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Research Note

Morphological and physiological identification of neurons in the cat motor cortex which receive direct input from the somatic sensory cortex

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Summary. The population of neurons in the cat motor cortex which receives monosynaptic input from a specific functional region of the somatic sensory cortex was identified with the techniques of intracellular recording and staining with HRP. Both pyramidal and nonpyramidal cells located in the superficial layers of the pericruciate cortex responded to stimulation of the sensory cortex with short latency, excitatory postsynaptic potentials. More than half of the labeled cells were classified as pyramidal cells and the remainder as sparsely spinous or aspinous nonpyramidal cells. The characteristics of the EPSP's of the 2 groups of cells, ie. latency, time from beginning to peak and amplitude were found to vary only slightly. The results suggest that input from the sensory cortex impinges upon neurons which may in turn have an excitatory or inhibitory effect on corticofugal neurons in the motor cortex.

Key words: Pyramidal cells – Nonpyramidal cells – Cortico-cortical fibers – Sensory-motor – Intracellular recording – Cat

Introduction

The flow of information from the periphery to the motor cortex is important for the execution of fine distal movements (Asanuma and Arissian 1984). Sensory input reaches the motor cortex in part through the primary somatic sensory cortex (SI). While the function of this pathway is not fully understood, it appears to play a specific role in motor behavior. When the sensory cortex is destroyed there is a resultant slight loss of motor skills (Asanuma and Arissian 1984) and the ability to learn complex motor tasks is impaired (Sakamoto et al. 1989). This indicates that SI input is involved in the acquisition of motor skills which necessitates long lasting changes in the cortical circuitry. The mechanisms by which lasting changes in the synaptic pathway might occur are beginning to be understood. Recently, it was shown that tetanic stimulation delivered to area 2 of the cat sensory cortex resulted in long lasting facilitation of excitatory postsynaptic potentials (EPSP's) recorded intracellularly from target cells in the motor cortex (Sakamoto et al. 1987). Only those cells which received short latency input from SI were affected by the tetanic stimulation. The population of motor cortex neurons which receives direct synaptic input from area 2 in the cat was described previously as being composed of stellate cells (Kosar et al. 1985). The smooth and sparsely spinous stellate cells of the motor cortex are interneurons which are thought to form inhibitory synapses. Therefore, facilitation of their response would decrease the excitability of their target cells, some of which might be the corticofugal neurons. Recent evidence (Porter and Sakamoto 1988) suggests that pyramidal neurons, which form excitatory synapses, also receive direct synaptic input from area 2 of the cat sensory cortex. In addition, pyramidal cells in the primate motor cortex receive direct synaptic input from somatosensory cortex (Ghosh and Porter 1988). In order to determine if pyramidal cells are also postsynaptic targets of the sensory to motor relay in cats, we used the techniques of intracortical microstimulation (ICMS) and intracellular recording and staining to identify cells in MI which receive direct input from SI.

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Methods

Eleven adult cats were used for this study. They were anesthetized with Nembutal (25 mg/kg) and Ketamine (10 mg/kg) and placed in a stereotaxic apparatus. An opening was made in the skull to expose the sensory-motor cortex and a closed double recording chamber (Waters et al. 1982) was placed over the opening. The chamber was filled with mineral oil and sealed. Three tungsten-in-glass stimulating microelectrodes were inserted through the lid into the posterior chamber and lowered into the rostral bank of the ansate sulcus, near its junction with the lateral sulcus, to a depth of 1.2 mm. Their locations corresponded to the site of the forelimb representation in area 2 of the primary somatosensory cortex (SI) (Iwamura and Tanaka 1978; Waters et al. 1982). Surface potentials were recorded from area 4 of the motor cortex with a silver ball electrode in response to ICMS (30 μA, 0.2 ms) delivered to the area 2 electrodes. The responses (20 sweeps) from each location were averaged on-line and then mapped on a photograph of the cortical surface. Micropipettes filled with a 5.0% solution of HRP in 0.5 M Tris buffered KCL, were then inserted into the motor cortex at the focus of the evoked potentials. The pipettes were lowered in 5 μm steps with a micromanipulator driven by a step

