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RESEARCH NOTE

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Somatotopical projections from the supplementary motor area to the red nucleus in the macaque monkey

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Abstract Direct projections from the supplementary motor area (SMA) to the red nucleus were investigated in the Japanese monkey (*Macaca fuscata*). The anterograde tracer, horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP), was injected into various regions of the SMA after intracortical microstimulation mapping. After WGA-HRP injection into the orofacial, forelimb, or hindlimb region of the SMA, anterogradely labeled axon terminals were found, respectively, in the medial, intermediate, or lateral portion of the parvocellular part of the red nucleus, bilaterally with an ipsilateral predominance. The results indicate the clear somatotopical arrangement of corticorubral projections from the SMA.

Key words Supplementary motor area · Red nucleus · Intracortical microstimulation · Anterograde tracing · Monkey

Introduction

The supplementary motor area (SMA) is considered to play important roles in many aspects of somatomotor control (for review, see Tanji 1994). The SMA, like other cortical motor areas, sends projection fibers to multiple subcortical motor structures, such as the putamen, subthalamic nucleus, red nucleus, pontine nuclei, reticular formation, and spinal cord (Wiesendanger and Wiesendanger 1984). Among these subcortical projections of the SMA, the projections to the red nucleus have been investigated repeatedly (Kuypers and Lawrence 1967; Catman-Berrevoets et al. 1979; Hartmann-von Monakow et al. 1979; Humphrey et al. 1984; Wiesendanger and Wiesendanger 1984, 1985a,b). However, only limited information has hitherto been available on the somatotopy of the corticorubral projections arising from the SMA (Wiesendanger and Wiesendanger 1985a,b). Therefore, it is necessary to analyze the pattern of distribution of axon terminals from the SMA region representing a particular body part. The present study aimed at examining the corticorubral projections from the orofacial, forelimb, and hindlimb regions of the SMA by anterograde tracing with horseradish peroxidase conjugated to wheat germ agglutinin (WGA-HRP), after intracortical microstimulation (ICMS) mapping of the SMA.

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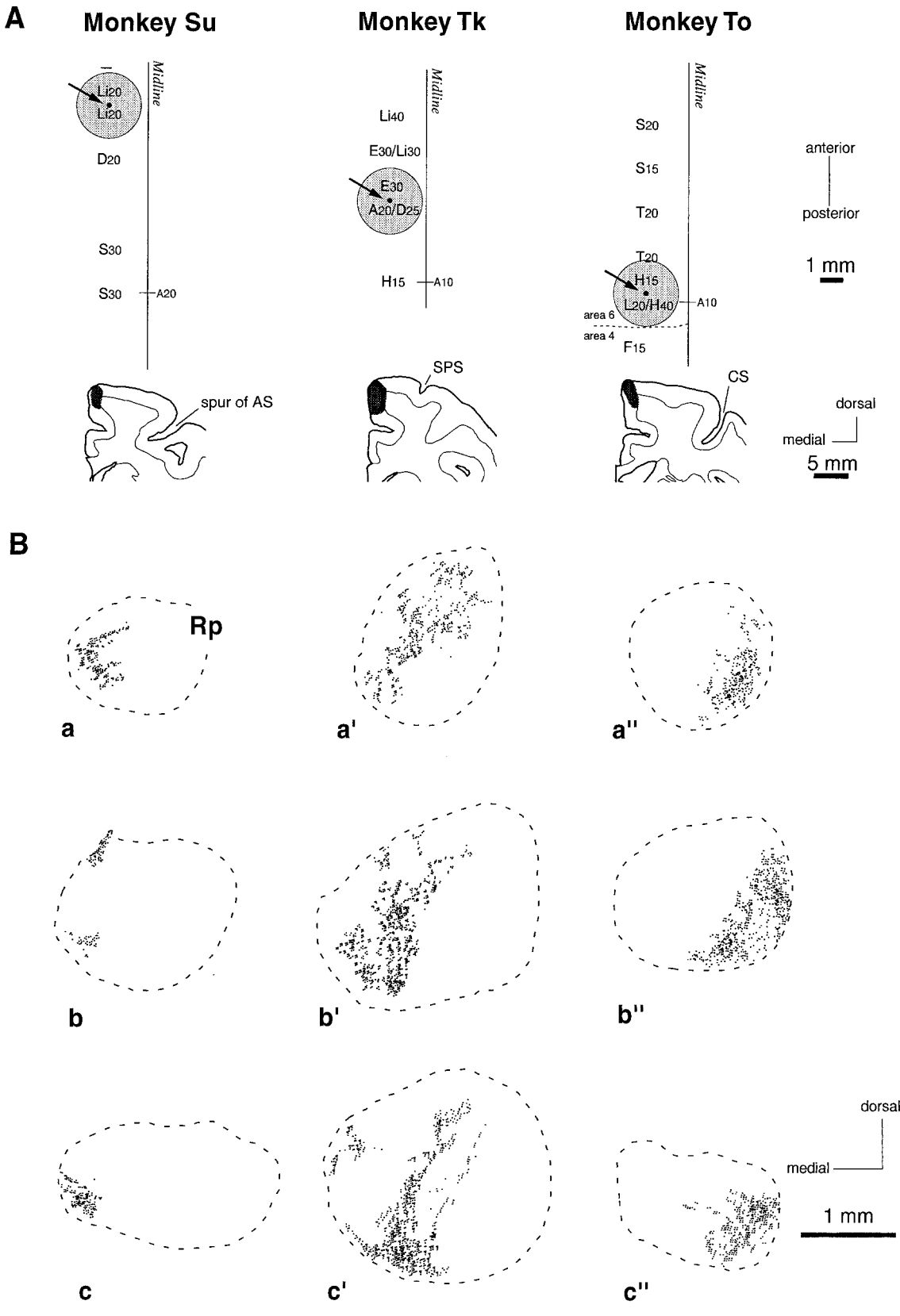
Materials and methods

