AFFERENT INPUTS TO THE MOTOR CORTEX

An intracellular and retrograde tracer study in the monkey (*Macaca fascicularis*) and in the cat

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STATEMENT

All the work described in this thesis is original and has been carried out by me. Some experiments were carried out by me in collaboration with Prof. R. Porter, Dr. R.E.W. Fyffe and Dr. C. Brinkman. Analysis of all data, and all the writing, including papers submitted for publication, have been my work.

(Dr. S. Ghosh)
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This work would not have been possible without the companionship and moral support of my wife, Lakshmi.
This thesis is presented as a series of four related papers based on the work carried out by the candidate. The broad theme of this investigation was the study of afferent connections to the motor cortex. The first paper relates to the study of these afferents by the use of the retrograde transport from the motor cortex of horseradish peroxidase in the monkey. The second paper describes the morphology of pyramidal neurones in the motor cortex of the monkey with observations on the distribution and the synaptic effects of recurrent collaterals of pyramidal tract neurones. The third paper reports the investigation of the synaptic influences of corticocortical afferents on pyramidal neurones of the motor cortex of monkeys. Finally, the last paper reports on observations of the intracellular studies made in the motor cortex of the cat. The studies were not done in that order. The mapping of neurones projecting to the motor cortex using the retrograde transport of HRP was done first in order to obtain a detailed localization of the sources of afferent input to the primate motor cortex. It was planned that the synaptic effects of these afferent sources would then be studied in individual motor cortical neurones by intracellular techniques. However, intracellular studies were initiated first in the cat to acquire expertise in this difficult technique using a species that was more readily available. From the initial studies it became evident that much more meaningful results could be obtained if identification of the neurones impaled could be extended to include as many kinds of cortical neurones as possible. Therefore in addition to neurones identified by antidromic stimulation of the pyramidal tract, other impaled neurones were also labelled by intracellular injections of HRP. Intracellular studies in the monkey were initiated after reliable penetration and injection
of neurones could be performed confidently. The two papers in the monkey report the results of different investigations but done simultaneously in the same animals. Some of the labelled neurones are also shared between the two investigations.

To avoid excessive repetition the papers have been modified as follows. The methods used in the three papers dealing with intracellular studies were more or less similar. Therefore all the details have been mentioned only once in the methods section of Chapter III. Thereafter only additional features, unique to that investigation, have been described. All the references from individual papers have been compiled to form a common bibliography. However, some repetitions in the introductions to and the discussions of individual sections is inevitable because of the presentation of the work in a form that can be presented for publication as separate papers.

In order to link all the four papers together a general introduction and discussion has been added. In individual chapters figures and tables have been numbered separately. The spelling of words and the citation of references in the individual chapters have followed the instruction to authors from the journal to which the papers have been submitted.
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ABSTRACT

Afferent projections to the precentral motor cortex in the monkey (*M. fascicularis*) were investigated by making injections of horseradish peroxidase (HRP) in the precentral motor representation of the forelimb and by counting a third of all retrogradely labelled cells in the cortex and the thalamus. The relative numbers of neurones labelled in the thalamus and in the cortex were found to depend on the size and location of the injection site. The densest labelling was seen in the forelimb area itself, within the same hemisphere or in the contralateral hemisphere, although the forelimb area was not found to receive afferent projections from other parts of the motor cortex representing hindlimb and head movements. Afferent input from other cortical areas were dominated quantitatively by bilateral projections from the secondary motor areas in the frontal lobe (postarcuate premotor area and supplementary motor area). However, most of the motor cortical representation of the forelimb in the anterior bank of the central sulcus did not receive any callosal projections. Other corticocortical afferents originated in the somatic sensory cortical areas I and II, and the posterior parietal cortex, ipsilaterally; these projections were somatotopically organized. Ten to twenty percent of all labelled neurones were located in the ipsilateral thalamus. These thalamocortical neurones were located in nuclei VPL₀, VL₀, VL₀, and the intralaminar nuclei CM and CL. In addition, thalamic nuclei VA, X, the intralaminar nucleus Pcn, and the hypothalamus showed labelling when rostral parts of area 4 (including adjacent parts of area 6) were included in the injection site.

The synaptic influences of these afferent inputs were investigated in individual neurones in the motor cortex by intracellular techniques. Impaled neurones were
identified by antidromic activation from the cerebral peduncles or the medullary pyramids, and by intracellular labelling with HRP. Intracellular labelling also allowed detailed examination of the morphology of those neurones that were stained in their entirety. Because of their large size and preponderance in the cortex, intracellular sampling was inevitably limited to pyramidal neurones.

The morphology of pyramidal neurones located in lamina III and lamina V was studied in monkeys. Those located in lamina V (including pyramidal tract neurones) had similar morphological features. Characteristic apical, basal and lateral dendritic arborizations were seen. Intracortical collateral branches from their axons were restricted to the deeper cortical laminae (V and VI) and arborized in the vicinity of the soma as well as in more distant areas (more than a mm away from the soma, usually). Exceptionally, local collateral branches were absent. The morphology of pyramidal neurones located in lamina III differed from those in lamina V. The lamina III pyramidal cells had dendritic arborizations limited to the superficial laminae, and these arbors were similar in individual neurones. Large variations were seen in the number and distribution of axonal collaterals, however, from branching limited to the vicinity of the soma to extensive arbors more than 3 mm wide and involving all cortical laminae.

The synaptic effects of the intracortical collaterals of PTNs were investigated in the same experiments, by antidromic volleys set up in the cerebral peduncles or the medullary pyramids. These investigations revealed extensive interactions between PTNs themselves, and between PTNs and other pyramidal neurones labelled in lamina III and V. Fast PTNs were excited by recurrent collaterals of other fast PTNs or slow PTNs, slow PTNs could be excited or inhibited by other PTNs (fast or
slow). Pyramidal neurones in lamina V identified only by their morphology and laminar location could also be either excited or inhibited by recurrent collaterals of PTNs, while lamina III pyramidal cells were inhibited by these collaterals.

Corticocortical synaptic influences on precentral cells originating from the postarcuate premotor area and the somatosensory cortical areas, were also studied in the same experiments as above. Bifocal cortical surface stimulation in these areas evoked monosynaptic epsps in pyramidal neurones located in lamina III and lamina V (including PTNs). These epsps were evoked at about the same latencies in these two groups of neurones and from the two cortical areas stimulated. These epsps were often followed by epsps of long duration (50 to 200 msec). Rarely only epsps without the preceding epsps were observed in pyramidal cells.

Intracellular studies of motor cortical neurones were also made in the cat. In these studies, neurones were identified by antidromic activation from the cerebral peduncles and by intracellular labelling with HRP. The detailed morphology of pyramidal neurones in all cortical laminae except lamina I were examined. Considerable variations in the morphology were found between pyramidal neurones located in different laminae, but neurones located in the same lamina were similar. All pyramidal neurones, except some in lamina VI, had apical dendrites terminating in lamina I. Lamina II pyramidal neurones were found to be atypical, they looked like spiny multipolar neurones. The axons of pyramidal neurones located in lamina II and VI were not found to give off any intracortical collaterals. Intracortical collaterals of lamina V pyramidal cells (including PTNs) were restricted to laminae V and VI, whereas those of lamina III pyramidal cells were found to arborize more widely in
all the cortical laminae.

Synaptic influences of the recurrent collaterals of PTNs were investigated in impaled neurones by antidromic volleys set up in the cerebral peduncles. Fast PTNs, slow PTNs, other pyramidal neurones in lamina V, and pyramidal neurones in lamina VI were found to be excited by the collaterals of PTNs at similar latencies; in some pyramidal neurones (all slow PTNs) an ipsp of long duration was the only response. Synaptic effects of thalamocortical afferents were also investigated in these neurones by stimulation of the ventrolateral nucleus of the thalamus. These afferents were found to excite, monosynaptically, pyramidal neurones located in lamina III, V (including slow and fast PTNs), and VI, at similar latencies.

In conclusion, the morphology of pyramidal neurones located in different laminae of the motor cortex of the cat and monkey, were described in detail. These descriptions revealed considerable differences in the morphological characteristics of pyramidal neurones in different laminae suggesting differences in intracortical connectivity. Less variation was seen of these characteristics among pyramidal neurones of a particular lamina. Some aspects of the interaction between these projection neurones through their intracortical collaterals were studied by activating collaterals of PTNs. Extensive connectivity was revealed between individual PTNs, and between PTNs and pyramidal neurones located in the same and different laminae, involving both excitatory and inhibitory effects. Extrinsic afferents from a number of sources were shown to excite pyramidal neurones in different laminae monosynaptically. This response was often followed by inhibition. It is suggested that while excitatory drives from extrinsic afferents activate projection neurones at different
laminar levels within the cortex, perhaps reinforced by intracortical recurrent excitation, inhibition, generated by intracortical collaterals as well as by corticocortical afferents, may provide sculpturing of the cortical output.
GENERAL INTRODUCTION

The earliest experimental studies of the motor areas of the cerebral cortex involved electrical stimulation to produce movement. These electroanatomical studies of the motor outflow from the brain provided the first clues regarding the close association of cortical motor areas with motor centres in the brain stem and the spinal cord. Sherrington (1906) referred to the zone of cortex which had this close association with the spinal cord motor machinery as the cord area of the cerebral cortex (see Phillips and Porter, 1977, for a description of the historical background). Since the earlier studies using stimulation methods, interest has focussed on the detailed organization of the connections between this area of the cortex and other brain regions and these have been studied extensively using electrophysiological and neuroanatomical techniques.

Of the descending motor projections from the cortex the corticospinal tract has been studied most rigorously. In the cat, the cells of origin of corticospinal fibres are restricted to the motor cortex (58.5%), somatic sensory cortex (34.5%), and area 5 (4.5%) (Groos et al., 1978). In the monkey, corticospinal cells are located mainly in area 4 (50.8%), area 6 (12.2%), somatic sensory cortex (13.2%), and posterior parietal cortex (11.4%) (Toyoshima and Sakai, 1982). In humans, an even greater proportion of the pyramidal tract (60%) arises from area 4 (Jane et al., 1967). The distribution and termination of corticospinal fibres differs in different mammals. Armand (1982) has distinguished four groups of mammals in this context. In one group (e.g. rabbit, goat) the fibres extend as far as the cervical or midthoracic level of the cord and terminate chiefly in the dorsal horn. In a second group (e.g. dog, cat, rat) the fibres extend throughout the spinal cord and terminate in the dorsal
horns and the intermediate zone of the spinal grey matter. In a third group (e.g. monkey, raccoon) the fibres also extend throughout the length of the spinal cord and terminate in the dorsal horn and intermediate zone. However, in addition, fibres in these animals terminate in the ventromedial part of the ventral horn and in the dorsolateral motoneuronal cell group. Finally, in a fourth group (e.g. chimpanzee, humans) these fibres in addition terminate more profusely among the ventromedial and dorsolateral motoneuronal cell groups. In all species, the cells of origin of the projections to the dorsal horn appear to be located predominantly in the somatosensory areas of the cortex, whereas those projections to the intermediate region and the ventral horn are predominantly from the motor cortex (Armand, 1982).

The progressive expansion of the distribution and termination of corticospinal fibres into the intermediate zone and ventral horn in "higher" mammals is clearly associated with increasing motor skills. In those primate species, where a proportion of corticospinal fibres make direct monosynaptic connections with motoneurones (Phillips and Porter, 1964) these projections are critically important for the dexterity and manipulative ability of the distal joints of the limbs (Lawrence and Kuypers, 1968; Lawrence and Hopkins, 1976). Corticospinal fibres terminating in the dorsal horn are probably involved in the control of ascending sensory pathways (Lundberg, 1964). Those fibres that terminate in the intermediate zone influence interneurones in segmental reflex pathways (Lloyd, 1941; Preston et al., 1967) and propriospinal neurones (Illert et al., 1974). In primates, corticomotoneuronal fibres bypass segmental reflex pathways. Bypassing the interneurones is probably of crucial importance, since individual interneurones distribute collateral branches to many motoneuronal
species (Jankowska and Roberts, 1972) and may therefore disseminate their influence over a relatively large population of neural elements. Selection of particular motor outputs may be possible by means of corticomotoneuronal actions which do not have to operate through this interneuronal distributing system.

Comparison of the terminal distribution within the spinal cord of single fibres of descending projections from phylogenetically older regions of the brainstem (reticulospinal, and projections from the nucleus raphe magnus and ventrolateral pontine tegmentum) to those of corticospinal projections show that single corticospinal (and rubrospinal) fibres have a more restricted distribution. In cats and monkeys, about 66% of individual reticulospinal fibres, 40 to 60% of fibres from the nucleus raphe and about 30% of pontospinal fibres provide collaterals to both the cervical and lumbar enlargements (Huisman et al., 1982), whereas very few individual corticospinal fibres are distributed to both enlargements (Shinoda et al., 1976; Hayes and Rustioni, 1981). Within one spinal enlargement of the monkey, single corticospinal axons distribute collaterals to different segments and one or more motor nuclei (Asanuma et al., 1979; Shinoda et al., 1979). It is possible for a single corticomotoneuronal fibre to have a "muscular field" including more than one muscle (Fetz and Cheney, '78), but those that synapse on motoneurones of intrinsic hand muscles may have a field restricted to only one muscle (Lemon and Muir, 1983). Each corticomotoneuronal fibre makes very few synaptic contacts with any one motoneurone (Lawrence et al., 1985). It seems likely that the specific connectivity of corticomotoneuronal projections may be even denser in anthropoids and in humans.

These, and other studies on the corticospinal projections
in different mammals, indicate that, during evolution, there is a progressive increase in the power and specificity of cortical control over spinal motor centres. This is reflected in the motor skills possessed by the animal and in the motor deficits that occur after interruption of this pathway.

The pyramidal tract is made up of fibres which have a wide range of diameters and conduction velocities. In the cat most of the fibres are thinner than 2 μm and only 6% of fibres are thicker than 6 μm (Van Crevel and Verhaart, 1963). Their conduction velocities range from 7 to 70 m/sec (Lance and Manning, 1954). The two peaks in the range of conduction velocities, at about 14 m/sec and 42 m/sec, led to the differentiation of slow and fast pyramidal tract neurones (PTNs). These two classes of PTNs have also been distinguished with respect to the passive electrical properties of their soma membranes (Takahashi, 1965). This concept of the existence of two separate classes of PTNs was strengthened by differences observed in the interactions between these two groups through their recurrent collaterals. It was found (in the cat) that fast PTNs are monosynaptically excited by the recurrent collaterals of slow PTNs while slow PTNs are disynaptically inhibited by the recurrent collaterals of fast PTNs (Armstrong, 1965; Takahashi et al., 1967). It is not known whether these two classes of PTNs, divided with respect to the different conduction velocities of their respective axons (in the cat the division being placed at 20 m/sec), also have similar distinguishing characteristics in the primate.

Cortical projections to the brainstem include those terminating on relay nuclei of ascending spinocortical pathways (trigeminal nucleus and dorsal column nuclei), on precerebellar nuclei (the inferior olivary nuclei, pontine nuclei and lateral reticular nucleus), and on
cells of origin of descending brainstem pathways (red nucleus and cells in the medial reticular formation). Electrophysiological and anatomical studies in the cat and monkey indicate that only a small proportion of these terminations are provided by collaterals of corticospinal axons (Endo et al., 1973; Jones and Wise, 1977; Rustioni and Hayes, 1981; Berrevoets and Kuypers, 1975; Humphrey and Corrie, 1978; Catsman-Berrevoets and Kuypers, 1976; but see Bentivoglio and Rustioni, 1986). These projections from the motor, sensory and parietal cortical fields to the brainstem have been studied in less detail than corticospinal projections.

Cortical projections to relay nuclei of ascending spinocortical pathways arise predominantly from somatosensory cortical areas. Corticonuclear axons enter the dorsal column nuclei and influence primary afferent axons (Andersen et al., 1964a), interneurones and second order relay neurones (Andersen et al., 1964b). These projections provide the anatomic basis for interactions between cortical output and afferent input, and may be important in tactile and stereognostic exploration, in maintaining awareness of the positions of parts of the body and of the direction, range and velocity of movements (Phillips and Porter, 1977).

The axons of slow and fast pyramidal tract neurones (PTNs) have been shown to send collaterals into the red nucleus; fast PTN collaterals inhibit rubrospinal neurones disynaptically whereas slow PTN collaterals excite rubrospinal neurones monosynaptically (Tsukahara et al., 1968). Apart from PTN collaterals the red nucleus also receives a separate projection of corticorubral axons; their synaptic influences remain to be investigated in detail. Corticoreticular fibres monosynaptically excite the large cells in the pontine and bulbar reticular formations which give rise to the
reticulospinal tract (Magni and Willis, 1964).

The effects of electrical stimulation of the motor cortex have been investigated in monkeys following pyramidotomy (where these effects must now be largely mediated by the corticoreticulospinal and corticorubrospinal pathways). Thresholds for producing movements at the periphery are increased and the responses never involve the distal joints of the forelimb and hindlimb (Woolsey et al., 1972). Observations in animals with bilateral pyramidotomy support conclusions regarding the considerable importance and functional competence of pathways other than the pyramidal tract in motor control: after a brief period of flaccid paresis of the extremities most motor deficits gradually diminish, and only the capacity to execute relatively independent finger movements is never recovered (Lawrence and Kuypers, 1968).

Projections from the frontal and parietal cortices to the precerebellar nuclei are part of the cerebro-cerebello-cerebral loops involved in the execution of and in the programming of motor tasks (Wiesendanger, 1983; Allen and Tsukahara, 1974). Projections from large areas of the neocortex to the basal ganglia are also involved in motor control. Recent studies indicate that loops from the neocortex to the basal ganglia, thalamus and back to the cortex are segregated throughout the pathway with regard to information relevant to motor and other complex functions (Delong et al., 1983) and that, within these two divisions, there is a finer grain for movements of individual body parts (leg, arm, face etc.) and for more complex behaviour (e.g. delayed alternation, delayed response, object reversal).