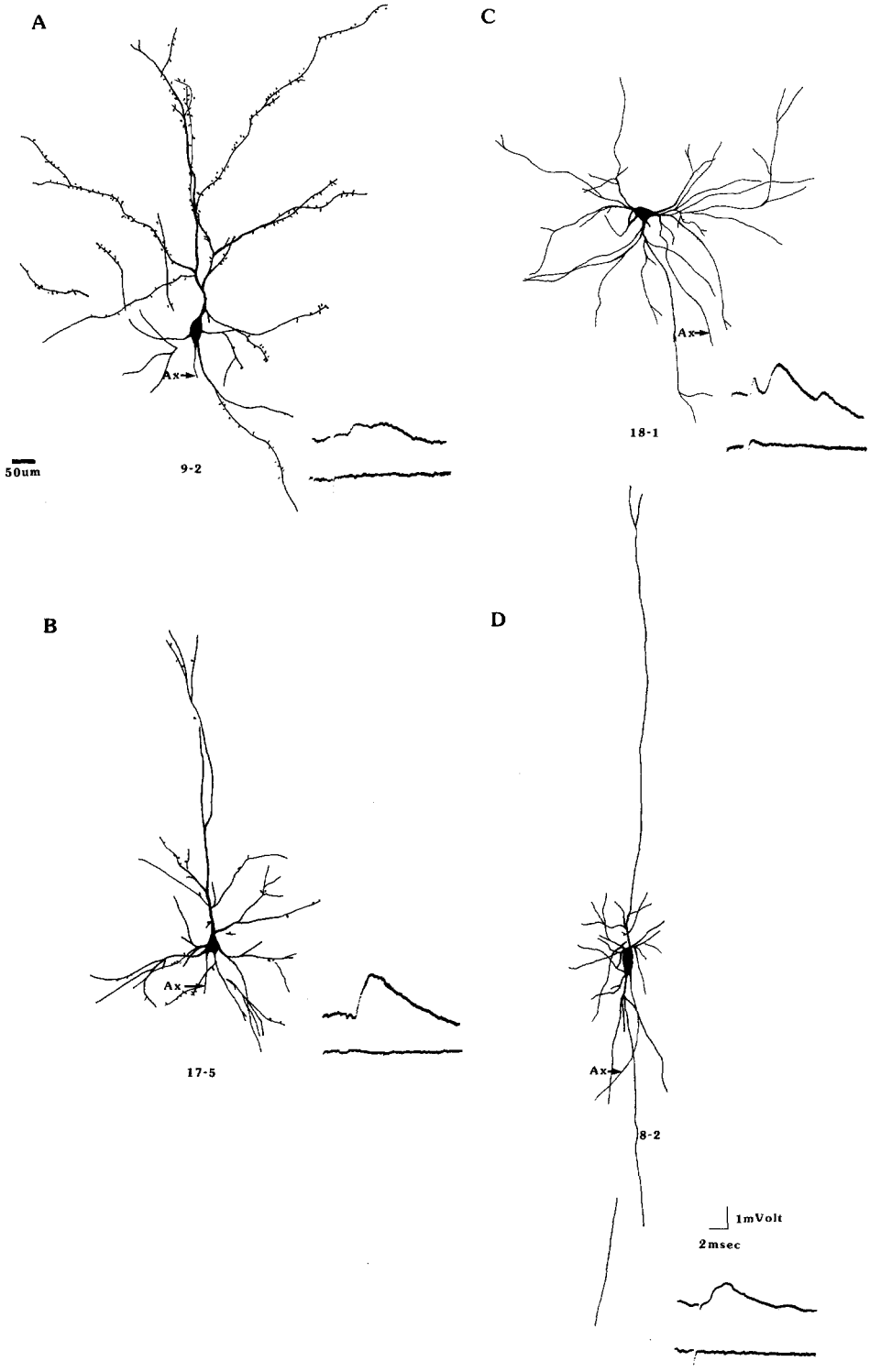

Fig. 1A–D. Camera lucida drawings of 4 cells stained by intracellular injection of HRP are shown in the figure. **A, B** Represent pyramidal cells which were located in cortical layers II and III respectively. The diameters of the cells' somata were 20–30 μm. Membrane potentials recorded prior to HRP injection were −45 mV for cell **A** and −35 mV for cell **B**. A stellate shaped cell with smooth dendrites, located in cortical layer III is represented in **C**. The membrane potential of this cell was −40 mV. The nonpyramidal cell shown in **D** exhibited an elongated soma and a bitufted dendritic morphology. The cell was located deep in layer III near its border with layer V. Its membrane potential was −60 mV. The upper EPSP traces adjacent to each cell represent 20 averaged sweeps elicited by SI stimulation and recorded intracellularly. Lower traces represent 20 averaged sweeps of the field potential recorded from the neuropil surrounding the neuron. Cell designations correspond to those in Table 1. (Ax, axon)

