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Research Note

Pallidal inputs to thalamocortical neurons projecting to the supplementary motor area: an anterograde and retrograde double labeling study in the macaque monkey

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Summary. The relationship between thalamocortical neurons projecting to the supplementary motor area (SMA) and pallidothalamic projection fibers was examined with an anterograde and retrograde double labeling technique in macaque monkeys *(Macaca fuscata)*. In each monkey, Fast Blue (FB) was injected into the hand-arm area of the SMA after mapping the somatotopy using intracortical microstimulation, and horseradish peroxidase conjugated with wheat germ agglutinin (WGA–HRP) was injected into the ipsilateral internal segment of the globus pallidus (GPi). As a result, numerous projection neurons labeled with FB were distributed in pallidal terminal areas labeled with WGA–HRP in the ventral nuclear group of the thalamus. The present findings indicate that the SMA receives strong indirect projections from the GPi via the thalamus.

Key words: Supplementary motor area – Thalamus – Globus pallidus – Double labeling technique – Monkey

In recent years, it has been shown that there exist multiple cortical motor areas in the frontal lobe of primates (for review, see Wiesendanger 1981; Tanji and Kurata 1989; Wise et al. 1991). These cortical motor areas include the primary motor cortex, frontal eye field, supplementary motor area (SMA), supplementary eye field, premotor cortex and cingulate motor area. To reveal functions of these multiple motor areas, it is necessary to know which subcortical nuclei send information to each of these motor areas via the thalamus. Many anatomical studies have shown the distribution of thalamic neurons projecting to the motor areas, and also afferent terminals in the thalamus. However, there is still a great deal of controversy (see Table 1 in Darian-Smith et al. 1990) concerning the connections between the motor areas and subcortical motor nuclei such as the internal segment of the globus pallidus (GPi), the substantia nigra pars reticulata, and the deep cerebellar nuclei.

We consider that the controversy has arisen for two reasons. One reason is that the cortical injection sites of tracers were not identified electrophysiologically in most studies. Because functional and somatotopical characteristics of the cortex differ from area to area, it is necessary to examine the cortex electrophysiologically before injecting tracers. Moreover, cortical sulcal patterns alone are by no means sufficient as a criterion for defining motor areas. Recent tracing studies on fiber connections of the cortical areas related to eye movements (Huerta et al. 1987; Huerta and Kaas 1990; Shook et al. 1990) and hand movements (Luppino et al. 1990; Huntley and Jones 1991; Holsapple et al. 1991) have used intracortical microstimulation (ICMS) to identify these areas accurately.

The other reason is that previous morphological studies on thalamic connections have mainly used a single tracer to label thalamic neurons projecting to motor areas, or to label pallidal, nigral or cerebellar terminals. Due to disagreements on classification and nomenclature of thalamic subregions, it has been difficult to determine precisely the spatial relationship between projection neurons and afferent terminals. A recent study by Darian-Smith et al. (1990) made injections of multiple retrograde tracers into various areas of the cerebral cortex to analyze the spatial relationship among thalamocortical neurons directly. Their report successfully indicated important effectiveness of multiple labeling techniques in individual animals. However, a combination of anterograde and retrograde tracing technique has not been used to reveal subcortical, transthalamic inputs to the motor areas.

The present double labeling study was undertaken to confirm the pathway from the GPi to the SMA via the