The study of neuronal activity in the motor areas of the cerebral cortex in conscious animals has provided new
insights into cortical motor control. Activities of individual neurones have been found to be related to specific movements at the periphery. This neuronal activity precedes the earliest indicators of movement (e.g. electrical events associated with muscle activity) by 50 msec or more in the motor cortex; activity of neurones in the somatic sensory cortex usually occurs after the onset of movement (Evarts, 1972). Motor cortical neurones are influenced at short latency by sensory receptors in the periphery, mainly by joint movement and muscle palpation (Lemon and Porter, 1976). There is often a close somatotopic relationship between this afferent input and the movement to which neuronal activity is related (Lemon et al., 1976). This short latency peripheral input to the motor cortex is carried through the dorsal columns (Brinkman et al., 1978) and relays in the thalamus (Horne and Tracey, 1979; Lemon and Van der Burg, 1979). Neuronal activity in the motor cortex has also been found to be related to anticipation and preparation for movement (Evarts and Tanji, 1976).

Many studies have related the activity of individual neurones in the motor cortex to different parameters of movement: force, rate of change of force, limb position etc. (Evarts, 1968; Cheney and Fetz, 1980; Smith et al., 1975; Hepp-Reymond and Diener, 1983). These studies indicate that the relationships between movement parameters and neuronal activity in the motor cortex vary for different neurones (including PTNs) and for the same neurone when different motor programmes are involved. Even in an unchanging motor task, temporal correlations between activity of precentral cells and motor responses (muscle activity, force, limb position) may be altered by reinforcement (Fetz and Finocchio, 1972). It is also relevant to add here that often the most intense activity of motor cortical neurones is seen in association with fine movements of the fingers (Lemon and Muir, 1983). The
patterns of activity of motor cortical neurones during rest and movement also differ in different neurones - phasic, tonic and ramp discharges and combinations of these have been reported in relation to steady state and dynamic motor performance (Cheney and Fetz, 1980; Evarts, 1964). Neuronal discharges in the motor cortex are affected at short latency by perturbations imposed on motor tasks (Evarts, 1981), confirming Phillips's (1969) proposal that there may be a transcortical loop functioning to automatically sense errors occurring during precisely controlled movement. The above brief survey of a variety of studies serves to emphasize the complex relationships that exist between motor cortical activity and the movements they influence.

Afferent synaptic influences to the motor cortex originate from other cortical areas as well as the thalamus. The synaptic inputs delivered to the motor cortex by thalamocortical and corticocortical afferents will determine to a large degree the spatial and temporal correlates with movement performance of neuronal activity in the motor cortex because the outputs from particular cells in particular locations will be generated by the sum of these inputs.

The details of the anatomical pathways that provide synaptic input to the motor cortex have been investigated in the monkey by the use of axonal degeneration and newer tracing methods. Projections to the precentral motor area originate from the postarcuate premotor and supplementary motor areas bilaterally, from the homologous primary motor area contralaterally, and the somatic sensory and posterior parietal cortical areas as well as the thalamus ipsilaterally (Jones et al., 1978; Jones et al., 1979b; Jenny, 1979). Thalamic nuclei that project to the precentral motor area are targets of ascending somatosensory pathways as well as projections from the
cerebellar nuclei (Asanuma et al., 1983b). Taken together these studies indicate a large variety of afferent sources of input to motor cortical neurones. These include somatosensory feedback pathways from the thalamus and the somatosensory cortical areas, cerebellar projections associated with the coordination and programming of movements, and pathways with more complex functional associations from the secondary motor areas in the frontal lobe. Studies of the functional associations of the neurones of the secondary motor areas suggest that these neurones are influenced by a variety of sensory modalities (Jones and Powell, 1970; Bignal and Imbert, 1969) and may be involved with control of movement performance in response to external as well as internal cues (Roland, 1984). However, the cell to cell connectivities that are established and the excitatory or inhibitory influences that are exerted by such afferents on individual neurones in the motor cortex are not understood in detail.

There are some electrophysiological studies of the synaptic influences from afferents on cells of the motor cortex of the cat. Thalamocortical fibres have been shown to excite slow and fast PTNs monosynaptically (Deschenes et al., 1982) as well as other pyramidal neurones (Kosar et al., 1985). Corticocortical fibres from the somatosensory cortex excite neurones throughout the depths of the motor cortex (Herman et al., 1985). In most cases, however, the identity of neurones postsynaptic to corticocortical and thalamocortical afferents in the motor cortex is not known.

Anatomical studies have revealed the vast variety of morphologically distinguishable neurones that comprise the cellular elements of the cerebral cortex. These neurones have been loosely divided into pyramidal and nonpyramidal neurones. Pyramidal neurones are the
projection neurones of the cortex, whereas nonpyramidal neurones have a restricted field of action within the cortical region in the vicinity of their somata and dendrites. Detailed studies of the morphology of pyramidal neurones (reviewed by Feldman, 1984) and of nonpyramidal neurones (reviewed by Fairen et al., 1984), especially by the newer intracellular labelling techniques, have revealed the extensive arborizations of the dendritic and axonal collateral branches of individual neurones. However, studies of the morphology of neurones in the motor cortex have been few, and restricted to PTNs (Deschenes et al., 1979b; Landry et al., 1984). Very little is known about the intrinsic connectivity of cortical neurones. It is likely that much more knowledge about morphology and intracortical connectivity of neurones will be necessary before we can begin to describe how the motor cortex processes information, how neurones in the motor cortex group to form functional units or modules, and what is the dynamic interplay between neurones of a module and between the modules themselves which must accompany the performance of even a single movement of a digit.

In the studies to be described in this thesis, an attempt has been made to investigate the afferent inputs to cells in the motor cortex using transport of retrograde tracer back to areas of input projection and intracellular recording techniques for single cells. To identify neurones that were recorded from intracellularly, these cells have been labelled by intracellular iontophoresis of horseradish peroxidase. This approach has also enabled examination of the morphology of those neurones which were filled completely by the tracer injected. The detailed examination of morphology of individual neurones is related to the study of afferent inputs. A part of the synaptic input to cortical neurones is provided by the axon projections from other neurones in the cortex
itself. Further, the dendritic morphology of the neurone determines the spatial dimensions over which afferent axons may exert their synaptic influence on that cell.

Most of the work was done in monkeys (Macaca fascicularis). Intracellular studies of motor cortical neurones were also made in the cat. The results of our studies in the cat are also included in this thesis, because the observations that were made are relevant to the overall understanding of organization of the motor cortex in mammals and because some new findings resulted which allow a more complete description of pyramidal cells in the motor cortex than has hitherto existed. However, the work on monkeys is presented first, in Chapters III to V inclusive, while the studies on the cat have been assembled in Chapter VI.
A Quantitative Study of the Distribution of Neurons Projecting to the Precentral Motor Cortex of the Monkey (*M. fascicularis*)

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ABSTRACT

The relative numbers and locations of neurons projecting to the "forelimb" region of the precentral motor cortex were studied in three monkeys by using the retrograde transport of horseradish peroxidase. Within the forelimb area of the motor cortex itself, there are extensive and profuse interconnections. However, regions within this area receive afferents from very few neurons in other parts of the motor cortex representing hindlimb or head movements. Most of the motor cortical representation of the forelimb in the anterior bank of the central sulcus is devoid of callosal connections.

In both the ipsilateral and contralateral hemispheres, the premotor (lateral area 6) and supplementary motor (medial area 6) areas dominate quantitatively the inputs to the motor cortical representation of the forelimb. The afferents from the premotor area are restricted and come from a region immediately behind the arcuate spur and adjacent parts of the superior and inferior limbs of the arcuate sulcus in the floor, caudal bank, and caudal lip of that sulcus. From the supplementary motor area (SMA), afferents originate from its whole rostrocaudal extent. Thalamic nuclear regions projecting to a restricted zone in the anterior bank of the central sulcus are recipients of cerebellar and somatosensory outputs. Involvement of more anterior parts of the motor cortex by the tracer labels thalamocortical cells, which are targets of pallidal output also. Within the first somatosensory cortex, cytoarchitectonic areas 1, 2, and 3a project to area 4. The projection from area 3a may provide one pathway by which short latency peripheral inputs, especially from muscles, reach the motor cortex.
INTRODUCTION

Studies of the activity of individual neurons in the primary motor cortex (Brodmann's area 4) of conscious monkeys have shown that such activity may be closely related to both the preparation for and the execution of movements (Evarts, '81). It is also clear that the postarcuate premotor area and the supplementary motor area (medial part of area 6) are closely associated with these activities (Wiesendanger, '81; Brinkman and Porter, '83). These areas project bilaterally to the precentral motor cortex (MI), and symmetrical areas of the frontal lobes project to the motor cortex of each hemisphere (Muakkassa and Strick, '79; Matsumura and Kubota, '79; Kunzle, '78). However, quantitative data regarding afferents from these areas to the motor cortex, both in relation to other projection areas and to precentral topography, are lacking. Such information would be useful in understanding corticocortical relationships and the anatomical basis of motor activity. Within the premotor cortex, the region of the caudal bank of the arcuate spur is found to project to the "forelimb" motor area. However, neurons related to movement tasks performed by the forelimb have been located behind the arcuate "spur" (Brinkman and Porter, '83) as well as caudal to the superior limb of the arcuate sulcus (Wise and Mauritz, '85). Therefore, more extensive regions of the premotor area may be involved in control of forelimb movement. Activity of neurons in the premotor area may also be related to motor set and responses to visual signals (Wise and Mauritz, '85). Activities of neurons in the supplementary motor area (SMA) are related to movement performance and recognition of visual signals (Brinkman and Porter, '79) as well as movement planning (Roland, '84) and bimanual coordination (Brinkman, '84). These studies and stimulation experiments (Macpherson et al., '82) show that a very large proportion of the SMA may be
involved in the control of forelimb musculature. In both the premotor and supplementary motor areas, most neurons are related to movements performed by either hand in contrast to the primary motor cortex, where the relationship is mostly contralateral (Porter, '83).

Asanuma et al. ('83a) have defined a region in the thalamus comprising the nuclei VPL, VL, and X, which is characterized by a loose cell-sparse appearance and which contains small cells interspersed among larger cells. Cells of this region of the thalamus are retrogradely labeled following HRP injections in the motor cortex. These cells are targets of projections from the deep cerebellar nuclei (Asanuma et al., '83b) and from ascending somatosensory pathways in the spinal cord (Horne and Tracey, '79; Lemon and Van der Burg, '79). It has been stated that the nucleus VPL projects mainly to the more caudal regions of area 4, whereas the nuclei VL and X project to rostral parts of area 4 (Jones et al., '79b) as well as to arcuate premotor (Kunzle, '78) and supplementary motor areas (Wiesendanger and Wiesendanger, '85). Thus there may be a considerable overlap of thalamic territories projecting to the motor, premotor and supplementary motor areas (see Schell and Strick, '84) and the quantitation of thalamocortical inputs to area 4 could help to describe the extent of this overlap.

The corticocortical projection from the somatosensory cortex to the motor cortex arises mainly from area 2 (Jones et al., '78). Although connections have been reported between area 3a and 4 in the cat (Zarzecki and Asanuma, '79), they have been described only rarely in monkeys (Godschalk et al., '84). It is also not clear whether short-latency peripheral responses recorded in the motor cortex are relayed in the somatic sensory area (Evarts, '81). These responses have been shown to persist unchanged when area 2 and adjacent parts of area 1 and 5
have been cooled (Brinkman et al., '85). Similarly, surface potentials recorded from the motor area following stimulation of peripheral nerves do not change in amplitude and latency following ablation of the postcentral somatosensory cortex (Malis et al., '52). In all these experiments, area 3a, which has been shown to receive afferents from muscle receptors (Phillips et al., '71), has been left intact. Thus area 3a could be a potential relay for short latency somatosensory stimuli to the motor cortex.

Jones et al. ('78) found that corticocortical cells projecting from somatosensory, motor and parietal areas were aligned in mediolaterally oriented, discontinuous strips of variable length but relatively constant width (0.5 to 1 mm). Similar strips were also formed by callosal cells of the somatosensory and motor areas (Jones et al., '79a). On the other hand, Jenny ('79) noted that callosal projections were oriented in anteroposteriorly directed strips in the contralateral motor cortex. Such strip like patterns of connections may affect the quantitation of connections using small injections of tracer, as has been attempted in this paper, because of variable involvement of the strips. These errors may be minimized by avoiding large gaps between the cortical sections analyzed.

There are no precise, quantitative descriptions of the distribution of cells projecting within the motor cortex itself. After making lesions of the full thickness of the motor cortex using fine needles, Gatter and Powell ('78) described a cylindrical zone of diameter 400 μm to 600 μm surrounding the lesion, which showed intense finely granulated degeneration, whereas a further zone of 4 to 6 mm diameter (greater anteroposteriorly than mediolaterally) showed a moderate degree of terminal and fiber degeneration. Even greater connectivity in the
mediolateral dimension within the motor cortex has been described by Pandya and Vignolo ('71), implying interconnections between lower limb, trunk, upper limb, and face regions of representation. Such connections have been denied by other studies (Matelli et al., '84; Jones et al., '78; Godschalk et al., '84). It was expected that the present quantitative study would help to detail these connections more precisely.
METHODS

Three adult crab-eating monkeys (Macaca fascicularis), M₁, M₂, and M₃, weighing 1.92 kg, 1.66 kg, and 4.03 kg, respectively, were used for the experiments. The animals were anaesthetised using ketamine (10 mg/kg) and xylazine (1 mg/kg), injected intramuscularly and supplemented at regular intervals during surgery. Antibiotics were administered routinely.

HRP injection

A solution of 50% horseradish peroxidase (HRP, Sigma) and 5% lysolecithin in saline was used for the injections. The anaesthetised animal was fixed in a stereotaxic frame and a trephine hole made over the precentral primary motor cortical representation of the forelimb in the cerebral cortex. The dura was reflected and a Hamilton syringe (1 μl or 5 μl) containing the HRP solution was lowered onto the cerebral surface using a micromanipulator. In animal M₁, a single injection of 0.6 μl of HRP was made in the anterior bank of the central sulcus, behind the spur of the arcuate sulcus (left hemisphere). In animal M₂, a similar injection was made in the right hemisphere. In animal M₃, 3 injections of HRP, each of 0.6 μl, were made in the left hemisphere starting in the anterior bank of the central sulcus behind the arcuate spur and continuing at 1 mm intervals medially. It was hoped that in animal M₃ a major extent of the "forelimb" region in area 4 would be injected with the tracer. In all cases the needle of the Hamilton syringe was angled posteriorly and the injections made at a depth of 3 mm in the cortex of the anterior bank of the central sulcus. Since our injections were made in the anterior bank of the central sulcus, we did not attempt to try to stimulate the surface of the brain to obtain electroanatomical correlates of movements evoked. It was
also thought inadvisable to make repeated tracks in the cortex for intracortical microstimulation. The injections were made slowly (0.1 μl every 5 minutes) and the needle was withdrawn gradually (1 mm every 5 minutes). The dura and skin were then sutured and the animal returned to the cage. In addition to these animals, the brain of a fourth monkey (M. fascicularis), M₄, was available for study. In this animal 3 injections, each of 0.3 μl, of 2% HRP-WGA (Sigma) had been made in the motor cortex, in the same area as in animal M₃.

**Histological procedure**

Forty-eight hours after the HRP injection, the animals were perfused through the heart, initially with one litre of saline followed by two to three litres of a mixture of glutaraldehyde (2.5%) and paraformaldehyde (1%) in phosphate buffer. The cranium and underlying dura were removed and the head soaked in cold fixative overnight. Following fixation, the brains were removed from the skull, photographed, cut in half, and soaked in cold sucrose solution in phosphate buffer (in 10% sucrose solution for 24 hours followed by 30% sucrose solution for 3 to 4 days). The two hemispheres of all four animals were then sectioned serially on a freezing microtome, sagittally in animal M₁, and coronally in animals M₂, M₃ and M₄. The serial sections, each 80 μm thick, were divided into three batches in sequence and collected in section trays at 0 °C. The first batch was stained using tetramethylbenzidine (TMB), no counterstain (Lane, '78); the second batch was stained with diaminobenzidine (DAB) and counterstained with thionine; and the third batch was used for a repetition of any one of the above methods of staining if confirmatory material was required. All the sections from one animal, processed by a particular method, TMB or DAB, were stained simultaneously in two section trays. Thus the staining procedure was identical.
for all the sections in one animal stained by that
method. This avoided differences in staining density
between sections in the same animal, which could affect
the number of cells stained. After staining, sections
were mounted on gelatin-coated slides that were numbered.
The TMB-stained sections were passed through absolute
alcohol quickly (20 seconds each, 2 changes) and soaked
in xylene (5 minutes each, 2 changes) before
coverslipping. The DAB-stained sections were passed
through descending grades of alcohol, counterstained with
thionine, differentiated through ascending grades of
alcohol, cleared in xylene, and coverslipped.

**Counting of corticocortical and thalamocortical cells**

In all three animals, M₁, M₂, and M₃, sections stained
with the TMB method were used for counting cells.
Adjacent sections, stained with the DAB/thionine
technique, were used to make a projection drawing (using
a Zeiss projection microscope) of the cerebral section at
magnification of x13 or x17.5. Relevant cytoarchitectonic
areas and thalamic nuclei were outlined. In the cortex,
the upper border of lamina II and the lower border of
lamina IV (where present) were also marked out. The TMB-
stained section was then projected to overlap the
projection drawing of the adjacent DAB/thionine-stained
section. Blood vessels, pial contours, and other
identifying landmarks were then added to the projection
drawing from the TMB-stained section.

