Functional Organization of the Projection From Area 2 to Area 4\gamma in the Cat

MARCELLO A. CARIA, TAKESHI KANEKO, AKIHISA KIMURA, AND HIROSHI ASANUMA

The Rockefeller University, New York, New York 10021

Caria, Marcello A., Takeshi Kaneko, Akihisa Kimura, and Hiroshi Asanuma. Functional organization of the projection from area 2 to area 4\gamma in the cat. J. Neurophysiol. 77: 3107–3114, 1997. It has been shown that tetanic stimulation of area 2 of the somatosensory cortex produces long-term potentiation in neurons in area 4\gamma, and this has been suggested as the basis of learning new motor skills. The purpose of this study was to further elucidate the characteristics of this projection by the use of evoked potentials in area 4\gamma elicited by intracortical microstimulation of area 2. The experiments were carried out in cats and the following results were obtained. 1) In six animals, stimulation of a given site in area 2 elicited evoked potentials in a restricted region of area 4\gamma, the size of which ranged from 1 to 1.5 mm². These responses were labeled “localized responses.” The evoked potentials were recorded from the superficial layers of the cortex, and were positive monophasic in shape. 2) In 16 animals, stimulation of a given site in area 2 elicited a focal response that was surrounded by smaller positive and monophasic potentials of <50% amplitude that spread broadly over area 4\gamma. These responses were labeled “graded responses.” 3) The sites that produced focal evoked potentials in area 4\gamma extended along the direction of the radial fibers in area 2. These sites were defined as most effective segments (MESs). 4) The receptive fields of neurons along the MES in area 2 were similar to those of neurons recorded at the foci of the evoked potentials in area 4\gamma. The results demonstrate that some of the projections from area 2 to area 4\gamma are highly specific and that the somatosensory and motor areas that are connected by these specific projections receive functionally related peripheral input. These results are discussed in relation to possible mechanisms underlying motor learning.

INTRODUCTION

The projection from the somatosensory cortex (SCx) to the primary motor cortex (MCx) has been studied extensively both electrophysiologically and anatomically. Electrophysiological studies showed that the projection from area 2 to area 4\gamma is topographically organized (Waters et al. 1982), with neurons receiving short-latency input from area 2 located in layers II and III of the motor cortex (Kosar et al. 1985). Anatomic studies with horseradish peroxidase confirmed the topographic arrangement of the projection from somatosensory area 2 to the postcruciate region of area 4\gamma (Yumiya and Ghez 1984). A more recent study demonstrated that injection of PHA-L into area 2 of the SCx stained a few discrete bundles of projection fibers in area 4\gamma. Each of these bundles was located within a small area of the MCx, forming a columnar shape with a diameter consistent with that of the cortical efferent zones in the MCx (Porter and Sakamoto 1988).

Several studies have shown that the projection from area 2 to area 4\gamma is likely to participate in learning new motor skills (Asanuma and Keller 1991; Iriki et al. 1989; Pavlides et al. 1993; Sakamoto et al. 1987). It has been proposed that the process by which novel motor skills are acquired depends on the synaptic plasticity within the loop circuits between the motor cortex and the periphery, which include, as an important relay station, the SCx. The synaptic plasticity involved in this hypothesis is long-term potentiation (LTP), whereby high-frequency stimulation of a set of afferents produces an increase in subsequent synaptic activation. In support of this hypothesis it has been shown that tetanic stimulation of area 2 produces LTP in neurons located in the superficial layers (II–III) of area 4\gamma (Keller et al. 1990; Sakamoto et al. 1987). The recent finding of LTP in the synapses between the fibers of the pyramidal cells located in layers II–III and the giant pyramidal cells of layer V (Kimura et al. 1994) demonstrated that neurons in layers II–III in turn affect the activity of motor cortical output neurons.