motor while ICMS (30 μA, 0.2 ms) was delivered through the stimulus electrodes. When injury discharges appeared after penetrating a cell, they were suppressed by passing 1–5 nA of negative current. Whenever synaptic potentials appeared in response to area 2 stimulation, the responsible stimulating electrode was identified and the responses were averaged and stored. Input to cells which responded to individual stimuli with consistent short latency postsynaptic potentials (PSP's) of less than 2.4 ms and with little fluctuation in latency to double stimuli was considered to be monosynaptic. This latency was derived by adding 0.2 ms (for a minimum synaptic delay (Watt et al. 1976)) to the antidromic response times of cells in area 2 following stimulation of the motor cortex (1.0–2.2 ms, $n=117$ (Waters et al. 1982)). Thus, the PSP's of cells included in this study, all with response latencies of less than 2.0 ms, were likely to have been elicited monosynaptically. Cells which received direct input from the sensory cortex were labeled by intracellular injection of HRP by passing 3–5 nA of positive pulsed current (50 ms, 10 Hz) for 4–6 min through the recording electrode. At the end of the experiment a negative current (10 μA, 10 s) was passed through the stimulating electrodes to create lesions so that their locations could be assessed in histological sections.

The animals were perfused intracardially with saline and then aldehydes (2.0% glutaraldehyde and 0.5% paraformaldehyde). The sensory-motor cortex was cut into 60 μm thick sections in the parasagittal plane, reacted for HRP with the cobalt-chloride enhancement of the DAB method (Itoh et al. 1979) and counterstained with cresyl violet. HRP filled cells were identified in serial sections and drawn at 100× magnification with a drawing tube. The cells were classified morphologically according to previous descriptions (Peters and Redigor 1981; Peters and Jones 1984) and their laminar locations were determined.

Table 1. The table shows the EPSP characteristics of pyramidal and nonpyramidal neurons to stimulation of area 2. Response latency (Lat) and time from beginning to peak amplitude (TTP) and mean amplitude (Amp) of the EPSP's were similar for the 2 groups of cells

Cell No.	Lat (ms)	TTP (ms)	Amp (mV)
Nonpyramidal cells			
3-2	1.7	1.0	1.5
4-1	2.0	0.8	0.3
18-1	1.8	1.5	1.3
12-1	1.5	1.4	1.1
19-1	1.2	1.4	1.3
8-2	1.1	0.9	1.3
	$\bar{X}=1.55$	$\bar{X}=1.17$	$\bar{X}=1.13$
	SD=0.35	SD=0.3	SD=0.43
Pyramidal cells			
4-2	2.0	0.9	0.4
9-2	1.8	1.4	1.3
17-4	1.8	1.1	0.9
17-5	1.5	1.2	2.8
10-1	1.7	0.8	1.0
10-3	1.5	1.0	2.3
15-3	1.0	0.6	0.2
19-2	1.4	1.5	1.7
11-2	1.6	1.3	3.0
	$\bar{X}=1.59$	$\bar{X}=1.09$	$\bar{X}=1.49$
	SD=0.29	SD=0.29	SD=1.01

Results

Stimulation of the sensory cortex elicited the largest evoked potentials in the posterolateral bank of the cruciate sulcus, an area which corresponds to the forepaw representation of area 4 (Nieoullon and Rispal-Padel 1976). The distribution of evoked potentials was topographically related to the location of the stimulating electrodes. Intracellular recording electrodes lowered into the cortex at the foci of evoked potentials encountered cells that responded to SI stimulation. Many cells located in the superficial cortical layers responded at short latency to sensory cortex stimulation and all of these exhibited excitatory postsynaptic potentials (EPSP). These EPSP's seldom elicited an action potential in the postsynaptic cell. Some cells in the deep cortical layers also were found to receive SI input. These neurons exhibited either EPSP's or, less frequently, inhibitory postsynaptic potentials (IPSP). In both instances the response latency was long and unstable and the latencies varied with changes in stimulus intensity, suggesting that these were polysynaptic responses.