After completion of the projection drawing (which
included features from both the TMB- and DAB/thionine-
stained sections), TMB-stained cells were identified with
high power microscopy (Zeiss binocular) and plotted
accurately on the projection drawings. The following
criteria were used to identify and plot stained cells:
(1) The outline of the cell was roughly pyramidal in
Fig. 1. A. Photomicrograph of a sagittal section of the left hemisphere in animal M₁ showing the HRP injection site. The section has been stained with diaminobenzidine and counterstained with thionine. Calibration bar = 1 mm. B. Photomicrograph of a TMB-stained coronal section of the right thalamus in animal M₂ showing labeled thalamocortical cells. Calibration bar = 20 μm.
Fig. 2. A. Photomicrograph of a TMB-stained sagittal section of the left hemisphere in animal M1 showing a part of the precentral area of the cortex and labeled corticocortical cells. B. Enlargement of the marked rectangle in A to show TMB-stained corticocortical cells in detail. Calibration bars = 200 μm for A, 20 μm for B.
shape - this applied to cortical cells; thalamocortical cells were round or oval (Fig. 1B). Occasionally, crystalline deposits tended to mask the shape of a cell, but study at a higher magnification helped to resolve this. In most cases a magnification of 100 was adequate to identify stained cell bodies. (2) There was a lightly stained oval nucleus in the cell. In some very darkly stained cells, it was difficult to visualise the nucleus, but in these cells the cell's shape was so starkly outlined that a positive identification was not difficult (Fig. 2). Using these criteria, it was possible to avoid including stained dendritic and axonal profiles, blood vessels, or other artifacts in the count.

In animals M₁ and M₂, all the TMB-stained sections were analysed quantitatively. Thus, approximately one-third of all cells projecting to the injection site were counted and plotted on the projection drawings. In animal M₃, every alternate section in the TMB-stained series was analyzed. In this animal approximately one-sixth of all cells projecting to the injection site were counted and plotted on the projection drawings. Those TMB-stained (alternate) sections of animal M₃, which were not analyzed numerically, were carefully studied to ensure that there were no remarkable differences in the topography and density of labeling. Therefore, in all the animals, regions of the cortex between adjacent TMB-stained sections that were not studied for labeling measured 160 µm in width. The labeled cells in the brain of animal M₄ were not counted. They were studied for comparison whenever there was any doubt in the topography of labeling in animal M₃.

Identification of cortical cytoarchitectonic areas and thalamic nuclei

DAB-stained sections counterstained with thionine were
used to delineate cortical cytoarchitectonic areas and thalamic nuclei. The criteria of Mountcastle and Powell ('59) and Jones et al. ('78) were used to distinguish between the areas comprising the somatic sensory cortex and its boundary with areas 5 and 7. Area 3a was taken as that region of the floor of the central sulcus that had an attenuated lamina IV and that was encroached upon by large pyramidal cells anteriorly in the hand area (Jones and Porter, '80). The posterior boundary of area 8 was taken to be the middle of the anterior bank of the arcuate sulcus (Walker, '40). It was very difficult to distinguish between areas 5 and 7. For most of its length, the floor of the intraparietal sulcus was taken as the boundary between areas 5 and 7 (Mountcastle et al., '75). The second somatosensory area was demarcated using the descriptions and maps of Jones and Burton ('76). Thalamic nuclei were outlined using the descriptions of Olszewski ('52) and Asanuma et al. ('83a).
ABBREVIATIONS FOR FIGURES

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI,</td>
<td>inferior limb of arcuate sulcus</td>
</tr>
<tr>
<td>AIA,</td>
<td>inferior limb of arcuate sulcus, anterior lip</td>
</tr>
<tr>
<td>AIf,</td>
<td>inferior limb of arcuate sulcus, floor</td>
</tr>
<tr>
<td>AIP,</td>
<td>inferior limb of arcuate sulcus, posterior lip</td>
</tr>
<tr>
<td>AS,</td>
<td>superior limb of arcuate sulcus</td>
</tr>
<tr>
<td>ASA,</td>
<td>superior limb of arcuate sulcus, anterior lip</td>
</tr>
<tr>
<td>ASF,</td>
<td>superior limb of arcuate sulcus, floor</td>
</tr>
<tr>
<td>ASP,</td>
<td>superior limb of arcuate sulcus, posterior lip</td>
</tr>
<tr>
<td>AV,</td>
<td>anterior ventral nucleus</td>
</tr>
<tr>
<td>Caud,</td>
<td>caudate nucleus</td>
</tr>
<tr>
<td>Cdc,</td>
<td>central densocellular nucleus</td>
</tr>
<tr>
<td>CE,</td>
<td>central sulcus</td>
</tr>
<tr>
<td>CEa,</td>
<td>central sulcus, anterior lip</td>
</tr>
<tr>
<td>CEf,</td>
<td>central sulcus, floor</td>
</tr>
<tr>
<td>CEp,</td>
<td>central sulcus, posterior lip</td>
</tr>
<tr>
<td>CI,</td>
<td>cingulate sulcus</td>
</tr>
<tr>
<td>Cl,</td>
<td>central lateral nucleus</td>
</tr>
<tr>
<td>GLd,</td>
<td>lateral geniculate nucleus, dorsal part</td>
</tr>
<tr>
<td>Cn Md,</td>
<td>center median nucleus</td>
</tr>
<tr>
<td>GM,</td>
<td>medial geniculate nucleus</td>
</tr>
<tr>
<td>HRP,</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>Hyp,</td>
<td>hypothalamus</td>
</tr>
<tr>
<td>IP,</td>
<td>intraparietal sulcus</td>
</tr>
<tr>
<td>IPA,</td>
<td>intraparietal sulcus, anterior lip</td>
</tr>
<tr>
<td>IPf,</td>
<td>intraparietal sulcus, floor</td>
</tr>
<tr>
<td>IPP,</td>
<td>intraparietal sulcus, posterior lip</td>
</tr>
<tr>
<td>LA,</td>
<td>lateral sulcus</td>
</tr>
<tr>
<td>LAu,</td>
<td>lateral sulcus, upper lip</td>
</tr>
<tr>
<td>LD,</td>
<td>lateral dorsal nucleus</td>
</tr>
<tr>
<td>LP,</td>
<td>lateral posterior nucleus</td>
</tr>
<tr>
<td>MD,</td>
<td>medial dorsal nucleus</td>
</tr>
<tr>
<td>Ncom,</td>
<td>substantia nigra, compact part</td>
</tr>
<tr>
<td>Ndif,</td>
<td>substantia nigra, diffuse part</td>
</tr>
<tr>
<td>Pcn,</td>
<td>paracentral nucleus</td>
</tr>
</tbody>
</table>
Pulₙ, pulvinar nucleus, oral part
R, reticular nucleus
Sf pc, subfascicular nucleus, parvocellular part
SMA, supplementary motor area
Sub, subthalamic nucleus
SII, second somatosensory area
TS, superior temporal sulcus
VA, ventral anterior nucleus
VAₘₜ', ventral anterior nucleus, magnocellular part
VLₘₜ, ventral lateral nucleus, medial part
VLₜ, ventral lateral nucleus, oral part
VP, ventral posterior nucleus
VPLₜ, ventral posterolateral nucleus, caudal part
VPLₜ, ventral posterolateral nucleus, oral part
VPI, ventral posteroinferior nucleus
VPM, ventral posteromedial nucleus
VPMₚc, ventral posteromedial nucleus, parvocellular part
X, nucleus X
Zic, zona incerta
1,2,3a, numbered cortical areas
3b,4,5,
6,7
RESULTS

Extent of the injection sites in area 4

In TMB-stained sections, at the site of the tracer administration, two regions of dark staining can be discerned: an inner zone where the deposition of the reaction product is very dense and individual axons or perikarya cannot be identified, surrounded by a second region where the reaction product is less dense and where individual labeled axons and perikarya (both pyramidal and nonpyramidal) can be detected (Mesulam, '82). The former is the injection site or area of HRP uptake. It was impossible to count labeled cells in the second region - therefore, quantitation of labeling in this region has not been done. Beyond the zone of deposition of reaction product, labeled cells were all pyramidal in shape and have been counted.

In animals M₁ and M₂, the injection sites involved the full depth of the cortex. However, the maximum spread of the injection sites occurred in the middle layers of the cortex. In animal M₁, the injection site was confined to area 4 and located mainly in the upper half of the anterior bank of the central sulcus. It extended to involve the anterior lip of the central sulcus at the level of the spur of the arcuate sulcus (see Fig. 4). The injection site in animal M₁ was similar to that in animal M₂ (see Fig. 6). In animal M₃, the injection site was more extensive; it involved the full thickness of the cortex and spread into the underlying white matter. It also involved the full depth of the anterior bank of the central sulcus (area 4) from behind the inferior limb of the arcuate sulcus laterally to the superior precentral sulcus medially. Behind the spur of the arcuate sulcus and the adjacent part of the inferior limb of the sulcus, the reaction product filled the precentral gyrus, thus
<table>
<thead>
<tr>
<th>Cytoarchitectonic area</th>
<th>Animal M1</th>
<th>Animal M2</th>
<th>Animal M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 4 Lam III</td>
<td>2995 (47.2)</td>
<td>1738 (44.5)</td>
<td>191 (2.0)</td>
</tr>
<tr>
<td>Lam V</td>
<td>45</td>
<td>217</td>
<td>13</td>
</tr>
<tr>
<td>Area 6 Lam III</td>
<td>1516 (24.9)</td>
<td>1163 (31.2)</td>
<td>908 (11.1)</td>
</tr>
<tr>
<td>(lateral) Lam V</td>
<td>92</td>
<td>207</td>
<td>213</td>
</tr>
<tr>
<td>SMA Lam III</td>
<td>Not</td>
<td>244 (6.2)</td>
<td>2019 (26.2)</td>
</tr>
<tr>
<td>Lam V</td>
<td>Counted¹</td>
<td>29</td>
<td>621</td>
</tr>
<tr>
<td>Area 3a Lam III</td>
<td>543 (8.45)</td>
<td>318 (7.5)</td>
<td>75 (0.9)</td>
</tr>
<tr>
<td>Lam V</td>
<td>1</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Area 3b Lam III</td>
<td>93 (1.4)</td>
<td>10 (0.2)</td>
<td>102 (1.0)</td>
</tr>
<tr>
<td>Lam V</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Area 1 Lam III</td>
<td>206 (3.2)</td>
<td>157 (3.6)</td>
<td>632 (6.5)</td>
</tr>
<tr>
<td>Lam V</td>
<td>1</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>Area 2 Lam III</td>
<td>539 (8.4)</td>
<td>193 (4.6)</td>
<td>1418 (15.4)</td>
</tr>
<tr>
<td>Lam V</td>
<td>4</td>
<td>10</td>
<td>134</td>
</tr>
<tr>
<td>Area 5 Lam III</td>
<td>170 (2.7)</td>
<td>38 (0.9)</td>
<td>1581 (16.6)</td>
</tr>
<tr>
<td>Lam V</td>
<td>1</td>
<td>2</td>
<td>95</td>
</tr>
<tr>
<td>Area 7 Lam III</td>
<td>110 (1.7)</td>
<td>3 (0.1)</td>
<td>998 (11.2)</td>
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<tr>
<td>Lam V</td>
<td>0</td>
<td>1</td>
<td>132</td>
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<tr>
<td>SII Lam III</td>
<td>123 (1.9)</td>
<td>31 (0.7)</td>
<td>382 (5.0)</td>
</tr>
<tr>
<td>Lam V</td>
<td>0</td>
<td>0</td>
<td>126</td>
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<tr>
<td>Area 24 Lam III</td>
<td>Not</td>
<td>12 (0.3)</td>
<td>253 (4.0)</td>
</tr>
<tr>
<td>Lam V</td>
<td>Counted¹</td>
<td>0</td>
<td>155</td>
</tr>
<tr>
<td>Others</td>
<td>8 (0.1)</td>
<td>2 (0.1)</td>
<td>17 (0.2)</td>
</tr>
<tr>
<td></td>
<td></td>
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<tr>
<td>Total: Lam III</td>
<td>6303 (98)</td>
<td>3909 (89)</td>
<td>8576 (84.8)</td>
</tr>
<tr>
<td>(laminae) Lam V</td>
<td>134 (2)</td>
<td>483 (11)</td>
<td>1533 (15.2)</td>
</tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>TOTAL:</td>
<td>6437</td>
<td>4392</td>
<td>10109</td>
</tr>
</tbody>
</table>

1. In animal M₁, some of the most medial sections of the left hemisphere were lost – hence the counts of cells in the SMA and area 24 were not considered reliable.

2. In animal M₃, every sixth section of the series was examined for numerical analysis whereas in the other two animals (M₁ and M₂) every third section of the series was thus examined.
<table>
<thead>
<tr>
<th>Cytoarchitectonic area</th>
<th>Animal $M_1$</th>
<th>Animal $M_2$</th>
<th>Animal $M_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area 4 Lam III</td>
<td>116 (82.8)</td>
<td>5</td>
<td>3805 (59.5)</td>
</tr>
<tr>
<td>Lam V</td>
<td>24</td>
<td>0</td>
<td>622</td>
</tr>
<tr>
<td>Area 6 Lam III</td>
<td>22 (14.8)</td>
<td>2</td>
<td>1990 (32.5)</td>
</tr>
<tr>
<td>(lateral) Lam V</td>
<td>3</td>
<td>0</td>
<td>428</td>
</tr>
<tr>
<td>SMA</td>
<td></td>
<td></td>
<td>209 (5.6)</td>
</tr>
<tr>
<td>Lam III</td>
<td></td>
<td></td>
<td>204</td>
</tr>
<tr>
<td>Lam V</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 24 Lam III</td>
<td></td>
<td></td>
<td>61 (1.8)</td>
</tr>
<tr>
<td>Lam V</td>
<td></td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>Area 3a Lam III</td>
<td>3 (1.8)</td>
<td></td>
<td>16 (0.2)</td>
</tr>
<tr>
<td>Lam V</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Area 3b Lam III</td>
<td></td>
<td></td>
<td>5 (0.1)</td>
</tr>
<tr>
<td>Lam V</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Area 1 Lam III</td>
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</tr>
<tr>
<td>Lam V</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Area 2 Lam III</td>
<td>1 (0.6)</td>
<td></td>
<td>21 (0.3)</td>
</tr>
<tr>
<td>Lam V</td>
<td>0</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total: Lam III</td>
<td>142</td>
<td></td>
<td>6114 (82.2)</td>
</tr>
<tr>
<td>(laminae) Lam V</td>
<td>27</td>
<td></td>
<td>1325 (17.8)</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>169</td>
<td>7</td>
<td>7439</td>
</tr>
</tbody>
</table>

3. See footnote 2 of Table 1.
involving part of area 6 in that region. Posteriorly it extended for some distance into area 3a (see Fig. 8).

**Numbers of cells labeled in the cortex and thalamus**

The absolute number of cells labeled in such a study would depend upon the volume, concentration, and nature of the tracer injected as well as the sensitivity of the histochemical technique. This study aims to describe the distribution of the cells that were labeled and their relative numbers in various cortical cytoarchitectonic areas and thalamic nuclei. These descriptions are equally affected by the above parameters in any one animal.

Table 1 shows the number of cortical neurons that were counted in the hemisphere that was injected with the tracer. In both animals M1 and M2, nearly half of all labeled corticocortical neurons in the ipsilateral hemisphere were located in area 4 itself, about one-fourth were located in postarcuate area 6, about one-fifth in the somatic sensory area I, and the rest in the supplementary motor area, the second somatosensory area, and posterior parietal area. Within the first somatosensory area of animals M1 and M2, the majority of the labeled neurons were situated in areas 3a and 2, a smaller number in area 1, and very few in area 3b. In the injected hemisphere of animal M3, very few neurons were labeled in area 4 (2%) and area 3a (0.9%). In the postarcuate area 6 of this animal, labeled neurons were fewer than expected (11.1%). Instead, a higher proportion of neurons were labeled in the supplementary motor area (26.2%) and posterior parietal areas (27.8%). In all three animals, most labeled corticocortical neurons were located in lamina III, and only a small percentage of labeled neurons were found in lamina V.