Participation of SCx in motor learning has been demonstrated by behavioral studies in cats (Sakamoto et al. 1989) and monkeys (Pavlides et al. 1993) in which the acquisition of new motor skills was impaired by removal of the sensory cortex.

To obtain further information about the mechanism of motor learning, it is imperative to elucidate finer functional organization of the connections between area 2 and area 4\gamma. The present experiments were designed to examine whether the anatomically demonstrated specific corticocortical projection can also be detected electrophysiologically by recording evoked potentials in area 4\gamma following intracortical microstimulation (ICMS) of area 2. Furthermore, the physiological properties of the projection from area 2 to area 4\gamma were studied by examining the receptive fields of neurons located at the stimulating and recording sites in area 2 and area 4\gamma, respectively.

METHODS

Experiments were performed in 22 adult cats of either sex, weighing between 2.8 and 3.3 kg. These were divided in two groups. In the first group (n = 12), distribution of the evoked potentials elicited in area 4\gamma by ICMS of area 2 were studied. In the second group (n = 10), spread of the effective area in the SCx for producing focal evoked potentials in area 4\gamma as well as the receptive fields of neurons at the stimulating and recording sites were studied.

First group

Twelve animals were used. Under pentobarbital sodium (Nembutal, Abbott) anesthesia (initial dose, 35 mg/kg ip; maintenance
dose, 2 mg·kg\(^{-1}·h^{-1}\) iv), the cisterna magna was opened for drainage of cerebrospinal fluid and the motor and the sensory cortices of the left hemisphere were exposed by a craniotomy. Body temperature was kept at 38\(^\circ\)C throughout the experiment. A double-hung, closed chamber was installed on the skull surrounding the exposed cortical surface, and an array of three platinum-in-glass stimulating electrodes was inserted, guided by metal tubes that were made direction adjustable by universal joints. The electrodes were inserted through the posterior chamber into the rostral bank of the ansate sulcus to a fixed depth of 1 mm, just below the neurons projecting to the MCx (Fig. 1A). The cortical surface was photographed to map the sites of the electrode insertions. ICMS consisting of 0.2-ms negative pulses of 30 \(\mu\)A at 1 Hz was delivered sequentially through each of the stimulating electrodes.

Monopolar recordings were made from area 4\(\gamma\) (Hassler and Muhs-Clement 1964) with the use of one of two tungsten-in-glass electrodes that were inserted through the lid of the anterior chamber. One electrode was inserted perpendicularly into the cortex in 100-\(\mu\)m steps up to a depth of 1.2 mm with the use of a hydraulic microdrive. The second electrode was positioned on the cortical surface through a guide tube the direction of which could be freely adjusted by a ball joint. Only the tip of this electrode was inserted into the cortex located just beneath the pia. The distribution of the evoked potentials just underneath the surface of the MCx elicited by ICMS in area 2 was first mapped by moving the second electrode in 1-mm steps. The extent of the focal area surrounding the largest potential in the subpial surface was then examined by moving the recording electrode in smaller steps (0.2–0.3 mm) depending on the arrangement of the blood vessels on the cortical surface. The recorded evoked potentials were averaged (20 sweeps) by an online averager and stored for later analysis.

**Second group**

Ten animals were used. The femoral vein and the trachea were cannulated under ketamine anesthesia (Ketaset, Aveco, 1–2 mg/kg im). The right forelimb was shaved up to the shoulder to facilitate examination of the receptive fields. The animal was then ventilated with a mixture of 60% nitrous oxide-40% oxygen supplemented by 1–1.8% halothane (Fluothane, Ayest Labs). The brain chamber for stimulating and recording electrodes was then installed with the same procedure used for the previous group of animals. All the wounded areas were infiltrated with a long-lasting local anesthetic (Xilocaine, Astra), the gas anesthesia was discontinued, and pentobarbital sodium (Nembutal) was delivered intravenously at a rate of 2 mg·kg\(^{-1}·h^{-1}\). The animals were then paralyzed with the use of gallamine triethiodide (Flaxedil, Davis-Geck, 4 mg iv every 2 h) and artificially ventilated. Careful observation of the animals in the preliminary experiments showed that with this dose of Nembutal, the animals were quiet with occasional spontaneous movements, but without any sign of discomfort. With this level of anesthesia, it was possible to examine the receptive fields of neurons in the SCx and motor cortex.