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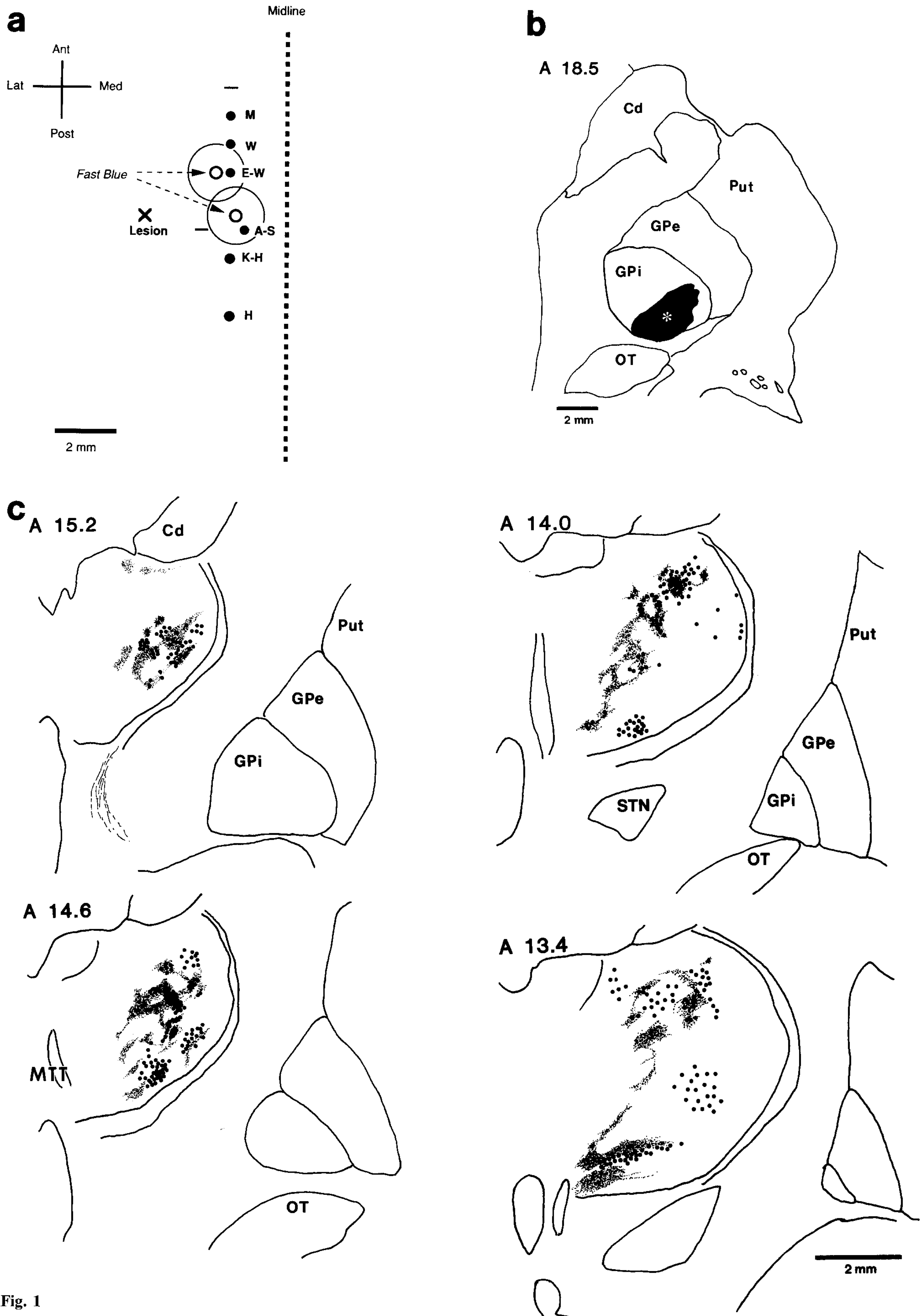