Table 2 enumerates the number of neurons counted in the
### Table 3

Numbers of thalamocortical cells counted.  
(Numbers in brackets indicate percentages)

<table>
<thead>
<tr>
<th>Thalamic Nuclei</th>
<th>Animal M1</th>
<th>Animal M2</th>
<th>Animal M3&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPL&lt;sub&gt;0&lt;/sub&gt;</td>
<td>579 (66.3)</td>
<td>756 (61.9)</td>
<td>1774 (31.9)</td>
</tr>
<tr>
<td>Vl&lt;sub&gt;0&lt;/sub&gt;</td>
<td>162 (18.6)</td>
<td>243 (19.9)</td>
<td>682 (12.3)</td>
</tr>
<tr>
<td>vl&lt;sub&gt;0&lt;/sub&gt;</td>
<td>88 (10)</td>
<td>86 (7)</td>
<td>433 (7.8)</td>
</tr>
<tr>
<td>X</td>
<td>-</td>
<td>-</td>
<td>432 (7.8)</td>
</tr>
<tr>
<td>VApc</td>
<td>-</td>
<td>-</td>
<td>449 (8.1)</td>
</tr>
<tr>
<td>VPL&lt;sub&gt;C&lt;/sub&gt;</td>
<td>15 (1.7)</td>
<td>9 (0.7)</td>
<td>61 (1.1)</td>
</tr>
<tr>
<td>VLM</td>
<td>-</td>
<td>4 (0.3)</td>
<td>-</td>
</tr>
<tr>
<td>VPM</td>
<td>1 (0.1)</td>
<td>12 (1.0)</td>
<td>141 (2.5)</td>
</tr>
<tr>
<td>CM</td>
<td>25 (2.8)</td>
<td>26 (2.1)</td>
<td>380 (6.9)</td>
</tr>
<tr>
<td>CL</td>
<td>1 (0.1)</td>
<td>83 (6.8)</td>
<td>688 (12.4)</td>
</tr>
<tr>
<td>Pcn</td>
<td>-</td>
<td>-</td>
<td>283 (5.1)</td>
</tr>
<tr>
<td>Cdc</td>
<td>-</td>
<td>-</td>
<td>45 (0.8)</td>
</tr>
<tr>
<td>Grenc</td>
<td>-</td>
<td>-</td>
<td>13 (0.2)</td>
</tr>
<tr>
<td>R</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>LD</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyp</td>
<td>-</td>
<td>-</td>
<td>129 (2.3)</td>
</tr>
<tr>
<td>MD</td>
<td>-</td>
<td>2 (0.2)</td>
<td>38 (0.7)</td>
</tr>
<tr>
<td>TOTAL:</td>
<td>873</td>
<td>1221</td>
<td>5549</td>
</tr>
</tbody>
</table>

4. See footnote 2 of Table 1
Fig. 3. Projection drawings of relevant parts of representative sections of the left hemisphere of monkey M1 showing labeled cells in the cortex following a small injection of HRP in area 4. Stained cells have been plotted accurately on the projection drawings as described in the Methods section. Open circles represent stained Betz cells. Each dot indicates 1 labeled corticocortical cell. The line within the cortical mantle is the lower border of lamina IV. Calibration bar indicates 2 mm for subfigures 1 to 7. A is a tracing of a photograph of the left hemisphere showing the sulcal pattern in this animal and the sagittal planes from which sections 1 to 7 have been obtained. The injection site is indicated by the dot on line 5. On the drawing of section 5, the location of the injection site is outlined and shaded. The darkly stained zone (in TMB-stained sections) is surrounded by a region of lighter staining for HRP, which is outlined by the interrupted line.
Fig. 4. A. Tracing of a photograph of the superior view of the left hemisphere of animal M1 showing that part of the cortex that was unfolded for presentation in B. B. Surface reconstruction from sagittal sections of the left hemisphere of animal M1 showing the distribution of labeled cells following a small injection of HRP in area 4. The cortex has been unfolded and the anterior and posterior lips and floors of the central sulcus, arcuate sulcus, and intraparietal sulcus marked with thick lines. The cytoarchitectonic boundaries have been delineated by thin lines. Each dot represents from 1 to 5 TMB-stained corticocortical cells. The extent of the injection site (darkly stained by TMB) is outlined by a continuous line and is surrounded by the lightly stained zone outlined by the interrupted line. The vertical and horizontal calibration bars each represent 2 mm in the mediolateral and anteroposterior planes, respectively.
contralateral hemisphere of animals $M_1$, $M_2$, and $M_3$. It is clear that few callosal neurons were labeled in the first two animals, $M_1$ and $M_2$. In contrast, a large number of such neurons were labeled in animal $M_3$, almost exclusively in area 4 (59.5%), in postarcuate area 6 (32.5%), and in the supplementary motor area (5.6%). Most of the labeled callosal neurons were located in lamina III of the cortex.

The number of labeled thalamocortical neurons counted in the three animals are detailed in Table 3. The distribution of these cells in animals $M_1$ and $M_2$ was similar: about two-thirds of the labeled cells were located in nucleus $VPL_0$; about one-fifth in nucleus $VL_c$; about one-tenth in nucleus $VL_0$; and the rest in the intralaminar nuclei, CM and CL. The labeled thalamocortical cells of animal $M_3$ were also mainly located in nucleus $VPL_0$, $VL_c$, and $VL_0$, but in this animal they accounted for only half of the labeled neurons. Neurons were now labeled in the nuclei $VA_{pc}$ and nucleus $X$ (7.8% and 8.1%, respectively) and a greater proportion of neurons (about one-fourth) were seen in the intralaminar nuclei CM, CL, and Pcn. A few neurons were also labeled in the hypothalamus and in nucleus MD.

**Distribution of labeled corticocortical neurons**

Figures 3 and 4 show the distribution of labeled neurons in the injected hemisphere of animal $M_1$. Within area 4, labeled cells were found in large numbers around the injection site both in the precentral gyrus as well as in the anterior bank of the central sulcus and extended as far medially as the superior precentral gyrus. This labeling was not uniformly dense anteroposteriorly or mediolaterally. Labeling was not seen further medially in area 4, in the convexity of the hemisphere or on the medial surface. Laterally, labeling was sparse in the
Fig. 5. Projection drawings of relevant parts of representative coronal sections of the right hemisphere of animal M₂ showing labeled corticocortical cells. Stained cells have been plotted accurately as dots on the drawings. The upper border of lamina II, the lower border of lamina IV, and the cytoarchitectonic boundaries have been marked within the cortical mantle. The extent of the injection site is outlined in subfigures 3 and 4. Calibration bar = 2 mm.
Fig. 6. A. Tracing of a photograph of the right hemisphere (sulci - thick lines) in animal M₂. The injection site (thick line in area 4), retrogradely labeled cells, and cytoarchitectonic boundaries (thin lines) have been mapped on the surface view of cortex from the coronal sections. Each dot represents from 1 to 5 labeled cells. A part of the cortex (marked by the rectangle) has been "unfolded" in B. Calibration bar = 2 mm. B. Surface reconstruction of the "unfolded cortex" from coronal sections of the right hemisphere of animal M₂. The floors and lips of relevant sulci have been marked with thick lines. The thin lines show the injection site - an inner zone of dark staining (uninterrupted line) surrounded by an outer zone of lighter staining (interrupted line). Each dot represents from 1 to 5 labeled corticocortical cells. Calibration bars = 2 mm.
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Although both Figures 4B and 6B show an area of "unfolded" cortex in the vicinity of the injection site, the perspectives in these two figures differ. This is because the "unfolding" of the cortex in animal M₁ has been done from parasagittal sections with the floor of the central sulcus as the reference point from one section to the next. (Thus, the cortex has been unfolded by stretching it rostrocaudally). On the other hand, the cortex of animal M₂ (and M₃) has been unfolded from coronal sections with the upper lip of the lateral sulcus as the reference point from one section to the next (i.e., the cortex has been "unfolded" by stretching it mediolaterally). Perspective obtained in Figure 6A (as well as 8A and 9A), where the distribution of neurons is shown on the surface of the "folded" cortex, is distorted by the fact that labeling in the banks and lips of the sulci overlap in the vicinity of the sulci.
motor cortex in front of the lateral end of the central sulcus. Labeled neurons in postarcuate area 6 were seen in the posterior bank and posterior lip of the spur and inferior limb of the arcuate sulcus as well as in the floor and posterior lip of the superior limb of the arcuate sulcus. The posterior bank of the superior limb of the arcuate sulcus was found to be devoid of labeled neurons, as was the floor of the inferior limb of the arcuate sulcus. In cytoarchitectonic areas 3a, 1, and 2, labeled neurons were found immediately behind the injection site as well as farther medially, in a mediolateral zone spanning about 10 mm. This zone was more or less aligned posterior to a similar zone of labeled cells seen in area 4. Labeling was sparse in areas 3b and 1. Labeling in areas 5 and 7 was confined to the anterior and posterior banks of the lateral third of the intraparietal sulcus and was continuous at the floor of the sulcus.

Figures 5 and 6 detail the labeling of corticocortical cells seen in the injected hemisphere of animal M2. As in animal M1, there was extensive labeling within area 4 in the region at the level of the spur and the superior and inferior limbs of the arcuate sulcus. Large parts of area 4, viz., the medial surface of the hemisphere, in the convexity, and the region in front of the lateral end of the central sulcus were devoid of label. Labeled neurons in postarcuate area 6 were seen in the posterior bank and posterior lip of the inferior limb of the arcuate sulcus. Unlike the findings in the previous animal (M1), labeled neurons were absent in the floor and posterior lip of the superior limb of the arcuate sulcus (Fig. 6A). Labeling in cytoarchitectonic areas 3a, 1, and 2 formed a patchy zone aligned (mediolaterally) posterior to the labeling in the motor cortex. The posterior bank of the central sulcus was more or less devoid of labeled cells. Labeling in area 5 was located in the anterior bank of the
Fig. 7. Projection drawings of representative coronal sections (1-5) of the left hemisphere of animal M₃ showing the distribution of labeled corticocortical cells. The cytoarchitectonic boundaries and the lower border of lamina IV are marked in each section. Each dot represents 1 labeled cell. The open circles mark the position of Betz cells. The extent of the injection site is outlined in subfigures 3 and 4 and in A. Calibration bar = 2 mm.
Fig. 8. A. Tracing of a photograph of the left hemisphere (sulci, thick lines) in animal M₃. The injection site (thick line in area 4), labeled cells (dots), and cytoarchitectonic boundaries (thin lines) have been mapped on the surface view of the cortex from coronal sections. Each dot represents from 1 to 5 labeled corticocortical cells. The injection site can be seen to encroach into areas 6 and 3a. The part of the cortex "unfolded" in B is marked by a rectangle. Calibration bar = 2 mm. B. Surface reconstruction of the unfolded cortex from coronal sections of the left hemisphere in animal M₃. The lips and floors of relevant sulci have been marked with thick lines. The thin lines demarcate the injection site - an inner zone of dark staining (uninterrupted line) and an outer zone of lighter staining (interrupted line). Each dot represents from 1 to 5 labeled corticocortical cells. Calibration bars = 2 mm.
intraparietal sulcus, whereas labeling in area 7 was located in the posterior bank of that sulcus and in the inferior parietal lobule. On the medial surface, labeled cells were seen in the rostral part of area 6 (rostral part of SMA) as well as in the adjacent part of area 24 in the floor of the cingulate sulcus.

Figures 7 and 8 show the location of labeled neurons in the injected hemisphere of animal M₃. Labeled neurons in area 4 were few and distributed sparsely in the medial part of the convexity and medial surface of the hemisphere as well as in the lateral end of the motor cortex, in the same areas that were devoid of label in animals M₁ and M₂. Those regions of area 4 that had showed large numbers of labeled cells in the previous two animals (M₁ and M₂) were completely filled with HRP reaction product in this animal (M₃). In postarcuate area 6 of animal M₃, labeled neurons were located on the floor, posterior banks, and posterior lips of the spur and the superior and inferior limbs of the arcuate sulcus. A part of area 6 immediately behind the spur and the adjacent inferior limb of the arcuate sulcus was filled with HRP reaction product and, therefore, did not reveal any labeling. Area 8 was devoid of label in this animal. Labeling in the somatic sensory and posterior parietal areas was more extensive. Labeled neurons in the parietal lobe formed a continuous zone of labeling in the lateral half of the postcentral gyrus, in the anterior and posterior banks of the intraparietal sulcus, and in the inferior parietal lobule, and extended farther laterally to the upper lip of the lateral sulcus into the second somatosensory area. On the medial surface of the hemisphere, labeling was seen throughout the rostrocaudal extent of area 6 (supplementary motor area).

The distribution of neurons in the right hemisphere (contralateral to the injection site) of animal M₃ is
Fig. 9. A. Tracing of a photograph of the right hemisphere (sulci, thick lines) in animal M₃. Labeled callosal cells (dots) and cytoarchitectonic boundaries (thin lines) have been mapped on the surface view of the cortex from coronal sections. Each dot represents 1 to 5 labeled corticocortical cells. Calibration bar = 2 mm. The part of the cortex marked by the rectangle has been unfolded in B. B. Surface reconstruction of unfolded cortex from coronal sections of the right hemisphere of animal M₃. The floors and lips of relevant sulci are shown. Each dot represents from 1 to 5 labeled corticocortical cells. Calibration bars = 2 mm.
Fig. 10. Projection drawings of sagittal sections of the thalamus showing most TMB-stained thalamocortical cells that were counted in animal M₁. Each dot represents 1 labeled cell. The number of labeled cells in each section is shown in parentheses. Calibration bar = 2 mm.
seen in figure 9. Large numbers of callosal neurons were labeled in the precentral gyrus behind the spur and inferior limb of the arcuate sulcus involving areas 4 and 6. The concentration of cells farther medially in areas 4 and 6, behind the superior limb of the arcuate sulcus, was less dense. Labeling in area 6 extended into the posterior banks of the spur and in the superior and inferior limbs of the arcuate sulcus. The anterior bank of the central sulcus was devoid of label except at its lateral end. Labeling in the medial surface of the hemisphere was confined to the rostral half of area 6 and adjacent part of area 24. The somatic sensory and posterior parietal areas were devoid of labeled neurons, except for a few in front of the tip of the intraparietal sulcus.

Distribution of labeled thalamocortical cells

Figure 10 shows the distribution, in parasagittal sections, of thalamocortical neurons labeled in animal M₁. In the sections F and G, where the concentration of labeling is densest, the labeled neurons occupy the whole rostocaudal and dorsoventral extent of nucleus VPL₀ and extend into the nuclei VL₀ and VL₁. In more lateral and medial sections (D, E, and H), labeled neurons are restricted to the posterior parts of nucleus VPL₀ and in sections B and C to the ventral part of that nucleus. Therefore, most of the labeled thalamic neurons in VPL₀ form a mediolaterally restricted slab with blurred borders posteriorly.

Figure 11 shows the distribution, in coronal sections, of thalamocortical neurons labeled in animal M₂. The mediolateral restriction of the band of labeled neurons is clear anteriorly (in sections A to I), but becomes less clear posteriorly where the number of neurons labeled also diminishes (in sections K to O). In most
Fig. 11. Projection drawings of coronal sections of the thalamus showing all TMB-stained thalamocortical cells that were counted in animal M2. The thalamic nuclear boundaries have been drawn using the criteria described by Olszewski ('52) and Asanuma et al. ('83). Each dot represents 1 labeled thalamocortical cell. The number of labelled cells in each section is shown in parentheses. Calibration bar = 2 mm.
Fig. 12. Projection drawings of coronal sections of the thalamus showing some TMB-stained thalamocortical cells that were counted in animal M. Each dot represents 1 labeled cell. The number of labeled cells in each section is shown in parentheses. Calibration bar = 2 mm.
sections the involvement of the entire dorsoventral extent of VPL is evident. This band of labeling is not uniformly dense anteroposteriorly (only 9 neurons labeled in section C).

Figure 12 shows some of the labeled neurones in coronal sections of the thalamus of monkey M\(^2\). The widening of the band of labeled neurons in nuclei VPL and VL is evident (sections B and C) and also the rostral and rostromedial extension of this band into nuclei VA, VL, and X (sections A and B).
DISCUSSION

Detailed studies of the representation of movements of the limbs in the motor cortex have been made using cortical stimulation (surface and intracortical) and recording of cortical neuronal activity in conscious behaving animals. These studies agree that the representations of movements of the head, forelimb, trunk, and hindlimb occupy separate zones in the motor cortex (Phillips and Porter, '77). Within the forelimb area of the motor cortex, populations of neurons related to the movements of individual joints and the activity of individual muscles and motor units may be widely dispersed over territories several square millimeters in area, which overlap extensively. Results of most studies (Anderson et al., '75; Kwan et al., '78; Sessle and Wiesendanger, '82; and Woolsey et al., '52) confirm that motor cortical neurons related to the movements of fingers are concentrated in the anterior bank of the central sulcus, whereas those related to movements at the wrist, elbow, and shoulders are concentrated in more rostral areas of the motor cortex in the precentral gyrus. However, the overlap of zones containing these populations of neurons is such that movements related to proximal joints may also be represented in the anterior bank of the central sulcus (Kwan et al., '78; Sessle and Wiesendanger, '82).

In animal M, the injection was small, restricted to area 4 in the anterior bank and the rostral lip of the central sulcus and situated in the lateral part of the forelimb representation. Following such an injection, large numbers of neurons were labeled in area 4 itself, restricted mostly to the forelimb representation of the motor cortex. It is not possible to quantify to what extent labeling in area 4 spread to the "face" representation laterally and to the "trunk"
representation medially. However, large areas of the motor cortex in the medial part of the convexity, the medial surface of the hemisphere, and the lateral end of the central sulcus, representing movements of the hindlimb, trunk, and face, were devoid of labeled cells. Outside the motor cortex, the most prominent labeling occurred in postarcuate area 6 (in the precentral gyrus as well as in the floor and posterior bank of the arcuate sulcus). In the somatic sensory area I, labeled neurons were found mainly in areas 3a and 2, to a smaller extent in area 1, and least in area 3b. These areas of SI that showed labeled cells were immediately posterior to the strip of labeling in MI and represent the forelimb regions of SI (Nelson, Sur, Felleman and Kas, '80). Labeling in the posterior parietal region was restricted to the banks of the lateral third of the intraparietal sulcus. Labeling in the contralateral hemisphere was very sparse.

It is quite clear that the distribution of labeled neurons in animal M₂, although essentially similar to that in M₁, differs slightly. As in animal M₁, large numbers of neurons are labeled in the forelimb region of MI and SI, and labeling in the opposite hemisphere is small. However, in animal M₂, there is a lack of labeling in the floor and posterior lip of the superior limb of the arcuate sulcus, and labeling is present in area 7 in the inferior parietal lobule. Significant involvement by the tracer of the face area of the motor cortex is precluded by the presence of very little labeling in the nucleus VPM of the thalamus (10 out of 1,221 thalamocortical neurons labeled; Jones, '81). On the medial part of the hemisphere, labeling is seen only in the rostral half of SMA.

