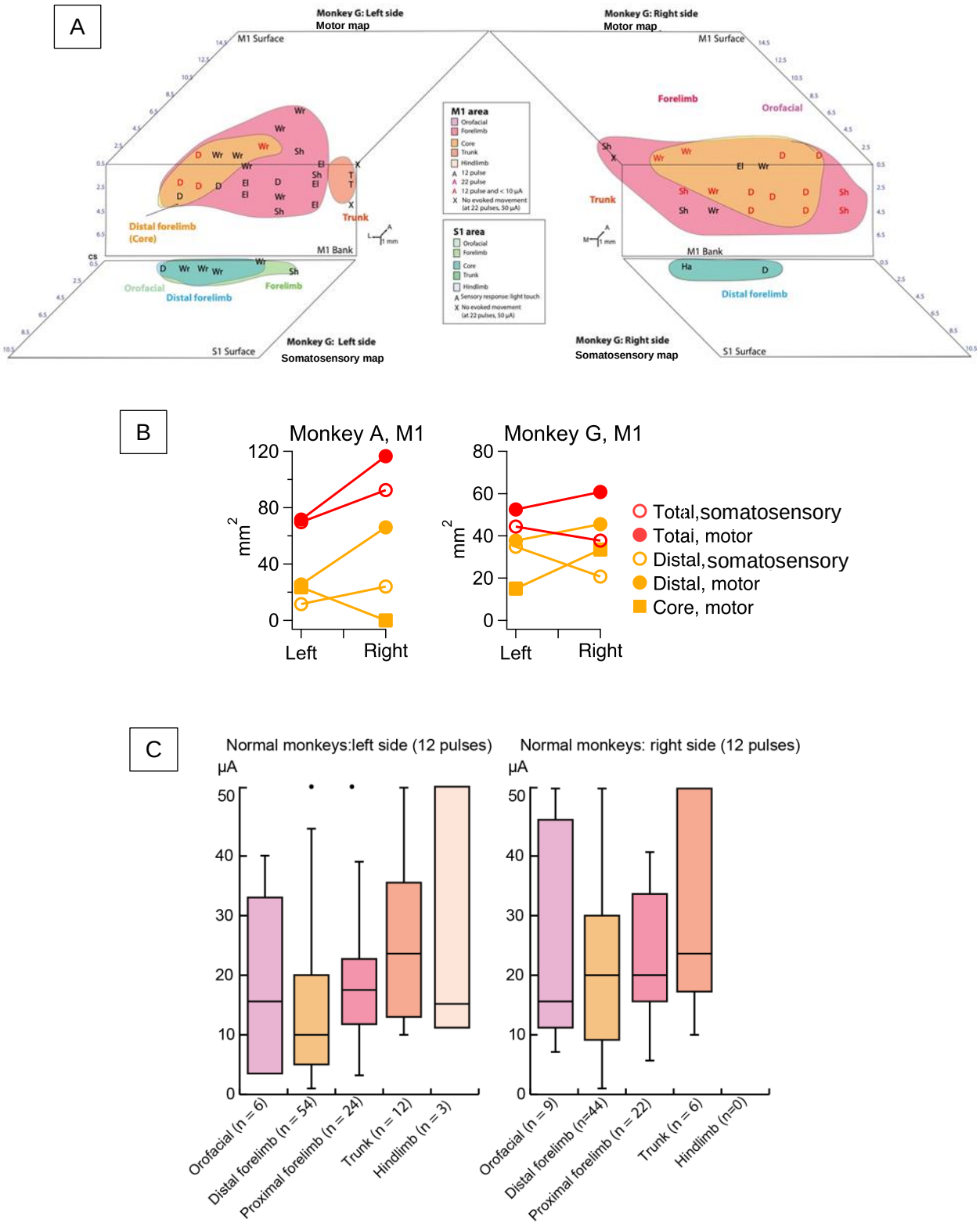

Figure 4 Mapping of the M1 and S1 in normal monkeys (*Monkeys A and G*). **A**, Somatotopic maps of the M1 (motor map) and S1 (somatosensory map) in left and right sides of *Monkey G*. D, forelimb digits; E, elbow; L, lips; Sh, shoulder; T, trunk, Wr, wrist; X, no evoked movement and no somatosensory response. **B**, The area of the core, distal, and total forelimb regions in the M1 examined by ICMS and distal, and total forelimb regions in the M1 examined by somatosensory inputs were compared between the left and right sides of normal monkeys. **C**,