Fig. 1

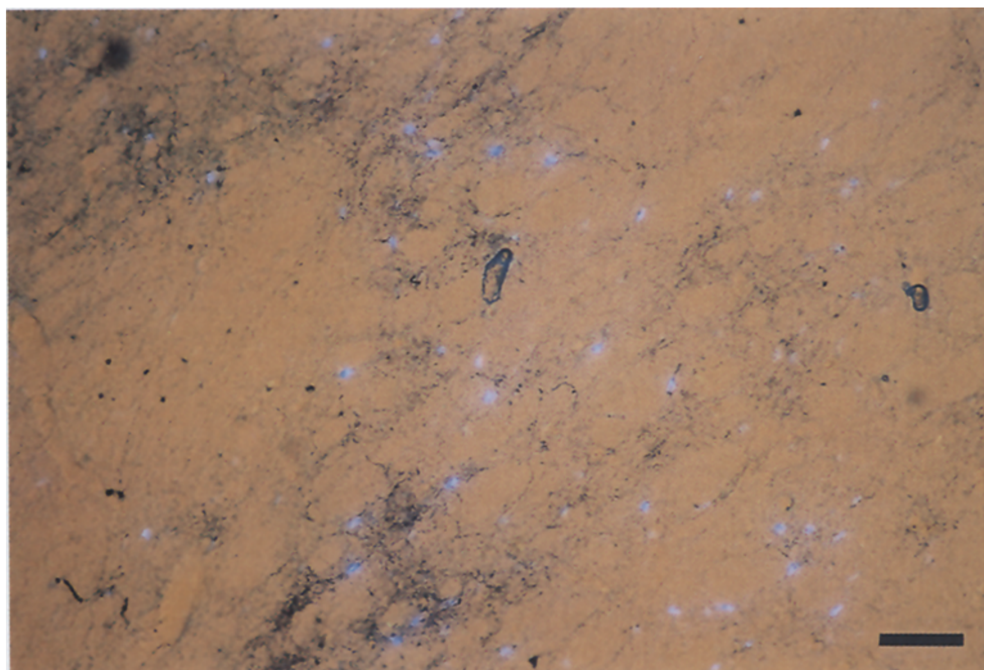

Fig. 2. Photomicrograph showing overlap of FB labeled neurons (light blue) and WGA–HRP labeled terminals (fine dark blue granules) in the thalamus. Combined epifluorescence and bright field illumination. *Top:* dorsal, *Left:* medial. Bar = 100 μm

thalamus, as the first step in a series of studies. We injected a retrograde fluorescent tracer, Fast Blue (FB), into the SMA after identifying it with ICMS, and also injected an anterograde tracer, horseradish peroxidase conjugated with wheat germ agglutinin (WGA–HRP), into the GPi in individual monkeys. We thereby expected to observe thalamocortical neurons projecting to the SMA and pallidothalamic terminals simultaneously.

Two male Japanese monkeys *(Macaca fuscata)* weighing 4.9 kg and 7.3 kg were used in the present experiment. In each monkey anesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and pentobarbital sodium (30 mg/kg, i.m.), a recording cylinder was attached to the skull under aseptic conditions, after a portion of the skull over the SMA was removed. After recovery from the anesthesia, the monkeys sat quietly in the chair during stimulation and recording. Glass-insulated Elgiloy-alloy microelectrodes, whose impedance measured 0.9-1.4 MΩ at 500 Hz, were used for stimulation as well as for recording extracellular unit activity. The cortex was stimulated through the electrode by using currents of 20–80 μA (12 or 22 cathodal pulses of 200 ms duration at 333 Hz through a constant-current stimulator), and evoked movements were observed. Afterwards, 2 μl of 5% FB (Sigma) dissolved in saline was injected into two points of the hand-arm area in the SMA using a 1-μl Hamilton microsyringe. For recording, stimulation and injection, we used a special manipulator which was designed to hold the electrode or the microsyringe in the same stereotaxic coordinate.

Two weeks later, the monkeys were reanesthetized with ketamine hydrochloride (10 mg/kg, i.m.) and xyladine (2 mg/kg, i.m.), and another cylinder was attached to the skull at an angle of 40 deg laterally from the vertical for recording neuronal activity and searching the extent of the ipsilateral GPi. After recovery from the anesthesia, the monkeys sat in the chair again. Extracellular-unit activity was recorded successively through the cerebral cortex, putamen and external segment of the globus pallidus. Neurons in the GPi were identified by both the depth profile of the microelectrode penetrations and their characteristic, sustained high frequency discharge rate (50–120 impulses)s) (DeLong 1971). A total volume of 0.08 μl of WGA–HRP (Toyobo) dissolved in 0.05 M Tris-HCl buffer (pH 7.6) was then injected into the GPi through a 1-μl Hamilton microsyringe.