The injection site in animal M₂, as expected, was much larger than in animals M₁ and M₂. It involved the full
depth of the anterior bank of the central sulcus throughout the mediolateral extent of the forelimb representation. From the number of cells labeled in nucleus VPM (141 out of 5,549 thalamocortical neurons labeled), it could be deduced that some amount of tracer spread to the "face" area of the motor cortex. Similarly, reaction product spread forward into area 6 behind the arcuate spur and backward into area 3a. The significant findings in the cortex of this animal were:

1. Only a small number of neurons were labeled in area 4.

2. A large number of neurons were labeled in the somatosensory and posterior parietal areas, which formed a continuous zone involving the forelimb and face representations of SI (Nelson et al., '80).

3. A large number of neurons were labeled in the supplementary motor area involving most of its rostrocaudal extent.

4. A large number of neurons were labeled in the contralateral hemisphere, in the motor, premotor, and supplementary motor areas. However, in the contralateral hemisphere, labeling was absent throughout the anterior bank of the central sulcus except at its lateral end; this labeling could have been due to involvement by the tracer of the "face" or "thumb" representation (Jenny, '79). It was clear that most of the motor cortical representation of the forelimb in the anterior bank of the central sulcus was devoid of callosal connections.

Labeled neurons in the thalamus comprised 11.7%, 21.7%, and 24% of all labeled neurons in animals M₁, M₂, and M₃, respectively. In animals M₁ and M₂, which had restricted injections of tracer in area 4, the thalamocortical neurons (located mainly in nucleus VPLo and adjacent parts of VLC and VLO) formed a continuous band of labeling, restricted mediolaterally (about half a millimeter wide) and occupying the full dorsoventral
anteroposterior extent of nucleus VPLo. However, this band was not uniformly dense or wide and its borders were blurred posteriorly. Similar bands have been described regarding thalamocortical neurons projecting to both the sensory and the motor cortex of monkeys (Jones et al., '79b). In animal M3, the aggregation of labeled thalamocortical neurons formed a wider continuous band in nuclei VPLo, VLo, and VLc, which extended into nuclei VA and X. The involvement of nuclei VA and X was due to the involvement by the tracer of rostral parts of area 4 and adjacent parts of area 6 (Jones et al., '79b; Kunzle, '78). Labeling in the thalamus of animal M3 was also marked by a larger proportion of labeled neurons in the intralaminar nuclei (24.4% of labeled thalamocortical neurons). This could be explained by the fact that neurons of the intralaminar nuclei project diffusely to the cortex of the frontal lobe and may be better labeled after a larger injection (Jones, '75a).

Electrical mapping of projections to the spinal cord from the motor cortex, using surface and intracortical stimulation, has shown extensive overlap of cortical areas that influence individual muscles and motor neurons (Phillips and Porter, '77). A reason for this could be an extensive interconnection and interplay between output elements and modules (function units) within the cortex. This study shows that within the forelimb representation of the motor cortex there are, indeed, profuse connections. When a small injection of HRP is made in a restricted part of the "forelimb" area of the motor cortex, large numbers of cells are labeled both anteriorly and medially, filling up the rest of the "forelimb" representation. However, very few connections derive from areas farther medially (trunk and hindlimb areas) or laterally (face areas). Even when the injection site involves most of the mediolateral and anteroposterior dimensions of the "forelimb" motor area
as in animal M_3, labeled cells in the other "body" regions (trunk, hindlimb, and face) of the motor cortex are sparse. It is not known how the interconnections within the forelimb region of the motor cortex influence the projection neurons of this area. These connections represent horizontal connectivity between input-output modules. Although these connections are extensive, they may also be specific; they may interconnect cells in different locations with the same or related functional specifications even though separated by considerable distances. Such connections have also been described in the visual cortex (Gilbert, '85) and somatosensory cortex (Shanks et al., '85). It is not known what proportion of these stained cells in area 4 have been labeled through axonal collaterals. Pyramidal cells in lamina III of the visual cortex have been shown to have collaterals that spread up to 2 mm away tangentially from the cell body (Gilbert, '85). Similar spread of collaterals have been described for pyramidal tract neurons in layer V of the motor cortex (Landry et al., '80).

Projections to the motor cortex from the cytoarchitectonic areas 1 and 2 of SI have been described before (Jones et al., '78), but these authors did not find any connections between areas 3a and the motor cortex. In our study, in both animals M_1 and M_2, there was a consistent projection from area 3a to area 4. The topography of this projection from area 3a matched that of the projections from areas 1 and 2. Thus it was only the "forelimb" regions of these areas of SI that showed retrograde labeling following injection of HRP limited to a part of the "forelimb" region of M_1. In animal M_3, which received a more extensive injection of HRP, only the "forelimb" and "face" regions of SI showed retrogradely labeled cells. Few neurons were labeled in area 3a of this animal since the reaction product spread to this area and could have masked any cells that were
labeled there.

Neurons in the precentral motor area respond at short latency to natural simulation of the limbs, mainly joint movement and muscle palpation (Lemon and Porter, '76). Precentral neurons, whose discharge can be related to movement about a particular joint of the contralateral forelimb, usually respond to passive movements about the same joint (Lemon et al., '76) - a proportion of such neurons do, however, respond to such movements from adjacent joints of that forelimb. These neurons are rarely influenced by natural stimulation of the ipsilateral forelimb, the trunk, or either hindlimb. In correlation with these findings, our anatomical studies also show that mainly the "forelimb" region of the ipsilateral somatosensory cortex projects to the "forelimb" region of the motor cortex.

Of areas outside the motor cortex, the largest proportion of labeled cells was seen in the postarcuate premotor area (area 6) of the same hemisphere (in all animals) and of the opposite hemisphere (in animal M_3). Fewer cells than expected were labeled in the left premotor area of animal M_3, presumably, because of the spread of the injection site into area 6 in this animal. Following injections of HRP in the "forelimb" regions of the primary motor cortex, cells were labeled in large numbers in the floor, posterior bank, and posterior lip of the spur and the inferior limb of the arcuate sulcus, both rostral and rostrolateral to the injection site. A similar pattern of projection from the premotor area 6 to the primary motor area (posteromedially directed) has also been described by Godschalk et al. ('84). In the same location in area 6, Brinkman and Porter ('83) found neurons related to forelimb movements. Fewer cells were labeled in the floor and posterior lip of the superior limb of the arcuate sulcus. Neurons related to forelimb
movement have also been located here (Wise and Mauritz, '85). This region has been shown to project to the "hindlimb" region of M1 by Muakkassa and Strick ('79) as well as to premotor regions (area 6) behind the spur and the inferior limb of the arcuate sulcus (Pandya and Vignolo, '71; Matelli et al., '84). This implies less strict segregation of forelimb-hindlimb topography within the postarcuate premotor area than in the primary motor cortex.

Using intracortical microstimulation to map the SMA, Macpherson et al. ('82) found that proximal motor effects in the forelimb were the predominant responses. Distal joint movements were found less commonly and more rostrally than proximal joint movements; the two effects overlapped extensively. Hindlimb movements were poorly localised and were obtained from caudal regions of the SMA, intermingled with forelimb motor effects. Although these motor effects of SMA may not be entirely through its projection to the motor cortex, our description of this projection complements the findings from the stimulation studies. A small injection of HRP in the anterior bank of the central sulcus labels a small number of neurons through the rostral half of SMA. A larger injection site in the whole "forelimb" region labels a relatively greater number of neurons in the whole rostrocaudal extent of SMA (see Muakkassa and Strick, '79). The unexpectedly large number of labeled cells in the SMA of animal M3 may also imply a diffuse projection from the SMA to MI.

In our study, injection of HRP limited to the anterior bank of the central sulcus labeled a few cells in the posterior parietal cortex (areas 5 and 7b). However, when the injection was extended more anteriorly (as well as medially) to include rostral parts of area 4, a much higher proportion of these cells was labeled. Neurons of
area 7b have been shown to project mainly to the premotor area 6 behind the inferior limb of the arcuate sulcus (Godschalk et al., '84), but our study shows that these neurons also project to area 4 (in greater numbers to rostral than caudal parts of area 4). Neurons of areas 5 and 7 have been related to both passive and active movements of the limb, manipulative movements of the hand, visual fixation and tracking, and visual coordination of hand movements (Mountcastle et al., '75).

Cells in the thalamic nuclei VPLO, VLC, and VLO are labeled following an injection of HRP restricted to the caudal region of the motor cortex. However, when the injection includes motor cortical areas anterior to it (which also project to the more posterior motor areas), labeling extents into nuclei VA and X. Now a smaller proportion of cells is labeled in nucleus VPLO. Thus, inputs from both the deep cerebellar nuclei (Asanuma et al., '83b) and basal ganglia (Kim et al., '76) are focused on a small region of the motor cortex and adjacent premotor region, through the thalamus.

Somatosensory inputs to the motor cortex arise from the postcentral cytoarchitectonic areas 1, 2, and 3a, as well as from the thalamus. There is an interesting similarity between the intrinsic connections within area 4 and its connections with the somatic sensory cortex, and there is considerable convergence of projections from the somatosensory to the motor cortex.

In summary, this study provides evidence that the premotor and supplementary premotor areas, which are found to be so closely related to movement execution, planning, and coordination, in response to both external and internal cues, dominate quantitatively the inputs to the precentral motor cortex. Although the number of thalamocortical neurons retrogradely labeled from the
motor cortex are fewer than labeled corticocortical cells, they form links through which extensive projections of the lateral cerebellum, basal ganglia, and ascending spinothalamic tracts could influence a small region of motor and adjacent premotor area, areas that are themselves profusely interconnected. However, more information regarding the fine anatomy of these projections to the motor cortex, and how these inputs interact to influence the output elements of the motor cortex, is required for better understanding of the control of movement execution by the motor cortex.
The Morphology of Pyramidal Neurones in the Motor Cortex of Monkeys and the Synaptic Actions of their Intracortical Axon Collaterals

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SUMMARY

1. Pyramidal neurones in the precentral motor area of the monkey were studied by intracellular techniques. Pyramidal tract neurones (PTNs) were identified by antidromic activation from the cerebral peduncles or medullary pyramids. Orthodromic responses were studied in PTNs and other pyramidal neurones to antidromic volleys set up by stimulation of the peduncles or pyramids. The neurones were then labelled by intracellular iontophoresis of horseradish peroxidase and their morphology examined.

2. Six pyramidal neurones located in lamina V were well stained; they included 2 fast PTNs and 2 slow PTNs. The morphology of all pyramidal neurones in this lamina (fast PTNs, slow PTNs and those pyramidal cells that were not antidromically characterized) was essentially similar. A single apical dendrite branched as it ascended and its terminals arborized subpially. Numerous lateral and oblique dendrites branched from the apical dendrites in lamina V and its border with lamina III. Short basal dendrites arborized in the vicinity of the soma in lamina V. Long basal dendrites had a wider field of arborization in lamina V and sometimes extending into lamina VI. Three to five collaterals arose from the axon in the cortex and arborized in laminae V and VI. Short collateral branches arborized in the vicinity of the soma in the region of the basal and lateral dendrites. Long collateral branches could be traced over long distances (often more than 1 mm). One pyramidal neurone in this lamina (a fast PTN) lacked short collateral branches from the axon.

3. Four pyramidal neurones in lamina III were stained well. The dendritic morphology of all these neurones was similar. Apical dendrites branched as they ascended and terminated subpially. Lateral and basal dendrites formed
a column of dendritic arbor around the soma. No long basal dendrites were seen. The number and arborization of intracortical collaterals from the axon varied widely; from 3 to 12 collaterals arose from the axon. The biggest arbor of collateral branches involved all the cortical laminae and was about 3 mm wide mediolaterally, while the smallest arbor was restricted mainly to lamina III in the vicinity of the soma. One neurone in this lamina also lacked short collateral branches from the axon.

4. Antidromic volleys in the pyramidal tract evoked excitatory responses in fast PTNs, predominantly inhibitory responses in slow PTNs and either excitatory or inhibitory responses in other pyramidal neurones in lamina V. Antidromic volleys in the cerebral peduncles evoked mainly excitatory responses in all the three types of neurones. Epsps evoked by PTN recurrent collaterals were probably monosynaptic and were mediated by the axons of both fast and slow PTNs. Ipsps evoked by these collaterals were probably disynaptic and clearly involved axons of fast PTNs in some cases.

5. Pyramidal neurones of lamina III were inhibited by the recurrent collaterals of PTNs when they were activated from the peduncles or the pyramids.

6. PTNs interact through their intracortical collaterals with other PTNs and other projection neurones in the cortex. Variations in the intracortical arborizations of these collaterals may represent variations in the potential for interactions initiated by individual neurones.
INTRODUCTION

Detailed studies of the morphology of pyramidal neurones in the cerebral cortex have revealed differences (and similarities) in the arborizations of dendritic and intracortical collaterals between neurones of different functional classes, in different cortical laminae, in different cortical areas and in the cortex of different species (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984; Deschenes, Labelle and Landry, 1979a; Landry, Labelle and Deschenes, 1980; Landry, Wilson and Kitai, 1984). These morphological differences suggest possible differences in the intracortical connectivities of such neurones. In the motor cortex, the morphology of pyramidal tract neurones (PTNs) has been described in the rat (Landry et al., 1984) and the cat (Deschenes et al., 1979a; Landry et al., 1980). In the monkey, the morphology of a few PTNs has been described by Hamada, Sakai and Kubota (1981); however, the dendritic arbors of some of these PTNs were not completely stained. Differences in the morphology of the two classes of pyramidal tract neurones (PTNs), fast and slow PTNs, have been found. These two classes are distinguished by differences in their conduction velocities and passive membrane properties (Lance and Manning, 1954; Takahashi, 1964). Differences in the structure of PTNs of the motor cortex of different species have also been found. However, equally detailed descriptions of other pyramidal neurones in the motor cortex, for example corticocortical neurones, are not available. Other projections from the motor cortex include those to cortical areas in the same hemisphere and to the contralateral hemisphere; subcortical projections terminate in the striatum, thalamus and brainstem as well as the spinal cord. The somata of origin of these supraspinal projections are segregated in different cortical laminae and sublaminae (Jones and Wise, 1977), and different projection neurones
are linked together through their recurrent collaterals and cortical interneurones.

The arborization of the intracortical collaterals of PTNs in the motor cortex is restricted to laminae V and VI (Landry et al., 1980; Hamada et al., 1981) except for some slow PTNs in the rat whose collaterals are more widely distributed in the cortex (Landry et al., 1984). The synaptic effects of these collaterals have been examined in cortical neurones by observing the effects of antidromic volleys set up in the cerebral peduncles and the medullary pyramids (Phillips, 1959). Studies of recurrent excitation and inhibition in fast and slow PTNs in the cat motor cortex produced by antidromic volleys in the medullary pyramids, support the conclusion that recurrent collaterals of slow PTNs excite fast PTNs monosynaptically while recurrent collaterals of fast PTNs inhibit slow PTNs disynaptically (Armstrong, 1965; Takahashi, Kubota and Uno, 1967). On the other hand, recurrent excitation and inhibition of PTNs produced by antidromic volleys from stimuli in the cerebral peduncles are not so restricted; both recurrent excitation and inhibition have been observed in both fast and slow PTNs (Stefanis and Jasper, 1964a). This could be due to the fact that additional corticofugal axons with terminations in the brainstem are activated by stimuli in the cerebral peduncles. Recurrent epsps in PTNs have been found to be of small amplitude and to be slowly rising (Deschenes, Labelle and Landry, 1979b). There is evidence that recurrent inhibition (when evoked by trains of stimuli) is powerful and that this synaptic effect may be mediated at the axon initial segment of PTNs (Stefanis and Jasper, 1964b). Synaptic actions of recurrent collaterals of PTNs in the rat (which species does not have a fast PTN population) produce recurrent inhibition as the predominant response in PTNs as well as other pyramidal neurones in lamina V (Landry et al., '84).
Stimulation experiments and investigations of neuronal activity of the primate motor cortex have shown that it is closely involved with the programming and execution of movement performance, especially in relation to the distal joints of the extremities (Phillips and Porter, 1977). However, the anatomical substrate that underlies neural activity in the motor cortex has not been fully investigated. One aspect of this intrinsic connectivity is the interaction between projection neurones through their recurrent collaterals and the spatial extent over which these collaterals influence other neurones. The morphology of pyramidal neurones (including PTNs) in the motor cortex of the monkey has therefore been studied by intracellular iontophoresis of horseradish peroxidase (HRP). The synaptic effects of PTN collaterals have been investigated in the individual penetrated neurones by antidromic volleys set up in the cerebral peduncles and the medullary pyramids.
METHODS

Nine young cynomolgous monkeys (M. fascicularis) of either sex, weighing between 1.54 and 2.36 kg, were used for the experiments. They were anaesthetized with ketamine (10 mg/kg) and xylazine (1mg/kg); anaesthesia was maintained with Nembutal (about 1.5 mg/kg/hour). The trachea and femoral artery and vein were cannulated. Normal saline was administered intravenously throughout the experiment. Arterial pressure and pulse rate were monitored continuously and the systolic pressure kept above 80 mm of Hg by administration of Dextran solution (MW 70,000) whenever necessary. The rectal temperature was maintained between 36° C and 38° C by using an electric blanket. Following initial surgery the animals were paralysed using Flaxedil (10 - 15 mg/kg/hour) and ventilated with a positive pressure pump to maintain an expiratory CO₂ of 3% to 4%. Bilateral pneumothorax was performed and end expiratory pressure kept at 2 cm of water.