The ICMS threshold currents to evoke orofacial, distal forelimb, proximal forelimb, trunk, and hindlimb movements in the left and right sides of normal monkeys.

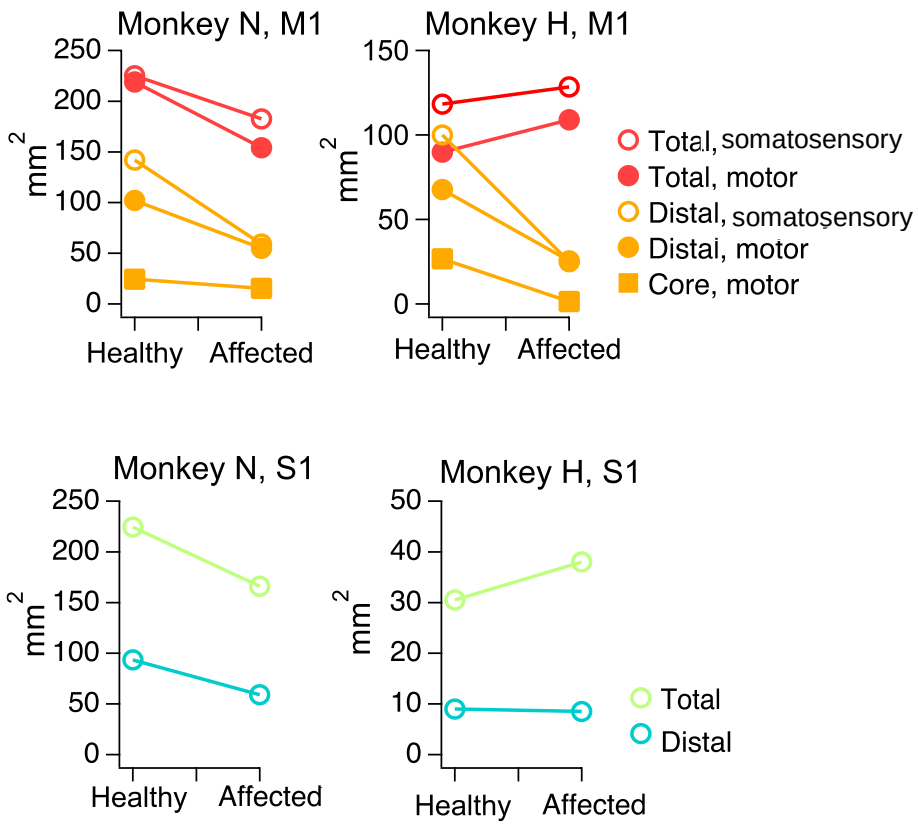

Figure 5 The area of the core, distal, and total forelimb regions in the M1 examined by ICMS evoked motor responses, of the distal and total forelimb regions in the M1 examined by somatosensory inputs, and of the distal and total forelimb regions in the S1 examined by somatosensory inputs were compared between the healthy and affected sides in *Monkeys N* and *H*.