Experiments were performed in three female Japanese monkeys (*Macaca fuscata*) weighing 4.6–5.2 kg. Each monkey was anesthetized with ketamine hydrochloride (10 mg/kg b.wt., i.m.) and sodium pentobarbital (30 mg/kg b.wt., i.m.), and underwent surgery to provide easy access for physiological mapping. Under aseptic conditions, the skull was widely exposed and small screws were attached to the skull for anchorage. The exposed skull and screws were completely covered with transparent acrylic resin. Two stainless-steel tubes were mounted parallel with each other over the frontal and occipital areas for head fixation. A few days after the surgery, the monkeys were anesthetized with ketamine hydrochloride (10 mg/kg b.wt., i.m.) and xylazine hydrochloride (1–2 mg/kg b.wt., i.m.). They then sat quietly in a primate chair with their heads fixed in a stereotaxic frame which was attached to the primate chair. Under aseptic conditions, skull portions over the midline and central sulcus were removed. Following recovery from the anesthesia, each monkey underwent ICMS to determine the boundaries of body part representations in the SMA. Glass-insulated Elgiloy alloy microelectrodes, whose impedance was 0.9–1.4 MΩ at 500 Hz, were used for ICMS and for recording extracellular unit activity.

The cortex was stimulated through the electrode by currents of 10–50 μA (22 cathodal pulses of 200 μs duration at 333 Hz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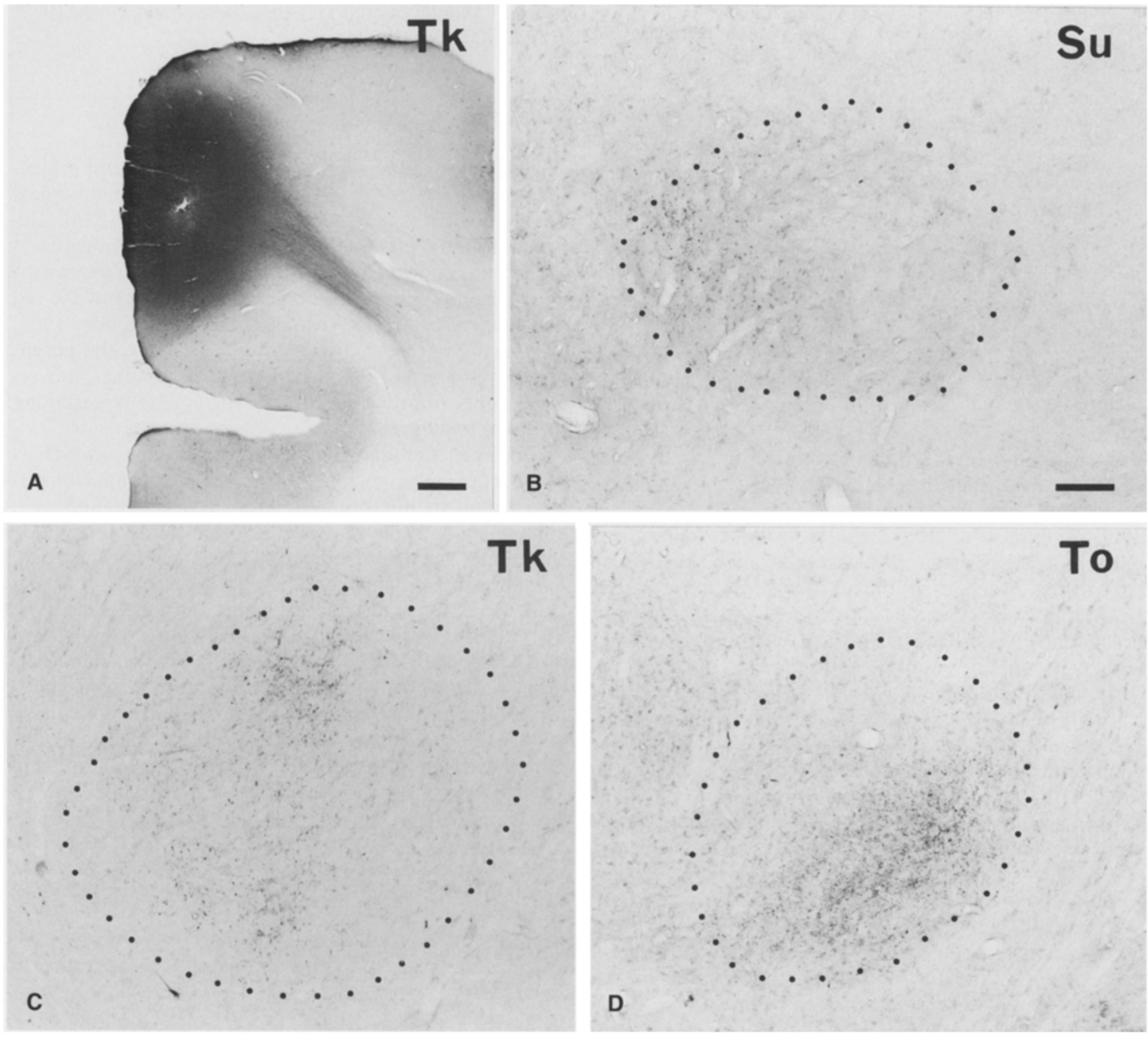