As in the first group, the distribution of the evoked potentials over area 4\(\gamma\) was mapped first. When a focus of evoked potentials was found, the electrical activity of single motor cortical neurons around the focus was examined with the use of the hydraulic micro-manipulator. Because the platinum-in-glass electrodes used for the ICMS could also be used to record single-unit activity, stimulation and recording could be made alternately along the electrode insertion in area 2; this was performed with the use of a manual micro-manipulator attached to the lid of the posterior chamber (Fig. 1A).

The focal evoked potentials elicited by the stimulating electrode during penetration of area 2 were recorded in area 4\(\gamma\) with the use of the subpial electrode. This technique allowed us to examine the receptive fields of somatosensory neurons along the electrode penetration.

At the end of the experiment, electrolytic lesions were made

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**FIG. 1.** A: experimental setup. Intracortical microstimulation (ICMS) (0.2-ms negative pulse, 30 \(\mu\)A, 1 Hz) was delivered through 3 platinum-in-glass electrodes inserted into ansate region through lid of posterior chamber. Recordings of evoked potentials were made from area 4\(\gamma\) with the use of 2 tungsten-in-glass electrodes inserted through lid of anterior chamber.

C.S., cruciate sulcus; A.S., ansate sulcus; Rostr., rostral; Caud., caudal. B: examples of localized evoked potentials (row b, column 7) not surrounded by diffuse responses. Calibration: 2 ms, 100 \(\mu\)V, positivity upward. C: schematic drawing of motor area with amplitude histogram of evoked potentials (B). Ans. Sul., ansate sulcus; Cru. Sul., cruciate sulcus; Med., medial; Lat., lateral; ss, stimulating site.

both in the SCx and MCx by passing negative current (10 \(\mu\)A, 20 s) through the recording and stimulating electrodes. The animals were then perfused with saline, followed by 10% Formalin. The brain block including the SCx and MCx was removed, stored overnight in 30% sucrose solution, and cut sagitally on a freezing
EVOKED POTENTIALS ELICITED IN AREA 4γ BY ICMS OF AREA 2

RESULTS

Evoked potential features

In 9 of 12 animals examined, the evoked potentials in area 4γ could be recorded only from the dorsolateral bank of the cruciate sulcus. In two animals the evoked potentials were also recorded in the anterior bank, and, in one animal, in the medial portion of the posterior bank of the cruciate sulcus. The evoked potentials were typically characterized by positive monophasic waves in the superficial layers of the cortex and disappeared at a depth of 600 μm, having their peak amplitude at between 200 and 400 μm. The evoked potentials showed two patterns of distribution in area 4γ. The first pattern showed the evoked potentials located only in a small area of the cortex that ranged from 1 to 1.5 mm² diam. Of 22 animals, 6 showed this pattern of distribution (Fig. 1, B and C). These responses were called “localized responses” according to the following criteria: 1) large amplitude of the potentials within small areas of the MCx (from 1 to 1.5 mm²), with amplitude declining to <65% at the boundaries of this area; and 2) a short and consistent latency, compatible with that of monosynaptic responses (i.e., between 1.2 and 2.5 ms) observed in previous intracellular studies (Kaneko et al. 1994a). The second pattern was characterized by responses with one or two foci located within a small area (1–1.5 mm²), which, however, were surrounded by a broader distribution of smaller-amplitude (<50%) potentials. These types of responses, which showed a focus within a broader arrangement of the evoked potentials over the MCx, were classified as “graded responses” and were observed in 16 of 22 animals, (Fig. 2, A and B).