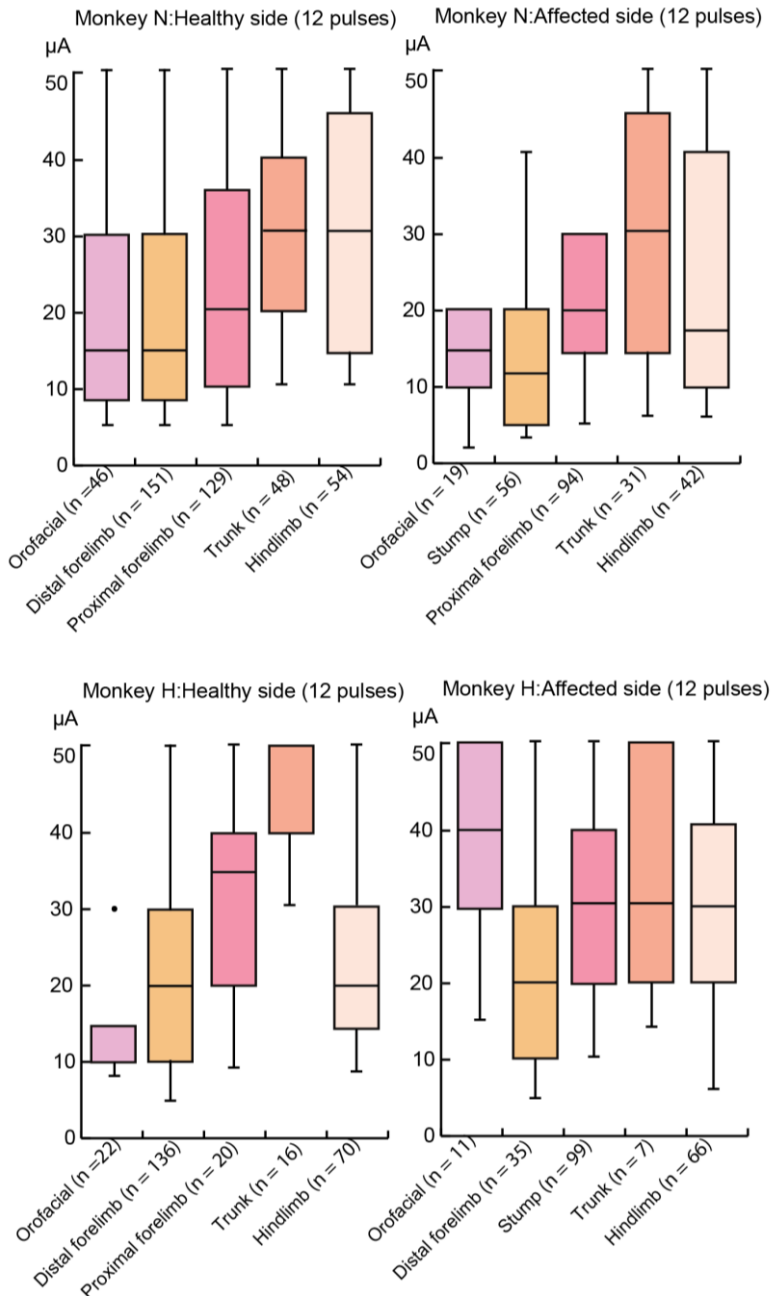

Figure 6 ICMS threshold currents to evoke orofacial, distal forelimb, proximal forelimb, trunk, and hindlimb movements in the healthy and affected sides of *Monkeys N and H* are shown in box plots (center lines, median; top and bottom of boxes, the first and third quartiles; whiskers, the minimum and maximum values excluding outliers; outliers outside 1.5 times the interquartile range from the first and third quartiles). n, number of stimulating sites; dot, outlier data.