Fig. 1 **A** *Upper row* Schematic diagrams of somatotopical representations obtained from the ICMS mapping and the sites of WGA-HRP injection (*hatched circles*) in the dorsal view of the brains of Monkeys Su, Tk and To. The *letters* represent the stimulation sites where movements were elicited from the following body parts: *Li* lip, *D* digit, *S* shoulder, *E* elbow, *A* arm, *T* trunk, *H* hip, *L* leg, *F* foot. Threshold current levels (μA) in each penetration are also shown. Each *arrow* points to the center of the injection site. The *dashed line* in Monkey To indicates the boundary between areas 6 and 4. *Lower row* WGA-HRP injection sites (*blackened areas*) in projection drawings of frontal sections. (*AS* arcuate sulcus, *SPS* superior precentral sulcus, *CS* central sulcus). **B** Schematic drawings of anterogradely labeled axon terminals in the parvocellular part of the red nucleus (*Rp*) ipsilateral to the injection sites shown in **A**. The parvocellular part of the red nucleus drawn from three equidistant (720 μm apart) sections are arranged rostrocaudally in *a–c* (Monkey Su), *a'–c'* (Monkey Tk) and *a''–c''* (Monkey To). *Dashed lines* indicate the contour of the parvocellular part of the red nucleus

Fig. 2 **A** Photomicrograph showing a site of WGA-HRP injection in the forelimb region of the SMA in Monkey Tk. *Top* dorsal. *Left* medial. *Bar* 1 mm. **B–D** Photomicrographs showing anterogradely labeled axon terminals in the parvocellular part of the red nucleus ipsilateral to the injection sites. In Monkey Su, Tk or To, the labeled terminals were seen in the medial (**B**), intermediate (**C**) or lateral (**D**) part of the parvocellular part of the red nucleus, respectively. *Dotted lines* indicate the contour of the parvocellular part of the red nucleus. These photomicrographs were taken from sections adjacent to those depicted in Fig. 1Bb, b' and b''. *Top* dorsal. *Left* medial. *Bar* 200 μm for **B–D**