A frontal craniotomy was made on the left side to expose the pre and post central gyri between the arcuate spur and tip of the intraparietal sulcus. The craniotomy was extended medially as far as the superior precentral dimple and laterally as far as the inferior precentral dimple. The dura was reflected and a drawing of the surface features (blood vessels and sulcal patterns) of the cortex was made. The exposed surface of the brain was kept moist at all times. A closed recording chamber was glued on to the surface of the skull over the craniotomy and sealed with silastic paste. The chamber was then filled with oil and all air bubbles expelled. A cervical laminectomy was performed at vertebrae C1 to C4. The dura was reflected over the roof of the fourth ventricle to allow free drainage of CSF and implantation of stimulating electrodes in the medullary pyramids.
Single focal tungsten stimulating electrodes (Clark Electromedical Instruments, impedance of 5 MΩ at 1 KHz) were implanted into the left cerebral peduncles (stereotaxically, Horsley-Clarke coordinates A 10.8, L 6.0, and V 3.0) in 6 animals and in the left medullary pyramids in 2 animals. In one animal stimulating electrodes were implanted at both sites. The pyramidal electrode was implanted through the dorsal surface of the medulla at the level of the obex, 1.5 mm lateral to the midline and angled 15° rostrally. The final position of the two electrodes was adjusted to obtain the best recording of tract waves (D waves of Patton and Amassian, 1954) following surface anodal stimulation of the motor cortex. The indifferent electrode was a silver wire implanted in the left temporal muscle. Rectangular cathodal pulses, 0.1 msec in duration, were delivered from an isolated stimulator (Digitimer DS2) to the stimulation sites. After the experiment the position of these stimulating electrodes was confirmed histologically. The distances from the peduncles and the pyramids to the motor cortex were estimated to be 27 mm and 50 mm respectively.

Thin walled glass micropipettes, with internal filaments for rapid filling, were used for intracellular penetrations. These were drawn into fine tips using a Brown Flaming microelectrode puller (model P-77). They were then filled with a solution of 0.2M KCl in 0.05M Tris buffer containing 7% HRP and bevelled (using a slurry beveller) at an angle of 45° to a tip diameter of about 0.5 μm (DC resistance between 20 and 50 MΩ).

All neurones studied were in the primary motor cortex (cytoarchitectonic area 4) of the left hemisphere. A stepping motor microdrive (J.K.M. Instruments, 1 μm steps) was used to advance the microelectrode under
a, b, c. Intracellular penetration and antidromic characterization of a fast PTN. Firing of the neurone to depolarizing current (a), unvarying latency of the antidromic response evoked from the pyramids (Pyr) (b) and the ability of the antidromic response to follow stimulus trains at 150 Hz (c) are illustrated by superimposed sweeps.

d, e. Averaged records of recurrent epsps recorded in a lamina V pyramidal neurone following stimulation of the cerebral peduncles (PP) and medullary pyramids (Pyr). These epsps followed stimulus trains at 50 to 100 Hz with unvarying latency. Calibration bars for each record are shown alongside and represent 10 mV (vertical) and 10 msec (horizontal).
microscopic control through the closed chamber. Following penetration of the pia the depth measurement of the microdrive was zeroed and the position of the track identified on the cortical surface map. DC potentials and signals from the microelectrode were fed into an Axoclamp microelectrode amplifier (Axon instruments) used in the bridge mode (bandwidth DC to 30 KHz). The signals from the amplifier were displayed on a dual beam storage oscilloscope (Tektronix 5103N). All electrophysiological events of interest were recorded on an FM tape recorder (bandwidth DC to 20 KHz).

Spontaneously active neurones or those responding antidromically or orthodromically to stimulation were penetrated by a few current pulses delivered through the microelectrode (+4 to +6 nA, duration 400 msec, repeated at 2 Hz). Following penetration, the depth of the cell in the cortex and the resting membrane potential were recorded. Antidromic responses to stimuli at the peduncles or the pyramids, if present, were confirmed by the following criteria: a) their latencies were unvarying even at threshold stimulation, b) the spike was an all or nothing response without underlying epsps at threshold stimulus, and c) they followed stimulus trains at 150 Hz with unvarying latency (Fig. 1). Orthodromic responses (epsps and ipspbs) from stimulation of the peduncles or pyramids were looked for in PTNs and other neurones at various stimulus strengths, and recorded when present. Recurrent epsps from both sites were tested with paired stimuli 10 to 20 msec apart (Fig. 1). The impaled neurone was then filled with HRP by iontophoresis (+4 to +8 nA, duration 400 msec, repeated at 2 Hz) for 5 to 10 minutes. Successful injection of HRP could often be predicted by a fall in electrode impedance and widening of the spike. Stable intracellular penetration could usually be maintained for between 10 and 15 minutes, rarely for more than 30 minutes. Following iontophoresis of HRP the
electrode was withdrawn and extracellular fields recorded using the same parameters of stimulation.

Two to five hours after the last intracellular penetration the animal was killed with an overdose of anaesthetic and perfused, initially with 2 litres of saline over 30 minutes, followed by 4 litres of fixative (0.1% glutaraldehyde and 1.0% paraformaldehyde in phosphate buffer) over 2 hours. The brain was then removed and soaked in 30% sucrose solution overnight. A block of the pre and postcentral gyri on the left side was sectioned parasagittally on a freezing microtome at 80 μm thickness. The sections were collected in phosphate buffer and stained by the Hanker-Yates method (Hanker, Yates, Metz and Rustioni, 1977). The sections were then mounted on slides, passed through absolute alcohol (2 changes, 5 minutes each), cleared in xylene and coverslipped. Stained cortical neurones were examined under the microscope and identified from data regarding their depth and location on the cortical surface map. All stained neurones were photographed and then reconstructed using a microscope (Zeiss, binocular) with a camera lucida attachment. The soma, dendrites and axon were usually reconstructed at a magnification of 400 (eyepiece X10, objective X25 and drawing tube X1.6), while the axon and its collaterals were reconstructed at a magnification of 800 (objective X50). Following reconstruction the coverslips were removed, the sections stained with thionin, coverslipped and examined under the microscope. Outlines of the cortical laminae were added to the drawings of the reconstructed neurones from the counterstained sections. These drawings were then used for morphometric analysis and photographed for illustration.

Satisfactory labelling with HRP was determined by the following criteria. The neuronal soma and dendrites were
darkly stained up to the farthest dendritic tips and spines could be seen clearly along even the finest and most extensive dendritic branches. The apical dendrites could be followed clearly up to lamina I where their terminal subpial branches were well stained. The axon could be traced into the white matter. Only those pyramidal neurones which satisfied these criteria were used for descriptions of morphology.

Apart from those neurones that were well stained and whose morphology has been described, there were other injected neurones, identifiable as pyramidal cells, which were not filled completely with HRP. Usually their basal dendrites were well stained but their apical dendrites became fainter as they ascended towards the pia with poorly defined or absent subpial terminations. The axons of these cells also became fainter as they descended and could not be followed into the white matter. These neurones were not included for descriptions of morphology, but electrophysiological responses in them have been described and attributed to pyramidal neurones of the layer in which they were located. After intracellular penetration and injection of a single neurone labelling of two or more neurones was never seen. However, around well stained pyramidal neuronal somata, one or two faintly stained pyramidal profiles were occasionally found. This could have occurred as a result of extracellular leakage of HRP from the electrode tip or from the filled neurone.

Electrophysiological data on magnetic tape were redisplayed on the oscilloscope and data of interest was transferred to a computer (Pyramid). Epsps and ipsps were averaged and extracellular fields subtracted from the averaged records. Individual sweeps and averaged records were plotted on an XY plotter (Hewlett Packard 7470A) and measured.
Fig. 2

Location of neurones labelled in both the morphological and electrophysiological studies in a representative sagittal section (A) and on a surface map (B). Labelled neurones were either in the precentral gyrus or the anterior lip of the central sulcus in the motor cortical representation of the forelimb.
RESULTS

Intracellular penetration of a neurone was accompanied by an abrupt change in membrane potential, action potentials more than 40 mV in amplitude, and firing of the neurone to injected depolarizing current. Only those penetrations where a stable membrane potential of at least -50 mV was maintained for more than 10 minutes were used for the study. The membrane potential in different penetrations varied from -50 mV to -60 mV. Almost all neurones that were successfully penetrated and satisfactorily filled with HRP were found to be pyramidal neurones located in either lamina III or lamina V. All labelled neurones were confirmed to be in cytoarchitectonic area 4 from counterstained sections.

Location of labelled neurones

Figure 2 shows the locations of the labelled neurones in the study (both morphological and electrophysiological). All the neurones labelled were either in the precentral gyrus or anterior lip of the central sulcus (Fig. 2A). They were located in the precentral area caudal to the arcuate spur in the motor cortical representation of the forelimb (Fig. 2B).

Morphology of pyramidal neurones located in lamina V

Thirty two PTNs whose axons had conduction velocities ranging from 6.9 to 47.4 m/sec were penetrated and injected out of which 4 PTNs (2 fast and 2 slow PTNs) were labelled satisfactorily for morphological description. Forty nine pyramidal neurones located in lamina V were not antidromically activated; two of these were stained well.

The two fast PTNs, F1 and F2, chosen for descriptions of
morphology had conduction velocities of 24.5 and 45.5 m/sec respectively (Table 1). Neurone F1 is illustrated in figure 3. A single apical dendrite ascended towards the pia and branched into 6 shafts in the middle of lamina III. These shafts terminated in fine branches under the pia. These terminal branches were sparsely spiny. Ten lateral and oblique dendrites branched out from the apical dendrite and arborized in lamina V and in the lamina III/V border. Seven basal dendrites originated from the lower part of the soma and arborized in lamina V. Two types of basal dendritic branches were seen: short and long. The short branches along with most of the lateral dendrites formed a sphere of dendritic arbor around the soma whose radial dimensions were similar to those of the subpial terminations of the apical dendritic branches. The long basal dendritic branches were either horizontal or descending and had a much more extensive arbor in lamina V. One lateral dendritic branch was also as long as these long basal dendrites. These long dendrites provided the asymmetry in the radial measurements of the dendritic arbors of apical terminals, basal dendrites and lateral/oblique dendrites (Table 1). The axon was traced for more than a millimeter into the white matter. Four intracortical collaterals arose from the axon and arborized in and contributed boutons to laminae V and VI. Two types of collateral branches could be distinguished. The short collaterals arborized around the soma in the region of the basal dendrites. The long collaterals had a wider extent of arborization and some could be traced for a millimeter or more away from the soma. Two of these collateral branches (the longest ones) were incompletely filled as they were not seen to terminate in boutons. Nevertheless the radial measurements of the axonal collateral arbor measured more than 2 mm in the anteroposterior plane and about 1.4 mm in the mediolateral plane (Table 1).
Fig. 3

Camera lucida reconstruction of a fast PTN (Fl) located in the anterior lip of the central sulcus.
A. Soma, dendrites and axon.
B. Soma, axon and axon collaterals.
The antidromic response to pyramidal (Pyr) stimulation is shown alongside.
Camera lucida reconstruction of a fast PTN (F2) located in the precentral gyrus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals. All collateral branches were long i.e. none of them arborized in the vicinity of the soma; two branches coursed anteriorly, one coursed posteriorly and the fourth coursed medially (arrowhead).
The antidromic response to pyramidal (Pyr) stimulation is shown alongside.
Plate 3

High power photomicrographs of a fast PTN (F2).
A. Soma, apical dendrite, basal dendrites, lateral dendrites.
B. Soma, basal dendrites.
C. Soma, proximal dendrites.
D. Apical dendrite, lateral dendrites.
E. Basal dendrite, axonal collateral.
Calibration bar represents 50 \( \mu \text{m} \) for A and 25 \( \mu \text{m} \) for B, C, D, and E.
This neurone did not have any axonal collateral branches that arborized and terminated in the vicinity of the soma and basal dendrites.
Camera lucida reconstruction of a slow PTN (S1) located in the anterior lip of the central sulcus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals.
The antidromic response to peduncular (PP) stimulation is shown alongside.
Table 1. Morphological dimensions of lamina V pyramidal neurons in the motor cortex of the monkey.

<table>
<thead>
<tr>
<th>No.</th>
<th>Location</th>
<th>A/D</th>
<th>Cond. vel. (m/sec)</th>
<th>Soma size (μm)</th>
<th>Apical dendrites**</th>
<th>Lat/Obl dendrites</th>
<th>Basal dendrites</th>
<th>Dia. (μm)</th>
<th>No. AP</th>
<th>ML</th>
<th>Laminae</th>
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<tr>
<td>F1</td>
<td>ALCS</td>
<td>PP/Pyr</td>
<td>24.5</td>
<td>25.0 x 40.0</td>
<td>6</td>
<td>500.0</td>
<td>10</td>
<td>712.5</td>
<td>600.0</td>
<td>800.0</td>
<td>200.0</td>
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<tr>
<td>F2</td>
<td>PCG</td>
<td>Pyr</td>
<td>45.5</td>
<td>20.0 x 32.5</td>
<td>9</td>
<td>422.5</td>
<td>7</td>
<td>420.0</td>
<td>600.0</td>
<td>800.0</td>
<td>100.0</td>
</tr>
<tr>
<td>S1</td>
<td>ALCS</td>
<td>PP</td>
<td>13.5</td>
<td>22.5 x 35.0</td>
<td>5</td>
<td>557.5</td>
<td>8</td>
<td>275.0</td>
<td>320.0</td>
<td>420.0</td>
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<tr>
<td>S2</td>
<td>ALCS</td>
<td>PP</td>
<td>7.3</td>
<td>20.0 x 47.5</td>
<td>5</td>
<td>512.5</td>
<td>14</td>
<td>367.5</td>
<td>720.0</td>
<td>900.0</td>
<td>150.0</td>
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<tr>
<td>N1</td>
<td>ALCS</td>
<td>No</td>
<td>20.0</td>
<td>42.5</td>
<td>4</td>
<td>717.5</td>
<td>11</td>
<td>1125.0</td>
<td>800.0</td>
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</tr>
<tr>
<td>N2</td>
<td>PCG</td>
<td>No</td>
<td>17.5</td>
<td>40.0</td>
<td>4</td>
<td>612.0</td>
<td>13</td>
<td>445.0</td>
<td>560.0</td>
<td>720.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Number of apical dendritic branches in lamina III.

**Dendrite measurements refer to arbor in lamina I.

Abbreviations: A/D = antidromic; Cond. vel. = conduction velocity; AP = anteroposterior; ML = mediolateral; Dia = diameter; PP = pes pedunculi; Pyr = pyramids;

ALCS = anterior lip of the central sulcus; PCG = precentral gyrus.
Figure 4 and plate 3 illustrate the morphology of neurone F2 which had the faster conduction velocity. Several differences were noticed in its morphology in comparison to neurone F1. The apical dendrite branched more proximally (close to the soma) and gave rise to a total of 9 shafts in lamina III. The apical terminals arborized under the pia and the radial dimensions of this arbor were similar to those of the arbor of the short basal and lateral dendrites. This neurone had only two long basal dendrites (one descending and one horizontal). This accounted for the relatively small tangential spread of its basal dendrites. The axon gave rise to 3 intracortical collaterals, none of which arborized in the vicinity of the soma. All collaterals were of the long type and none of them was completely filled. Where they could be seen their course was within lamina V.

Two slow PTNs were well stained and their morphology was similar to each other and to neurone F1. One slow PTN (neurone S1) is illustrated in figure 5. The apical dendrite branched in the middle of lamina III and formed 5 shafts. It had several long basal dendritic branches mainly confined to lamina V. The radial dimensions of the arbors of its dendrites were comparable to those of neurone F1. Three intracortical collaterals arose from the axon and arborized in laminae V and VI. Some collateral branches were short and arborized in the vicinity of the soma; the 3 long collateral branches were all incompletely filled.

Two well stained pyramidal neurones in lamina V were not antidromically activated (N1 and N2, Plate 1). Their morphology was similar to one another, to the slow PTNs, and to neurone F1. However one of them had exceptionally long basal dendritic branches and its axonal collateral branches (the long ones) were traced for much longer distances than for the cells described above. This
Camera lucida reconstruction of a pyramidal neurone (N1) that was not antidromically characterized. The neurone was located in the anterior lip of the central sulcus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals.
Fig. 7

Camera lucida reconstruction of a pyramidal neurone (C2) located in lamina III and in the anterior lip of the central sulcus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals.
Plate 2

High power photomicrographs of a pyramidal neurone (C2) labelled in lamina III.
A. Soma, basal dendrites, apical dendrite and lateral dendrites.
B. Soma, proximal dendrites, local axonal collaterals.
C. Apical dendrite, lateral dendrites.
D. Subpial apical terminals.
E. Basal dendrites, local axonal collaterals.
F. Axon and axonal collaterals.
Calibration bar represents 50 \( \mu \text{m} \) for A and 25 \( \mu \text{m} \) for B, C, D, E, and F.
Camera lucida reconstruction of a pyramidal neurone (C3) located in lamina III and in the anterior lip of the central sulcus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals.
Table 2. Morphological dimensions of lamina III pyramidal neurons in the motor cortex of the monkey.

<table>
<thead>
<tr>
<th>No.</th>
<th>Location</th>
<th>Soma size (um)</th>
<th>Dendritic Arbor (um)</th>
<th>Axon Axon collateral arbor (um)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Apical Dendrites**</td>
<td>Lat/Obl Dendrites</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>shafts* AP</td>
<td>ML</td>
</tr>
<tr>
<td>C1</td>
<td>PCG</td>
<td>30.0 x 40.0</td>
<td>3 100.0</td>
<td>80.0</td>
</tr>
<tr>
<td>C2</td>
<td>ALCS</td>
<td>22.5 x 42.5</td>
<td>2 462.5</td>
<td>400.0</td>
</tr>
<tr>
<td>C3</td>
<td>ALCS</td>
<td>17.5 x 32.5</td>
<td>3 320.0</td>
<td>320.0</td>
</tr>
<tr>
<td>C4</td>
<td>ALCS</td>
<td>17.5 x 37.5</td>
<td>2 225.0</td>
<td>160.0</td>
</tr>
</tbody>
</table>

*Number of apical dendritic branches in lamina II.

**Dendrite measurements refer to arbor in lamina I.

Abbreviations: AP = anteroposterior; ML = mediolateral; Lat/Obl = lateral and oblique; dia = diameter; PCG = precentral gyrus; ALCS = anterior lip of the central sulcus.
neurone (neurone N1) is illustrated in figure 6.