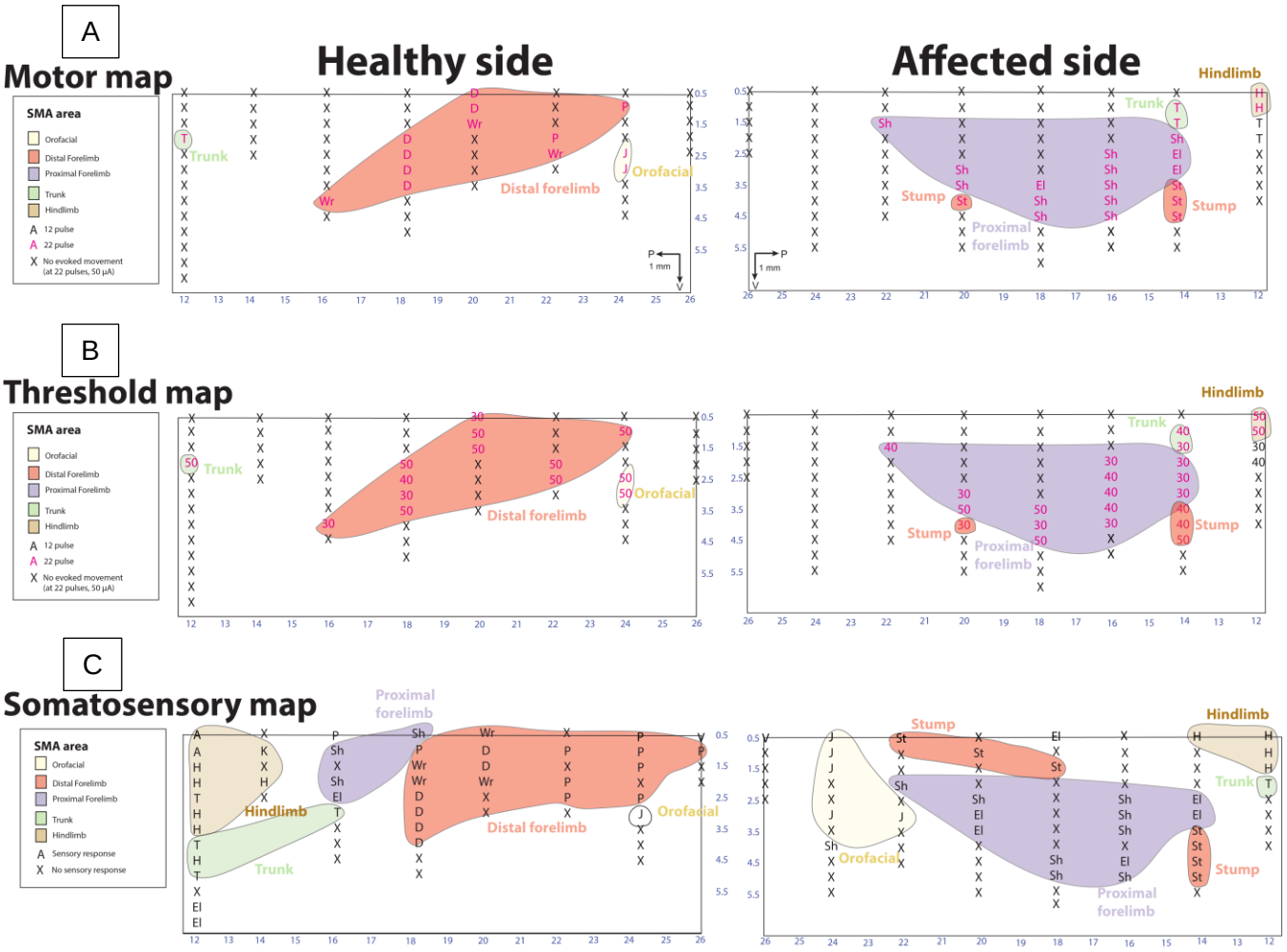

Figure 7 Somatotopic maps of the SMA in the healthy (left) and affected (right) hemispheres of *Monkey N*. **A,** SMA motor map representing body part movements evoked by ICMS. **B**, SMA threshold map representing the ICMS threshold (µA). **C**, Somatosensory map representing body parts whose somatosensory stimulation evoked neuronal activity. Letters in A and C represent the following body parts; D, forelimb digits; d, hindlimb digits; El, elbow; Ha, hand; L, lip; Or, orofacial; Pl, plantar, Sh, shoulder; T, trunk; V, visual response, Wr, wrist; X, no evoked movement or no somatosensory response.