After a survival period of 3 days, the monkeys were deeply anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially with 5L of 8% formalin in 0.1 M phosphate buffer (pH 7.3) followed by 3L of the same buffer containing 10% sucrose and 2L of the same buffer containing 30% sucrose. The brains were

Fig. 1a–c. Schematic representation showing injection sites of tracers (**a, b**) and resulting labelings (**c**) in a monkey. **a** Dorsal view of reconstruction of ICMS mapping and FB injection sites in the SMA. Each letter indicates penetration where movements were elicited predominantly from the following body parts. M, mouth; W, wrist; E, elbow; A, arm; S, shoulder; K, knee; H, hip; –, negative. Diffusion of FB is indicated by circles. Injections were localized in the SMA where movements of upper extremities were produced. Electrolytic lesion was placed at A = 21.0. **b** An injection site of WGA–HRP into the GPi (asterisk) in the same case. Frontal section. **c** Distribution of FB labeled neurons (filled circles) and WGA–HRP labeled terminals (fine dots) in anterior part of the thalamus. Note that substantial numbers of neurons are located in terminal fields in these sections. Frontal sections at intervals of 0.6 mm are arranged in anteroposterior order. *Abbreviations:* Cd, caudate nucleus; Put, Putamen; GPe, external segment of globus pallidus; STN, subthalamic nucleus; OT, optic tract; MTT, mammillothalamic tract

removed immediately, saturated with 30% sucrose in the same buffer at 4° C, and cut serially at 60 μm in the frontal plane on a freezing microtome. Every alternate section was processed with tetrametylbenzidine (Mesulam 1978), and mounted on gelatin-coated glass slides. The sections were examined with an epifluorescence microscope with ultraviolet excitation and/or bright field illumination. After charting neuronal cell bodies labeled with FB and terminal fields labeled with WGA–HRP in the thalamus, the sections were stained with Cresyl Violet and examined with a light microscope using a bright field illumination. We determined antero-posterior levels of the sections according to the atlas of the Japanese monkey (Kusama and Mabuchi 1970). However, we did not attempt to classify subregions of the ventral nuclear group of the thalamus, in order to avoid the confusion about nomenclature and delineation of subnuclei.

Fig. 1a summarizes the results of ICMS mapping and FB injection sites in the SMA of one monkey. Diffusion of injected FB was restricted within a part of the SMA where the forelimb was represented. In this monkey, the WGA–HRP injection site (Fig. 1b) was located in the ventral part of the GPi and no leak was observed in adjacent structures, except for a small deposit of WGA–HRP along the needle track in the external segment of the globus pallidus. These injections resulted in the labeling of both projection neurons and afferent terminals in the thalamus (Fig. 1c). Using combined epifluorescence and weak bright field illumination, we could easily observe cell bodies of retrogradely labeled neurons and anterogradely labeled terminals at the same time (Fig. 2). As shown in Fig. 1c, the distribution of projection neurons to the SMA largely overlapped with that of pallidal terminal fields at A = 13.4–15.2. Retrogradely labeled neurons were also distributed in more posterior areas of the ventral nuclear group of the thalamus at A = 12.2–12.8. Labeled terminals were not seen in these sections. In addition, labeled terminals were seen in the centromedian nucleus of the thalamus, where no FB labeled neurons were observed. The same results were obtained in the other monkey.

The present findings provide strong evidence that the hand-arm area in the SMA receives indirect pallidal inputs via the thalamus. This conclusion agrees with previous retrograde tracing studies (Schell and Strick 1984; Wiesendanger and Wiesendanger 1985a; Darian-Smith et al. 1990). However, it is still unclear whether the SMA receives other subcortical inputs via the thalamus. For instance, it is suggested that there exists a gradual shift of input sources in the SMA; i.e. an anterior portion of the SMA may receive cerebellar inputs via the thalamus (Wiesendanger and Wiesendanger 1985a, 1985b). Further double labeling studies on pallido-, nigro-, and cerebellothalamocortical connections are necessary to fully elucidate subcortical projections to the SMA.

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