Altogether, 24 evoked potential foci were identified. An example of localized responses is shown in Fig. 1, B and C, and the responses in the rest of five animals were similar. The evoked potentials shown in Fig. 1B were recorded just below the pia membrane in area 4γ in response to ICMS in area 2. The recording electrode was moved in 1-mm steps, forming a grid (Fig. 1C). As shown, ICMS elicited responses only in a restricted area of the postcruciate sulcus with a focus located on row b, column 7 (Fig. 1, B and C).

FIG. 2. A: examples of graded responses and their distribution in area 4γ. Widespread evoked potentials with a localized focus were elicited by ICMS of a given site of somatosensory cortex (SCx; ss). B: amplitude histogram of evoked potentials shown in A. Recording sites along cruciate sulcus were separated by 1 mm. Note localized focus on row b, column 6 in both A and B. Calibration: 2 ms, 100 μV, positivity upward.
Figure 2, A and B, shows an example of graded responses. The focus of evoked potentials located on column 6, row b was surrounded by a large area where smaller amplitude potentials could be recorded. The locations of these foci within the motor area were related to the location of the stimulating electrodes along the ansate sulcus. When the stimulating electrode was positioned at the medial or lateral part of preansate gyrus, the foci were usually found in the medial or lateral part of the postcruciate gyrus, respectively. Analysis of the electrolytic lesions at the site of ICMS showed the location of the electrode tips ranging from layer III to upper layer V, whereas the lesions found in the motor cortex showed that the “surface” evoked potentials were actually recorded at depths of 30–60 μm, in layer I.

The subpial distribution of the responses around the focus of the largest-amplitude potential was examined in all the identified foci both in the graded and localized responses. A schematic drawing of evoked potentials recorded on the cortical surface from 33 different positions, ranging between 0.3 and 2 mm from the focus (F), is shown in Fig. 3A. The largest evoked potentials were confined within the inner three circles. The same analysis, carried out on a total of 12 of 24 foci of evoked potentials, revealed that they were confined within a cortical area of 1 and 1.5 mm². It was also found that the localized responses were highly sensitive to damage of the cortex, because even very small bleeding at the surface of the focal area abolished the potentials.

The latencies for the onset and the peak of the evoked potentials were analyzed both for localized and graded responses (Fig. 4). The distribution of the latencies of ~200 localized responses is shown in Fig. 4A. The majority of the responses showed onset latencies between 1.5 and 2.5 ms and peak latencies between 2.3 and 4.5 ms. The same analysis carried out on ~300 evoked potentials outside the foci and classified as graded responses showed an onset latency between 3.0 and 4.0 ms in 85% of the cases and a peak

![Figure 3](http://jn.physiology.org/)
Figure 5D shows a typical example of the results. The evoked potentials elicited by ICMS delivered at different depths along the track are shown in Fig. 5E. The sites that produced the most effective responses were confined within a segment ~1 mm in length (thick line in Fig. 5D), the boundaries of this segment being established by the amplitude of the potentials ≥50% of the largest potential within the focus. This segment was labeled the most effective segment (MES). Its extent along the track was 0.5 ± 0.1 (SD) mm when the penetration was tangential to the radial fibers (n = 15) and 1.0 ± 0.3 (SD) mm when the penetration was parallel to the radial fibers (n = 7). Additional penetrations revealed that horizontal spread of the MES was limited to an area of <1 mm². Furthermore, the histological examination revealed that the MES in 7 of 10 penetrations ranged from layer III to upper layer V.

In the experiment shown in Fig. 5B, we examined the receptive fields of six neurons, five being located within the MES. The receptive fields of three neurons are delineated by the thick solid line, whereas the receptive fields of two neurons are indicated by the dashed area. All neurons, including the one outside the MES, showed the same modality and were activated by blowing on the hair or a light touch of the skin. In Fig. 5C, the receptive fields of two neurons that were located close together (within 200 μm) are shown at the focus of the evoked potentials in area 4γ. As shown, these receptive fields were similar to those of neurons within the MES (Fig. 5C).