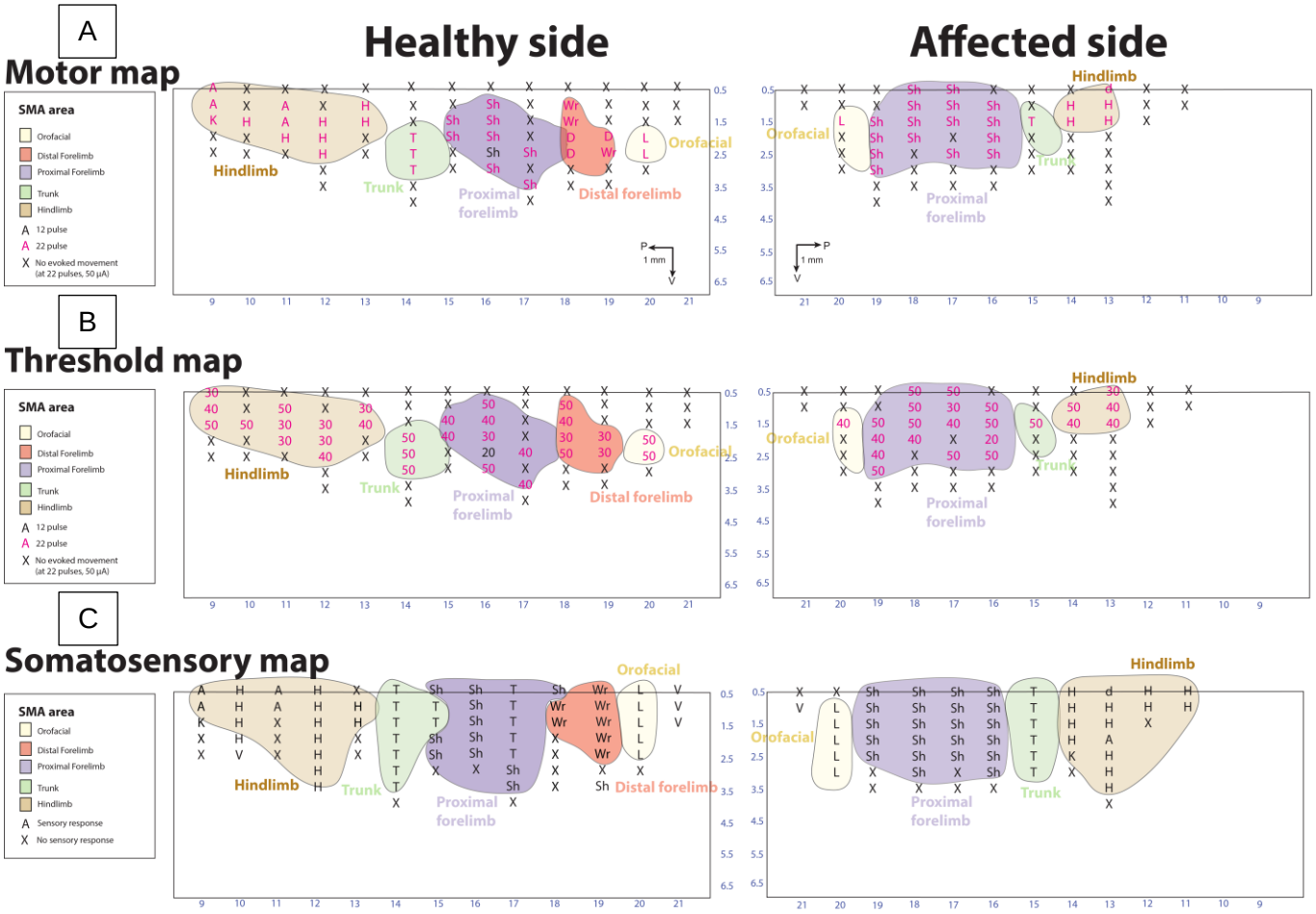

Figure 8 Somatotopic maps of the SMA in the healthy (left) and affected (right) hemispheres of *Monkey H*. All conventions are same as Figure 7.