Morphology of pyramidal neurones located in lamina III

Thirty pyramidal neurones were labelled in lamina III of which four were well stained. No pyramidal neurone in this lamina was antidromically activated from either the peduncles or the pyramids. One of them (neurone C2 of table 2) is illustrated in figure 7 and plate 2. The apical dendrite branched in lamina III to form 2 shafts; the terminal subpial branches were profusely spiny. Nine lateral and oblique dendrites were given off from the apical dendrite. Along with the lateral/oblique dendrites the basal dendrites formed a column of dendritic arbor whose radial dimensions were similar to those of the arbor of apical subpial terminals. No long basal or lateral dendritic branches were seen. The axon gave rise to 12 collaterals in the cortex which arborized extensively in both laminae III and V. A few branches were seen to ascend into and terminate in laminae I and II. One short collateral was seen at the lamina V/VI border and another in the border of the cortex and white matter. Although 3 of the collateral branches (the long ones) were clearly incompletely filled, axon collateral branches of this neurone were seen in 39 serial sagittal sections (mediolateral spread of more than 3 mm).

Another pyramidal neurone in this lamina (neurone C3) is illustrated in figure 8. The dendritic morphology of this neurone was similar to that of neurone C2. However its axon gave off only 6 collaterals in the cortex which arborized mainly in the lower third of lamina III in the vicinity of the soma. One short collateral was seen in the superficial part of lamina V. Only one long collateral was seen and traced for about 0.5 mm before terminating in a small string of boutons. All the collaterals of this neurone seemed to have filled
Fig. 9

Camera lucida reconstruction of a pyramidal neurone (C1) located in lamina III and in the precentral gyrus.
A. Soma, dendrites and axon.
B. Soma, axon and axonal collaterals.
Plate 1

Photomicrographs of pyramidal neurones labelled by intracellular iontophoresis of horseradish peroxidase.

A. Pyramidal neurone in lamina III (C1).
B, C. Pyramidal neurones in lamina V (N1, N2).
D. Slow PTN (S2).

Two incompletely stained superficial pyramidal cells are also seen in C and D.

Calibration bar represents 125 μm.
Table 3. Numbers of pyramidal tract neurones (PTNs) studied and their antidromic latencies.

<table>
<thead>
<tr>
<th>Stimulus site</th>
<th>No. of fast PTNs</th>
<th>A/D latency range (msec)</th>
<th>No. of slow PTNs</th>
<th>A/D latency range (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td>3</td>
<td>0.57 to 1.0</td>
<td>9</td>
<td>1.4 to 3.7</td>
</tr>
<tr>
<td>Pyr</td>
<td>7</td>
<td>1.1 to 1.75</td>
<td>5</td>
<td>2.9 to 4.1</td>
</tr>
<tr>
<td>Both sites:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP</td>
<td>3</td>
<td>0.7 to 1.1</td>
<td>5</td>
<td>1.4 to 3.57</td>
</tr>
<tr>
<td>Pyr</td>
<td>3</td>
<td>1.3 to 2.2</td>
<td>5</td>
<td>2.6 to 7.2</td>
</tr>
</tbody>
</table>

Abbreviations: PP = pes pedunculi, Pyr = pyramids, A/D = antidromic

The conduction distances from the cerebral peduncles and the medullary pyramids to the motor cortex were estimated to be 27 mm and 50 mm respectively.
completely since all were seen to terminate in boutons. The morphology of neurone C4 was similar to that of neurone C3 except for a short axonal collateral in lamina VI.

The morphology of neurone C1 was different from all the other neurones described in this lamina (Fig. 9 and Plate 1). The morphology of its dendrites was essentially similar except that the apical dendritic branches in lamina I did not give off any subpial terminals. The axon gave off 3 collaterals, none of which arborized in the vicinity of the soma (they were all long collaterals). One of these collaterals (Fig. 8, collateral labeled a) was traced coursing anteriorly for about 1.5 mm, while the other two collaterals (Fig. 8, collaterals b, c) were traced coursing medially and laterally (respectively) for about 0.5 mm. None of these collaterals had been completely filled.

**Antidromic activation of pyramidal tract neurones**

Pyramidal tract neurones (PTNs) were antidromically activated from either the cerebral peduncles, the medullary pyramids, or from both sites. Thresholds of currents for antidromic activation of PTNs from both the peduncles and the pyramids ranged from 50 μA to 500 μA. On average, this threshold was higher for activating PTNs antidromically from the peduncles than from the pyramids. Table 3 shows the numbers of PTNs that were satisfactorily penetrated in the study. Slow PTNs were more often identified from the peduncles and fast PTNs were more often identified from the pyramids. This provides an indication that descending axons activated from the cerebral peduncles included, in addition to pyramidal tract axons, a group of other axons, mainly slowly conducting, which probably terminated in sites in the brain stem rostral to the medullary pyramids.

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Fig. 10

Intracellular records from a fast PTN (F1) located in the anterior lip of the central sulcus.

a. Antidromic response to pyramidal (Pyr) stimulation.

b, c. Superimposed sweeps (b) and averaged response (c) following stimulation of the pyramids below antidromic threshold. The vertical bar represents 9 mV for b and 3 mV for c.

d, e. The initial portions of the records in b and c shown in an expanded time scale.

f, g. Superimposed sweeps (f) and averaged response (g) following stimulation of the cerebral peduncles (PP) below antidromic threshold.
Otherwise on the basis of relative sizes of the somata, fast PTNs should have been identified more commonly from both sites. In those cases where PTNs were activated antidromically from both the peduncles and the pyramids, the antidromic latency following pyramidal stimulation was found usually to be equal to, or slightly less than, twice the antidromic latency from the peduncles, consistent with the relative conduction distances from the two sites. Twenty nine pyramidal neurones which were also labelled in lamina V, could not be antidromically identified.

**Recurrent synaptic effects on lamina V pyramidal neurones**

Stimulus currents for investigating recurrent synaptic effects were always kept below 500 μA. Of the thirteen fast PTNs that were satisfactorily penetrated, reduction of the stimulus current below antidromic threshold revealed recurrent epsps in 4 neurones. Recurrent epsps were evoked from the peduncles in one neurone (conduction velocity 30 m/sec) at a latency of 2.1 msec. Recurrent epsps were evoked in two neurones (conduction velocities 38.6 and 45.5) from the pyramids at latencies of 1.7 and 5.0 msec (respectively). In one neurone (conduction velocity 24.5) recurrent epsps were evoked from both sites at latencies of 1.3 msec (pyramid) and 5.4 msec (peduncles) (Fig. 10). Because of the longer latency of the peduncular effect, this must have been due to collateral influences of different axons to those producing the pyramidal collateral influence. These epsps were usually small, followed stimulus trains at 50 to 100 Hz at unvarying latency, and were often followed by ipspss.

Recurrent pspss were seen in 5 slow PTNs. Recurrent pspss in response to pyramidal stimulation were seen in two neurones; in both cases the response was an ipsp of long
Fig. 11

Intracellular records from a slow PTN (S1) located in the anterior lip of the central sulcus.
a. Antidromic response to peduncular (PP) stimulation.
b, d. Superimposed sweeps (b) and averaged record (d) of the response following peduncular stimulation below antidromic threshold.
c, e. Superimposed sweeps (c) and averaged record (e) of the response following pyramidal (Pyr) stimulation.
Horizontal bar represents 10 msec for a and 40 msec for the other records.
Fig. 12

Intracellular records from a pyramidal neurone located in lamina V and in the precentral gyrus.
A. Camera lucida reconstruction of the neurone.
a, b, c, d. Superimposed sweeps (a, c) and averaged records (b, d) of the response following stimulation of the peduncles (PP) with 300 μA (a, b) and 200 μA (c, d) of current.
e, f. Superimposed sweeps (e) and averaged record (f) of the response following stimulation of the pyramids (Pyr) with 200 μA of current.
Fig. 13
Histogram showing the distribution of latencies at which pyramidal neurones in lamina V were excited (epsp) and inhibited (ipsp) by recurrent collaterals of PTNs activated from the peduncles (PP) and from the pyramids (Pyr).
Hatched bars represent fast PTNs, filled bars represent slow PTNs, and open bars represent those lamina V pyramidal cells that were not antidromically activated from the peduncles or pyramids.
Table 4. Latencies of psp's evoked following stimulation of the cerebral peduncles and medullary pyramids in lamina V pyramidal cells.

<table>
<thead>
<tr>
<th>Response type</th>
<th>PP latency (msec)</th>
<th>Pyr latency (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± SD (range)</td>
<td>mean ± SD (range)</td>
</tr>
<tr>
<td>Epsp</td>
<td>2.23 ± 1.25 (1.1 to 5.4)</td>
<td>3.37 ± 1.43 (1.3 to 5.0)</td>
</tr>
<tr>
<td>Ipsp</td>
<td>5.76 ± 3.18 (2.4 to 9.0)</td>
<td>3.18 ± 1.89 (1.5 to 5.4)</td>
</tr>
</tbody>
</table>

Abbreviations: PP = pes pedunculi, Pyr = pyramids
duration (latencies 2.5 and 5.0 msec). Recurrent pspss were seen in one neurone in response to peduncular stimulation; the response in this case was excitatory (latency of epsp 2.4 msec). Recurrent pspss were seen in the two other neurones in response to stimuli at both sites; one of them was recurrently excited from both sites (latencies of the epsps were 4.4 msec from pyramidal stimulation and 3.2 msec from peduncular stimulation) while the other was recurrently inhibited from both sites at latencies of 1.5 msec (pyramid) and 2.4 msec (peduncles) (Fig. 11). Recurrent epsps in slow PTNs were also small, followed stimulus trains of 50 to 100 Hz, and were often followed by ipsps. Recurrent ipsps increased in amplitude when evoked by paired stimuli and when the membrane was depolarized.

Recurrent synaptic effects were seen in nineteen neurones that were not antidromically activated but found to be pyramidal neurones located in lamina V on subsequent histological examination of the labelled neurone. Eight were recurrently excited from the peduncles at latencies between 1.14 and 3.6 msec. Three neurones were recurrently inhibited from the peduncles at latencies of 3.9, 4.3 and 9.4 msec. Three neurones were recurrently excited by pyramidal stimulation at latencies of 2.7, 2.8 and 4.7 msec. Four neurones responded to stimuli at both sites. Two were recurrently excited from both sites at latencies of 1.5 and 3.9 msec (peduncles), and 2.7 and 5.0 msec (pyramids) (respectively). One neurone was recurrently inhibited from both sites at latencies of 8.9 msec (peduncles) and 5.4 msec (pyramid) (Fig. 12). Finally, one neurone was recurrently excited from the peduncles (latency 1.6 msec) and recurrently inhibited from the pyramids (latency 5.36 msec).

Figure 13 illustrates the distribution of the latencies of recurrent pspss seen in fast PTNs, slow PTNs, and other
Table 5. Comparisons of the latencies of psp's evoked following stimulation of the peduncles and the pyramids in the same neurone.

<table>
<thead>
<tr>
<th>Neurone type</th>
<th>PP evoked psp latency (msec)</th>
<th>Pyr evoked psp latency (msec)</th>
<th>CV of mediating axon</th>
<th>Estimated intracort. delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>fPTN</td>
<td>5.4 (epsp)</td>
<td>1.3 (epsp)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sPTN</td>
<td>3.2 (epsp)</td>
<td>4.4 (epsp)</td>
<td>19 m/sec</td>
<td>1.8 msec</td>
</tr>
<tr>
<td>nPTN*</td>
<td>2.4 (ipsp)</td>
<td>1.5 (ipsp)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>nPTN*</td>
<td>1.5 (epsp)</td>
<td>2.7 (epsp)</td>
<td>19 m/sec</td>
<td>0.1 msec</td>
</tr>
<tr>
<td>nPTN*</td>
<td>1.5 (epsp)</td>
<td>2.7 (epsp)</td>
<td>21 m/sec</td>
<td>2.6 msec</td>
</tr>
<tr>
<td>nPTN*</td>
<td>3.9 (epsp)</td>
<td>5.0 (epsp)</td>
<td>21 m/sec</td>
<td>-</td>
</tr>
<tr>
<td>nPTN*</td>
<td>8.9 (ipsp)</td>
<td>5.4 (ipsp)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>nPTN*</td>
<td>1.6 (epsp)</td>
<td>5.4 (ipsp)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: fPTN, sPTN, nPTN = fast, slow and non PTN
* Lamina V pyramidal neurones not antidromically characterized.
pyramidal neurones located in lamina V. Table 4 shows the mean latencies of these psp's evoked from the two sites. Given the differences in the conduction distance from the motor cortex to the peduncles and the pyramids, these results show that psp's evoked in the neurones from the peduncles occurred on the average at relatively longer latencies than similar responses evoked from the pyramids. Table 5 shows the latencies of psp's evoked from both the peduncles and the pyramids in the seven neurones, where responses were obtained from both sites of stimulation. In four neurones the data are incompatible with the ability to estimate conduction velocities of the axons mediating the response (epsp or ipsp). In three neurones this estimate was possible because the respective latencies were sensible, i.e. longer for the longer conduction distance. The conduction velocities of the axons which could be deduced to mediate recurrent excitation ranged from 19 to 21 m/sec, if the same axons were responsible for the effect from both stimuli. The estimated intracortical delays ranged from 0.1 to 2.6 msec. Taken together all data of tables 4 and 5 indicate that psp's studied in impaled neurones, in response to activation of recurrent collaterals of PTNs, were probably mediated through different (possibly overlapping) population of axons which could be activated from the cerebral peduncles and the medullary pyramids.

Recurrent synaptic effects in lamina III pyramidal neurones

In 4 neurones recurrent synaptic effects were recorded in cells that were subsequently shown to be pyramidal neurones located in lamina III. In all cases the recurrent synaptic response observed was an ipsp. Two were recurrently inhibited from the peduncles at latencies of 2.9 msec and 5.0 msec, one was recurrently inhibited from the pyramids at a latency of 5.8 msec.
Fig. 14

Intracellular records from a pyramidal neurone located in lamina III and in the precentral gyrus.
A. Camera lucida reconstruction of the neurone.
a, b. Superimposed sweeps (a) and averaged record (b) of the response following stimulation of the pyramids (Pyr).
c. The same record in a shown in an expanded time base.
Horizontal bar represents 10 msec for c and 40 msec for a and b.
(Fig. 14), and one was recurrently inhibited from both the peduncles (latency 5.2 msec) and the pyramids (latency 11.4 msec). The observations in the last neurone allowed an estimate of the conduction velocity of the axon(s) potentially mediating this recurrent inhibition (with the assumption that the responses were evoked by the same axons activated from the two sites). The estimated conduction velocity of the presumed mediating axon was 3.7 m/sec but an impulse travelling along this axon would arrive in the cortex at least 7.3 msec after its initiation in the peduncles, revealing the impossibility of its involvement in causing the epsp.
DISCUSSION

In intracellular studies there is a bias towards sampling of neurones with larger somata. Since pyramidal neurones in the cortex are the most populous type and have the biggest somata, it was not surprising that all neurones examined in this study proved to be pyramidal neurones.

Six pyramidal neurones located in lamina V were well labelled and their morphology was studied in detail. Four of these neurones were identified as PTNs, two of which (both slow PTNs) were antidromically activated from the cerebral peduncles only. Therefore the designation of these two neurones as PTNs is not strictly accurate since all descending fibres from the motor cortex contained in the peduncles do not reach the medullary pyramids. Two pyramidal neurones in lamina V were not antidromically identified. It is possible that they were PTNs since we cannot be sure that all fibres in the peduncles or the pyramids were activated by the stimuli used.

The morphology of all the pyramidal neurones located in lamina V was essentially similar with one exception. In all cases the apical dendrite branched to form numerous shafts which ascended to lamina I; their terminal branches arborized under the pia. The radial dimensions of the subpial arborization were similar in both slow and fast PTNs and similar to the radial dimensions of slow PTNs reported by another study of PTNs in the monkey (Hamada et al., 1981). However, the observations in that earlier report that these dimensions are much smaller in fast as compared to slow PTNs could not be confirmed by the present study. The lateral and oblique dendrites arising from the apical dendrites were restricted to lamina V and its border with lamina III and this is clearly a characteristic of lamina V neurones. Two kinds of basal dendrites could be distinguished. The short
basal dendrites along with the lateral and oblique dendrites had a small field of arborization around the soma whose radial dimensions matched those of the apical terminal arbor. Several long dendritic branches of the basal dendrites (and occasionally of the lateral dendrites) had a more extensive field and extended to more distant sites in lamina V. In one neurone (N1) the long basal dendrites also extended into the full depth of lamina VI. In contrast, in another neurone (F2), which had only two long basal dendritic branches, these branches did not expand the total basal dendritic territory appreciably. From 3 to 5 intracortical collaterals arose from the axon and their field of arborization was restricted to lamina V and VI. The short collaterals branched in the vicinity of the soma and contributed collaterals to the region of the basal and lateral dendrites. The long collateral branches coursed for much longer distances and provided a field of influence that was measured up to 3 millimetres in the radial plane (and was probably more extensive since in most cases the branches were incompletely filled). In one neurone (F1), these collaterals were the only ones present. No short axon collaterals in the vicinity of the soma and basal dendrites were observed in this neurone. This was the only one of the six neurones studied in lamina V that showed a significant variation in morphology.