through a constant-current stimulator), and evoked movements were observed. After mapping of the SMA, electrolytic microlesions (anodal direct current of 10–15 μA, 20–30 s) were placed at selected sites. The electrode was then removed, and a site was selected for injection of WGA-HRP into the orofacial (Monkey Su), forelimb (Monkey Tk) or hindlimb (Monkey To) region of the SMA. A volume of 0.08–0.1 μl of a 4% solution of WGA-HRP (Toyobo, Japan) dissolved in 0.1 M Tris-HCl buffer (pH 7.0), was injected slowly through a 1-μl Hamilton microsyringe which was

attached to the same manipulator as the electrode. After a survival period of 3 or 4 days, the monkeys were deeply reanesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially with 5 l of 8% formalin in 0.1 M phosphate buffer (pH 7.3), followed by 3 l of the same buffer containing 10% sucrose and 2 l of the same buffer containing 30% sucrose. The brains were removed immediately, immersed in the same buffer containing 30% sucrose at 4°C for 2–5 days, and then cut serially at 60 μm in the frontal plane on a freezing microtome. Every third or sixth section was reacted with tetramethylbenzidine (Mesulam 1978), incubated with a 3% aqueous solution of ammonium molybdate to stabilize the reaction product (Fujii and Kusama 1984), mounted onto gelatin-coated glass slides, and counterstained with 1% Neutral Red. Histological reconstruction of both the electrode and injection needle tracks was made with the aid of electrolytic microlesions as reference points.

Results

ICMS mapping of the medial wall of the hemisphere (Fig. 1A) confirmed the somatotopical representations of the SMA as previously reported (Mitz and Wise 1987; Luppino et al. 1991): the orofacial, forelimb, and hindlimb regions of the SMA were arranged rostrocaudally in this order. Histological reconstruction of serial sections revealed that the site of WGA-HRP injection in each case was localized to the cytoarchitectonically defined Brodmann's area 6 and to the electrophysiologically identified orofacial (Monkey Su), forelimb (Monkey Tk), or hindlimb (Monkey To) region of the SMA (Figs. 1A, 2A). In the Monkey which received the injection into the orofacial region of the SMA (Fig. 1A, Monkey Su), retrogradely labeled neurons were found in the lateral part of the primary motor cortex (MI), while no labeled neurons were found in the prefrontal cortex. It was thus suggested that WGA-HRP injection in Monkey Su was localized to the SMA proper (Matsuzaka et al. 1992) or area F3 (Luppino et al. 1993), but did not infringe upon the pre-SMA or area F6, which is situated just rostral to the SMA proper or area F3. In the monkey which received the injection into the hindlimb region of the SMA (Fig. 1A, Monkey To), the boundary between Brodmann's area 6 and area 4 was determined by observing the distribution of the giant layer V pyramidal neurons (Wise and Tanji 1981; Mitz and Wise 1987); no diffusion of the tracer into area 4 was seen in this case.

Densely labeled axon terminals were seen in the parvocellular part of the red nucleus ipsilateral to each injection. After WGA-HRP injection into the orofacial, forelimb, or hindlimb region of the SMA, the labeled axon terminals were found in the medial, intermediate, or lateral portion of the parvocellular part of the red nucleus, respectively (Figs. 1B, 2B–D). In each case, the labeled axon terminals were distributed into a band which extended dorsoventrally. The labeled axon terminals were observed much more sparsely in the homotopical portions of the parvocellular part of the red nucleus contralateral to the injection. No labeled axon terminals were found in the magnocellular part of the red nucleus; the findings were consistent with previous data with retrograde axonal tracing (Catman-Berrevoets et al. 1979; Humphrey et al. 1984).

Discussion

The present results indicate that the corticorubral projections from the SMA are arranged in a somatotopical manner, which is quite similar to those from the MI: The fibers from the orofacial, forelimb, or hindlimb region of the MI are known to terminate in the medial, intermediate, or lateral portion of the parvocellular part of the red nucleus, respectively (Kuypers and Lawrence 1967; Hartmann-von Monakow et al. 1979). Thus, the parvocellular part of the red nucleus perhaps receives convergent inputs from the SMA and MI regions representing the same body parts.

The parvocellular part of the red nucleus, which is phylogenetically more developed and influential in primates than in non-primates, has been considered as a component of the dentato-rubro-olivo-cerebellar loop (for review, see Oka 1988; Ten Donkelaar 1988). The somatotopically organized corticorubral projections arising from the SMA in primates, together with those from the MI, seem to exert direct control over the dentato-rubro-olivo-cerebellar loop at the level of the parvocellular part of the red nucleus. It is thus conceivable that the dentato-rubro-olivo-cerebellar loop is also organized somatotopically, and that each somatotopical subloop may individually participate in the control of orofacial, forelimb, and hindlimb movements.

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