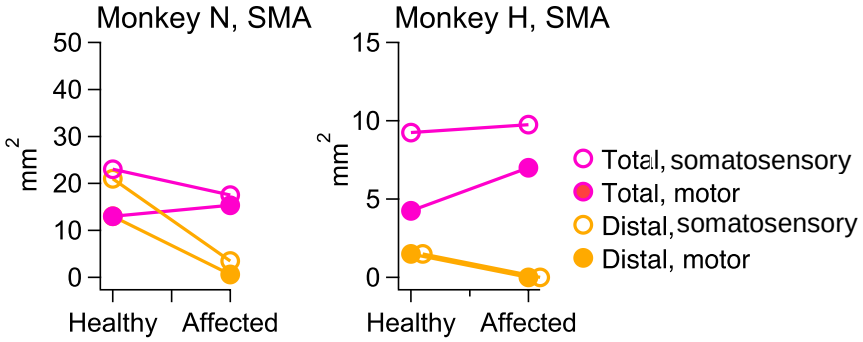

Figure 9 The area of the distal and total forelimb regions in the SMA examined by ICMS-evoked motor responses or by somatosensory inputs were compared between the healthy and affected sides in *Monkeys N and H*.

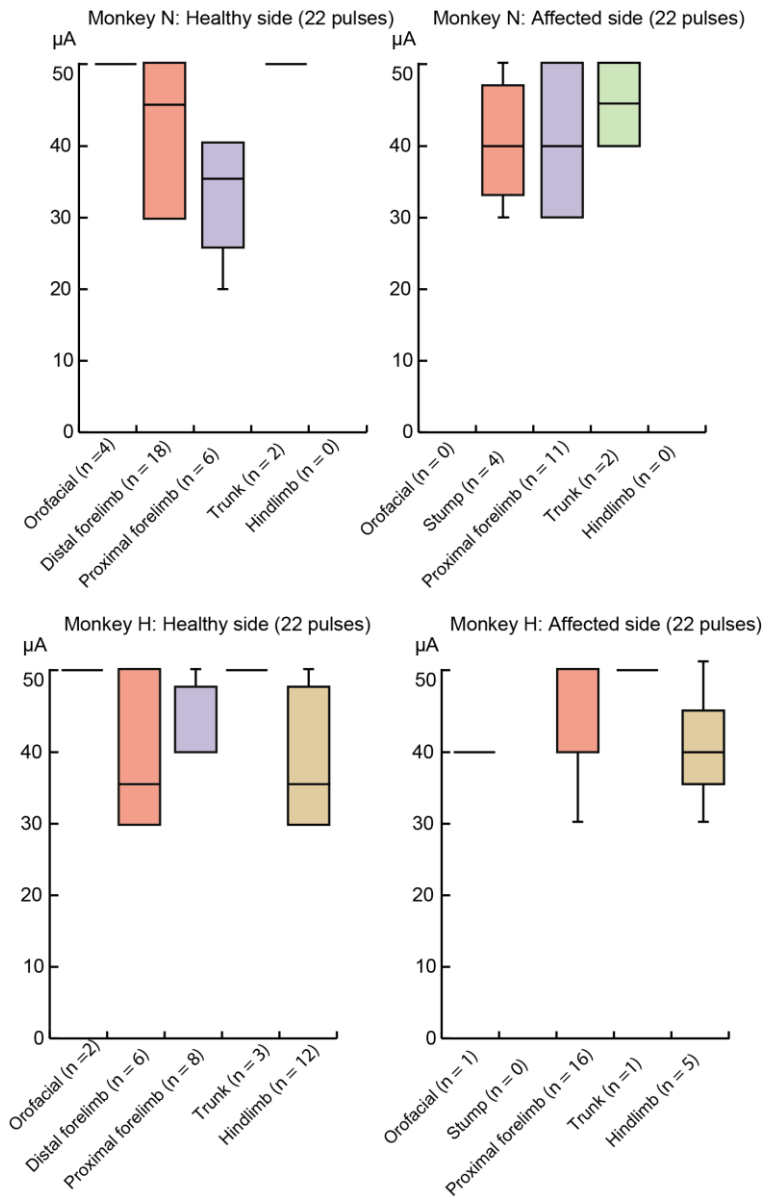

Figure 10 ICMS threshold currents in the SMA to evoke orofacial, distal