Table 1 summarizes the characteristics of the receptive fields of 39 somatosensory neurons recorded within the MES in eight tracks from eight animals. These receptive fields are compared with those of 26 motor cortical neurons recorded in penetrations made at the focus of the evoked potentials. The receptive fields of 16 somatosensory neurons overlapped with the receptive fields of 17 motor cortical neurons located at the focus of the evoked potentials. The receptive fields of the remaining neurons were located at the contiguous areas. With regards to the modality, all the somatosensory neurons showed the same modality and they were activated by an air puff or a light touch of the skin.

Similar features were observed in 16 of 17 motor cortical neurons, which responded to light touch of the skin. In one case, the receptive fields of two motor cortical neurons recorded along the same track responded to joint movement, i.e., extension of the second digit.

**DISCUSSION**

The distribution of evoked potentials elicited by ICMS of the SCx on the pericruciate gyrus of the cat MCx has been previously described by Kosar et al. (1985). Those researchers found that ICMS of area 2 elicited a diffuse distribution of positive monophasic potentials along the pericruciate gyrus, with the largest focus always located in the posterior bank of the cruciate sulcus. However, they could not find focal potentials and no further attempt was made to characterize these responses. The more recent anatomic finding showing “columnlike” arrays of labeled fibers in the cat MCx, after injections of a small amount of PHA-L into the rostral bank of the ansate sulcus, demonstrated the existence of a specific projection from area 2 to area 4γ (Porter and Sakamoto 1988). The present experiments were designed to

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**Fig. 4.** A: percentage distribution of mean onset latencies (filled bars) and peak latencies (hatched bars) for localized responses. B: percentage distribution of mean onset latencies (filled bars) and peak latencies (hatched bars) for graded responses. Histograms were constructed with 0.5-ms bins.
identify these specific projections electrophysiologically and to further delineate their functional characteristics, with the hope that this information may increase our understanding about the neural mechanisms involved in motor learning. The present results demonstrate that the neurons located within the foci in the MCx and SCx, which are connected by corticocortical fibers, receive input from the same or similar areas in the periphery. The results furthermore opened new perspectives in the study of motor learning, because it now becomes possible to examine the excitability changes of specific motor cortical neurons by delivering tonic ICMS to the MES in area 2.

In the previous study (Kosar et al. 1985), this specific projection could not be detected by recording the evoked potentials from the surface of the motor cortex. This was probably due to the relatively high resistance of the pial membrane, which diffused the focal potentials when recorded from outside the brain. In the present experiments monopolar recordings of the evoked potentials were performed from just below the pia. Even though the electrophysiological technique used in these experiments has a lower degree of resolution than the anatomic technique, the localized foci of evoked potentials supports the previous anatomic findings (Porter and Sakamoto 1988).

In all of these experiments, 30-μA intensity was used for ICMS. The effective spread of this current is known to be ~600 μm diam (Stoney et al. 1968). The evoked potentials recorded in the MCx were, therefore, elicited by stimulation of a tissue volume possibly limited to a single sensory column, or at most a few adjacent sensory columns. Furthermore, the finding that ICMS within a segment in area 2 that extended along the direction of radial fibers elicited larger-amplitude evoked potentials in area 4γ supports the notion that a single sensory column projects to a single motor column. As for the broad distribution of evoked potentials over area 4γ, it might be due to the intrinsic connections of neurons within the SCx. Schwark and Jones (1989) demonstrated that some pyramidal cells in the supragranular layers of the SCx have long axon collaterals that project to distant neurons within the same cortex. It is possible that stimulation of a given site in the SCx activates orthodromically and/or antidromically distant cortical neurons, which in turn project to a wide area of the MCx. Another possibility is related to the intrinsic connections between columns within the MCx.