More than 40 cells, which received short latency input from SI, were impaled and injected with iontophoretic current for HRP staining. However, the pipette often withdrew from the cell during the current injection, resulting in inadequate labeling. Fifteen cells were stained satisfactorily with HRP and identified histologically. The depths of labeled cells were determined from histological sections. The mean depth of these cells was 0.72 mm and all of the cells were located in superficial layers of the motor cortex.

Nine of these cells were pyramidal cells with small to medium sized somata (20–30 μm in diameter). Five cells were stellate in shape with smooth or sparsely spinous dendrites. One other nonpyramidal cell exhibited an elongate soma and an aspinous, bitufted dendritic morphology oriented perpendicular to the cortical surface. All labeled cells lie in cortical layers II and III; cells 8–2 and 4–2 were located deep in layer III near its border with layer V. Examples are shown in Fig. 1. Computer averaged sweeps of the EPSP's for individual cells were analyzed and the response properties were determined and listed in Table 1. There was no significant difference between the 2 groups of cells in latency ($T=-0.235$, $p=0.68$), TTP ($T=0.498$, $p=0.627$) and amplitude ($T=-0.858$, $p=0.41$).

Discussion

The goal of this experiment was to determine the morphological identification and location of neurons in the motor cortex which receive direct input from area 2 of the sensory cortex.

Neurons throughout the depth of motor cortex responded to microstimulation of area 2. However, in accordance with findings of Kosar et al. (1985) only those cells in the superficial cortical layers fit the predetermined criteria for receiving direct input from area 2. On the other hand, neurons which receive short latency input from area 3a of the sensory cortex are found in all laminae of the motor cortex except layer I (Herman et al. 1985). This suggests that axon terminals of cells in areas 2 and 3a differ in their distribution in motor

cortex. Thus, muscle spindle input which is relayed through area 3a (Oscarsson and Rosen 1966) is more likely to impinge directly on the output neurons in deep layers of the motor cortex whereas area 2 input may affect the output cells indirectly.

All cells with short latency responses to area 2 stimulation exhibited EPSP's. This corresponds to our previous findings that area 2 axon terminals formed asymmetric or excitatory synapses (Porter and Sakamoto 1988) with their target cells in the motor cortex. Morphological studies showed that these synapses were formed primarily with dendritic spines of motor cortex neurons (Ichikawa et al. 1985; Porter and Sakamoto 1988). Stellate cells in the motor cortex are aspinous or sparsely spinous (Ramon-Moliner 1961) and so could not account for the high percentage of axospinous contacts evident in this pathway. Therefore, it seemed unlikely that stellate cells were the sole recipients of SI afferents as reported by Kosar et al. (1985). To reconcile this discrepancy, the population of neurons targeted by SI afferents was reexamined. We have now identified 9 postsynaptic neurons as small to medium sized pyramidal cells and 6 as nonpyramidal cells. In many of these cells, perhaps because of their small size, axonal filling was incomplete. However, only those cells with adequate label extending to the distal dendritic processes were included. Therefore, it is unlikely that incompletely filled pyramidal cells, exhibiting neither apical dendrites nor dendritic spines, may have been mistakenly classified as nonpyramidal cells. In fact, the polysynaptic IPSP's exhibited by cells in the deep cortical layers are more likely to be generated by input from those multipolar cells than from superficial pyramidal cells.

The EPSP characteristics of identified cells were compared to those of unidentified cells which Kosar et al. (1985) found to be targeted by SI afferents. The time from onset to peak of the EPSP (TTP) is distorted by the high resistance of the HRP filled electrodes but amplitude and latency are thought to be unaffected (Kosar et al. 1985). These latter parameters were similar for the 2 groups of cells.

Our findings indicate that pyramidal cells, as well as multipolar cells, receive direct synaptic input from the sensory cortex. Both types of neurons exhibit local axonal ramifications (Feldman 1984; Peters and Saint Marie 1984) and so are likely to impinge upon corticofugal cells within their cortical efferent zone (Asanuma and Sakata 1967). Pyramidal cell axons form asymmetric synapses and smooth stellate cell axons form symmetric synapses (Feldman 1984; Peters and Saint Marie 1984) with their postsynaptic targets. This is evidenced by recordings of both EPSP's and IPSP's from cells in the deep cortical laminae. Therefore, it is likely that each group has a different role, one which is facilitatory and one which is inhibitory, in cortical information processing and hence, in motor performance.

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