forelimb, proximal forelimb, trunk, and hindlimb movements in the healthy and affected sides of *Monkeys N* and *H* are shown in box plots.

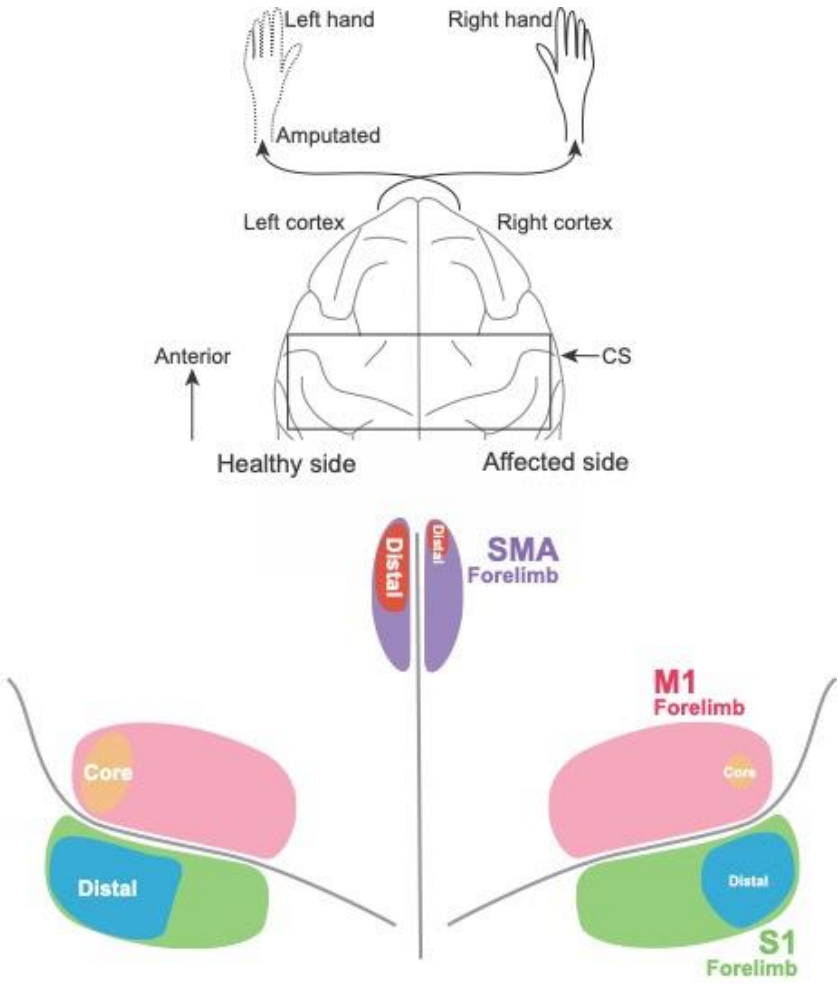

Figure 11 Summary of results. The distal and total forelimb regions in the M1, S1, and SMA in the healthy and affected sides are schematically shown.

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