It has been shown that neurons in the MCx send long horizontal axons within the MCx (Keller et al. 1990) and therefore it is possible that the input from the SCx spread to wider areas within the MCx.

The electrode penetration through the motor cortex showed that positive field potentials could be recorded throughout the superficial layers (I–III), having their peak amplitude in layers II–III. These data agree with previous electrophysiologic and anatomic studies (Ichikawa et al. 1985; Kosar et al. 1985) that revealed that neurons receiving
short-latency corticocortical input were located in layers II and III. Anatomic studies in which intracellular biocytin injections were used (Kaneko et al. 1994a; Keller et al. 1990) demonstrated that the majority of these cells were pyramidal cells having spines. These pyramidal cells are different from deep layer pyramidal cells, which have well-developed apical dendrites and can produce electrical dipoles (Chang 1959). The dendritic arborization of these pyramidal cells is spherical in shape (Peters and Jones 1984). It has been postulated that depolarization of neurons having a spherical dendritic arborization produces only a positive field potential outside of the arborization (Tasaki et al. 1954). It seems reasonable to assume, therefore, that the positive monophasic potentials that we have observed were generated by these pyramidal cells located in layers II–III.

An interesting finding concerns the characteristics of the receptive fields of neurons located along the MES. Previous studies demonstrated that projections from the ansate area to area 4γ are topographically organized (Jones and Powell 1968; Waters et al. 1982). The latter authors in particular studied the receptive fields of neurons antidromically activated by ICMS of the MCx and of neurons located around the site of ICMS in area 4γ. They compared the receptive fields of MCx and SCx neurons and classified these neurons into three different categories: neurons having overlapping, contiguous, and noncontiguous receptive fields. The present study confirms these data, further elaborating the preceding findings by demonstrating that the receptive fields of neurons recorded within the MES were similar to those of the neurons in the MCx to which these neurons project. With regard to the modality in our experiments, the dominating input was from the skin, although it is known that area 2 receives exteroceptive as well as proprioceptive input (Mountcastle 1957). A possible explanation of these differences could be due to the anesthetics used. It is known that the anesthetics can limit the activation of neurons by natural stimulation (Mountcastle 1957), and we used moderately deep anesthesia. Because it is known that a given motor column receives peripheral input produced by the contraction of the target muscle (Asanuma et al. 1979), it is likely that the input that arrives at a sensory column that in turn projects to a single motor column also arises from receptors activated by the contraction of muscles to which that motor column projects.

As for the functional role of this projection, it is likely to participate in learning of new motor skills. To acquire a new motor skill, repeated practice of a particular movement is necessary. Repeated practice of a particular movement sends a set of somatosensory inputs back to SCx and subsequently to those efferent zones of the MCx responsible for that movement. It is known that the projection from the ventrolateral nucleus of the thalamus to the MCx (Massion and Rispal-Padel 1973; Strick and Sterling 1974) is diffuse. It has been shown that the input from the SCx can produce associative LTP at the selected terminals of the ventrolateral nucleus (Iriki et al. 1991). Therefore the diffuse thalamocortical projection becomes specific and capable of activating selected motor cortical columns (Asanuma and Keller 1991). The specific projection from area 2 to area 4γ may therefore play an important role in the process of learning new motor skills by producing plasticity only in those selected motor cortical columns involved in the execution of a new motor skill.

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Present addresses: M. A. Caria, Institute of Human Physiology, Faculty of Medicine, Sassari University, V.le S. Pietro 43/B, 07100 Sassari, Italy; T. Kaneko, Dept. of Morphological Brain Science, Faculty of Medicine, Kyotou University, Kyoto 606, Japan; A. Kimura, Dept. of Physiology, Wakeyama Medical College, Wakayama Shi, Kyubuncho 27, 640, Japan.

Address for reprint requests: H. Asanuma, The Rockefeller University, 1230 York Ave., New York, NY 10021.

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