The present description of the morphology of PTNs in the monkey was compared with the description of PTNs in the cat (Deschenes et al., 1979a) and found to be similar with a few exceptions. The apical dendrites of PTNs in the cat tend to branch into ascending shafts more proximally than those in the monkey, a comment that is also made by Hamada et al. (1981) for fast PTNs in the monkey. The apical dendritic terminals occupy a much wider area in lamina I in the cat. Similarly the basal
dendritic arbor is much more extensive in the cat if the long basal dendrites are not included in the measurements. If they are, then the dimensions of the basal dendritic territory in both species are comparable. In both animals, 3 to 5 recurrent collaterals are given off from the axon within the cortex and the arborization of these collaterals is limited to the deeper laminae (V and VI). Both long and short varieties of axon collaterals are seen in cat PTNs and exceptions to this morphology have not been described previously (as it has in the description of neurone F2 in this study).

Four pyramidal neurones in lamina III were well stained. The somata of all four neurones were located in the deep half of lamina III. Therefore they are likely to be corticocortical or callosal neurones (Jones, Coulter and Wise, 1979). The dendritic morphology of these neurones was essentially similar. However the number, branching and arborization of the intracortical collaterals showed wide variation. In one neurone a large number of collaterals originated from the axon and arborized in all the cortical laminae (neurone C2). In two others (C3 and C4) fewer collaterals were given off from the axon and the arbor of these collaterals was much smaller. These collaterals also seemed to have filled completely since they were seen to terminate in boutons. However, it is still possible that these collaterals were incompletely visualized. That would not explain their small number. In the fourth neurone (C1) an equally small number of collaterals was found to originate from the axon, but all the collateral branches were of the long variety. These findings suggest wide variations in the fields of influence of the recurrent collaterals of individual pyramidal neurones located at similar depths in the cortex, and therefore possibly having similar targets of projection.
The synaptic influences of the recurrent collaterals of PTNs in the motor cortex were studied by stimulating PTN axons in the cerebral peduncles and the medullary pyramids. These effects could conceivably have been mediated by ascending pathways to the motor cortex in the medial lemniscus and the internal capsule. Stimulating currents were, however, always kept below 500 μA. Further, excitatory responses studied were always tested with trains of stimuli at 50 to 100 Hz and were found to follow these trains at unvarying latencies. Therefore, these excitatory responses are likely to be monosynaptic within the cortex and unlikely to be mediated by fibres in the medial lemniscus (which relay in the thalamus). The peduncular site of stimulation was more than 2 mm posterior to possible thalamocortical fibres in the internal capsule. Hence, the parameters of stimulation used in this study make it most likely that the synaptic effects observed were mediated by the intracortical collaterals of PTNs.

Detailed analysis of the nature and latency of evoked excitatory and inhibitory responses observed following pyramidal and peduncular stimulation indicated that these responses, even when evoked from both sites in the same neurone, were probably not mediated by the same axon or group of axons. This is consistent with studies in the cat, which have also found differences in the nature and latency of these responses, when evoked from the peduncles instead of from the pyramids (Stefanis and Jasper, '64a). Therefore estimates of the conduction velocity of the axons mediating recurrent excitation and inhibition, and the intracortical delays, could not be calculated, and in so far as a few estimates were made, they are likely to be misleading.

In the relatively small number of observations made in this study all varieties of pyramidal neurones - fast
PTN, slow PTN, morphologically identified pyramidal neurones in lamina V, and pyramidal neurones in lamina III - were found to be influenced by the recurrent collaterals activated. When the pyramidal tract was stimulated, fast PTNs were found always to be recurrently excited, while slow PTNs were predominantly inhibited (3 out of 4 studied). This finding is similar to that reported by Armstrong (1965) and Takahashi et al. (1967) in the cat. Recurrent excitation and inhibition was seen in the other morphologically identified pyramidal neurones located in lamina V.

When the cerebral peduncles were stimulated fast PTNs were also always excited. But both recurrent excitation and inhibition were observed in slow PTNs. This finding has also been reported for the cat motor cortex (Stefanis and Jasper, 1964a). In lamina V, pyramidal neurones that were identified only morphologically were found to be predominantly excited by peduncular stimuli.

The latencies of recurrent collateral excitation and inhibition give an indication of the conduction velocity of the axons mediating the synaptic effect. This must also take into account conduction along the thin collaterals (which may occur slowly) and synaptic delays. Therefore short latencies indicative of a fast conducting pathway are more reliable indicators of the conduction velocity of the mediating axon. There is even greater uncertainty for recurrent inhibition where considerable delays might occur in the disynaptic pathway. Latencies of recurrent excitation initiated from the cerebral peduncles and reported in the study indicate that both fast and slow axons may be involved. Similarly recurrent excitation initiated from the pyramids was also found to involve both fast and slow axons. This finding is different from that observed in the cat where recurrent excitation has been found to be mediated predominantly by
slow axons (Armstrong, 1965; Takahashi et al., 1967). Recurrent inhibition initiated from the pyramids was indicative in two cases of the involvement of fast PTN axons. In all other cases the latencies were longer but due to the uncertainties involved in transmission through a disynaptic pathway the conduction velocities of the mediating axon cannot be accurately assessed.

Recurrent collateral excitation and inhibition in the motor cortex of the monkey was found to be similar to that in the cat in all ways except one. In the monkey it was found that, in addition to the interactions seen in the cat, recurrent collaterals of fast PTNs were capable of exciting other fast and slow PTNs. The functional significance of this finding is difficult to assess but could be of great theoretical importance in recruiting relatively synchronous discharge of associated groups of fast PTNs. Records of the activity of PTNs in the monkey during movement have shown that these neurones have high rates of firing during brief periods (Porter and Lewis, 1975). Further, the power of the corticomotoneuronal synapses is enhanced during high rates of discharge of the fibres due to synaptic facilitation (Muir and Porter, 1973). It is possible that PTNs in the monkey initiate such high rates of activity by extensive mutual excitation and that the recruitment of additional PTNs contributes to temporal facilitation. Moreover, the large postspike facilitation effects seen in cross correlation studies by Fetz and Cheney (1980) and Lemon and Muir (1983) could be, in part, due to linked action of numbers of fast PTNs, connected by excitatory recurrent collaterals.

In this study pyramidal neurones located in lamina III were found to be inhibited by the recurrent collaterals of PTNs. This effect must have been exerted via interneurones. The axon collaterals of PTNs were more
restricted, for the most part to the deeper layers of the cortex (lamina V itself). Even so, interactions between pyramidal neurones through their recurrent collaterals is not restricted within a single lamina. The identity of the inhibitory interneurone involved in recurrent inhibition is not known. Electrophysiological evidence indicates that such inhibition may be mediated at the axon initial segments of pyramidal neurones (Stefanis and Jasper, 1964b). Therefore it is likely that axoaxonic cells or basket cells are the interneurones involved, since their axonal branches have been shown to terminate on the axon initial segments of pyramidal neurones (Somogyi, Freund and Cowey, 1982; Somogyi, Kisvarday, Martin and Whitteridge, 1983). However, since the axoaxonic cells have dendritic and axonal arbors usually located in the superficial cortical layers, basket cells are more likely to be involved. Basket cells have been described in the motor cortex (Jones, 1975b), they occur in lamina V, have a large dendritic arbor spanning many laminae, and the branches of their axons arborize in lamina V (where they synapse on pyramidal cells including PTNs) as well as in lamina III, where they synapse on more superficially located pyramidal neurones (Jones and Hendry, 1984).

In summary, this study provides detailed descriptions of the morphology of pyramidal cells located in lamina III (which are likely to be corticocortical neurones) and those located in lamina V (PTNs). A study of the synaptic effects of PTN collaterals in the motor cortex has shown interactions between PTNs similar to those observed in the cat. In addition mutual excitatory synaptic interactions between fast PTNs has been found. This study has also shown interactions between PTNs and other projection neurones located in lamina V and III which is most likely to be mediated through recurrent collaterals. Finally, variations in the spatial distribution of
recurrent collaterals have been recorded in pyramidal neurones both those located in lamina III and in lamina V, which may determine variations of such interactions between individual neurones. It remains to be demonstrated in other experiments, whether the morphology of cells with different axonal destinations, e.g. to the striatum or the pons or the contralateral cortex may exhibit some unifying morphological features. But the fact that variation exists even among PTNs may be indicative of a variety of axonal collateral connectivities and dendritic architectures within one class.
Corticocortical Synaptic Influences on Morphologically Identified Pyramidal Neurones in the Motor Cortex of the Monkey

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Running Title:
Corticocortical Inputs to the Motor Cortex

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postarcuate premotor area, somatosensory area, corticocortical afferents, pyramidal tract neurones

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SUMMARY

1. Corticocortical synaptic influences on pyramidal neurones in the primary motor cortex of monkeys were studied by intracellular techniques. Corticocortical afferents from the postarcuate premotor area and the somatic sensory cortical areas were activated by bifocal stimulation of the cortical surface. Neurones that were found to respond orthodromically to such stimuli were labelled by intracellular iontophoresis of horseradish peroxidase.

2. Almost all neurones that were penetrated satisfactorily and labelled successfully were found to be pyramidal neurones located in lamina III or lamina V. Some labelled neurones in lamina V were also characterized as pyramidal tract neurones (PTNs) by antidromic activation from the cerebral peduncles or medullary pyramids.

3. Pyramidal neurones located in lamina III and lamina V (including PTNs) were excited at short latency by stimulation of the premotor cortex (1.1 to 4.0 msec) and somatosensory cortex (1.1 to 6.5 msec). There were no statistical differences in the distribution of latencies of corticocortical epsps between those evoked in lamina III neurones and those recorded in lamina V neurones, or between corticocortical epsps evoked from the premotor cortex in comparison with those from the somatosensory cortex. Excitatory responses to stimulation of the premotor area were usually more difficult to evoke and smaller in amplitude than those produced by stimulation of the somatosensory areas.

4. Corticocortical epsps were often followed by ipsps. The amplitudes of the epsps and ipsps could be increased by increasing the stimulus intensity. In a few neurones
ipsps that were not preceded by epsps were recorded.

Different synapses in thalamus were evoked by stimulation of the primary motor cortex. Ipspsps that were not preceded by epsps were recorded.
INTRODUCTION

Afferent synaptic influences exerted by corticocortical fibres on neurones of the primary motor area (cytoarchitectonic area 4) of monkeys provide one determinant of the spatial and temporal distribution of activities generated in these cells in relation to movement performance. Corticocortical projections to area 4 arise from the postarcuate premotor area (lateral area 6) and supplementary motor area (medial area 6) bilaterally, from the homologous primary motor area (area 4) contralaterally, and from topographically organized regions of the somatosensory cortex (areas 3a, 1, 2) and posterior parietal cortex (areas 5 and 7) ipsilaterally (Jones, Coulter and Hendry, 1978; Jones, Coulter and Wise, 1979a; Jenny, 1979; Ghosh, Brinkman and Porter, 1987). A great majority of corticocortical fibres to the motor cortex make synapses on dendritic spines (Sloper, 1973). However, the identity of the neurones postsynaptic to corticocortical afferents is largely unknown. There are also no intracellular studies of the excitatory and inhibitory synaptic effects of these afferents on individual precentral cells in the monkey.

In the cat, a few observations have been made of the synaptic effects of afferents from the somatosensory cortex to the motor cortex. Herman, Kang, MacGillis and Zarzecki (1985) found that motor cortical neurones in all laminae except lamina I were excited at short latency by intracortical microstimulation of area 3a; these neurones were not identified in any other way. In contrast, Kosar, Waters, Tsukahara and Asanuma (1985) found neurones only in laminae II and III of the motor cortex that were excited by stimulating area 2; five neurones were identified as multipolar spiny cells by intracellular iontophoresis of horseradish peroxidase.
In the present work the synaptic effects of stimulation of the postarcuate premotor and somatosensory areas have been studied in pyramidal neurones of the primary motor area by intracellular techniques. These neurones have been identified by intracellular iontophoresis of horseradish peroxidase (HRP) and subsequent examination of their morphology. Some pyramidal neurones located in lamina V have also been identified as pyramidal tract neurones (PTNs) by antidromic activation from the medullary pyramids or cerebral peduncles.
METHODS

The investigations described in this section were carried out concurrently with those described in the previous chapter (IV) in the same animals. The method of anaesthesia, recording of and maintenance of vital signs, and operative procedures followed are therefore not repeated here. As was described in the previous chapter a craniotomy over the left hemisphere was followed by implantation of the closed recording chamber. In addition to the stimulating electrodes implanted in the cerebral peduncles or the medullary pyramids, bifocal stimulating electrodes were also positioned on the cortical surface over the postarcuate premotor area (area 6) and somatosensory area (area 2). The placements of these two electrodes will be described below. Penetration of neurones, recording of intracellular potentials, and intracellular injections of HRP were accomplished in the manner already described. After the experiment, the animal was perfused and the brain processed for microscopic study of the labelled neurones. Electrophysiological data was analysed using a computer.

The wires of two bifocal silver ball electrodes were led through grooves under the chamber for electrical stimulation of the surface of the postarcuate and postcentral cortices. The silver ball stimulating electrodes were positioned over the posterior lip of the arcuate sulcus and the anterior lip of the intraparietal sulcus to press lightly on the cortical surface. They were adjusted to lie over regions of the cortex that the previous mapping study (Chapter III) had shown to have the highest density of corticocortical neurones projecting to the area of motor cortex under study (Ghosh et al., 1987). The electrode wires were then glued on to the skull and the chamber sealed on the skull with silastic paste. The chamber was filled with oil and all
Plate I

Photomicrographs of pyramidal neurones labeled in lamina III (A, B) and lamina V (C, D) of the primary motor cortex. These neurones responded to stimulation of the somatosensory or premotor cortices or both sites and were intracellularly labeled with HRP. The neurone in B is the same that is illustrated in Fig. 6. Calibration bar represents 25 μm for A and C, and 125 μm for B and D.
Fig. 1

Corticocortical epsps recorded in a pyramidal neurone located in lamina V.
A. Camera lucida reconstruction of the soma, dendrites and axon of the pyramidal cell
a, b, c. Suprathreshold epsps (a, 4 superimposed sweeps), subthreshold epsp (b, averaged record, 9 sweeps) and extracellular field (c, averaged record, 9 sweeps) recorded following stimulation of the somatic sensory cortex.
d, e, f. Individual epsps (d, 4 superimposed sweeps), averaged epsp (e, 11 sweeps) and extracellular field (f, averaged record, 11 sweeps) recorded following stimulation of the postarcuate premotor area.
Averaged records of epsps are shown after subtraction of the extracellular field.
air bubbles expelled. In initial experiments each of the two silver balls over the premotor and somatosensory cortices were tried independently as unifocal surface cathodal and surface anodal stimulating electrodes. However, the smallest stimulus currents needed to evoke synaptic responses in the impaled neurones were obtained using surface bifocal stimulation. Therefore in all later experiments only bifocal stimulation was used for cortical surface stimulation. Rectangular pulses, 0.1 msec wide, were delivered from an isolated stimulator (Digitimer DS2) to the cortical and bulbar stimulation sites.

In histological sections, pyramidal neurones were identified by the following criteria. There was a distinct apical dendrite arising from the upper pole of the soma and ascending towards the pia, there was an axon descending from the lower pole of the soma towards the white matter, there were several basal dendrites arising from the base and lateral sides of the soma, and the dendrites were spiny. The completeness and degree of staining neurones varied considerably. At one end of the range all intracortical processes of the pyramidal neurones were darkly filled up to their ends including subpial apical terminals and axon collaterals; at the other end of the range only the soma and proximal dendrites were faintly stained. Only those neurones which were unequivocally proved to be pyramidal neurones by the above criteria were included in the study (Plate 1).

Electrophysiological data on tape were transferred to a computer (Pyramid). Epsps and ipsps were averaged and extracellular fields subtracted from the averaged records (Fig. 1). Individual sweeps and averaged records were plotted on an XY plotter (Hewlett Packard 7470A) and analysed.
Location of labeled neurones in lamina III (A, B) and lamina V (C, D) that responded to stimulation of the somatosensory or premotor cortex.

Abbreviations: CE = Central sulcus; AS = Superior limb of the Arcuate sulcus; AI = Inferior limb of the Arcuate sulcus; IP = Intraparietal sulcus.
RESULTS

Intracellular penetration of a neurone was accompanied by an abrupt change in membrane potential, large spikes more than 40 mV in amplitude, and firing of the neurone to injection of depolarizing current. Only those penetrations in which a stable membrane potential of at least -50 mV was maintained for more than 10 minutes were included for analysis. The membrane potential in different penetrations varied between -50 mV and -60 mV. Inclusion in the study was also restricted to those neurones which were unequivocally identified to be pyramidal neurones on histological examination. Almost all the neurones that were penetrated satisfactorily and subsequently identified histologically were found to be pyramidal neurones located in either lamina III or lamina V (one neurone was identified to be nonpyramidal and one pyramidal neurone was located in lamina VI). In counterstained sections, all labeled neurones were confirmed to be in cytoarchitectonic area 4 (Fig. 2).

Location of labelled pyramidal neurones

The locations of the labelled pyramidal neurones which responded to stimulation of the premotor and somatosensory cortices are shown in Fig. 2. They consist of 53 pyramidal neurones located in lamina III and 38 pyramidal neurones located in lamina V. Eight of the latter were characterized as PTNs - 3 fast PTNs and 5 slow PTNs. A number of other pyramidal neurones were also labelled in both lamina III and V which did not respond to activation of the corticocortical afferents stimulated. A few impaled neurones that were shown to be influenced by corticocortical afferents could not be identified unequivocally as pyramidal cells (since they were too faintly stained or not stained at all). The results from these neurones have not been reported.
Fig. 3

Corticocortical epsp recorded in a fast PTN following stimulation of the somatic sensory cortex
a. Antidromic activation of the PTN from the medullary pyramids
b. Corticocortical epsp (superimposed sweeps)
Fig. 4

Corticocortical epsps recorded in a pyramidal neurone located in lamina V following stimulation of the postarcuate premotor area.

A. Camera lucida reconstruction of the labeled neurone.

a1, b1, c1. Superimposed sweeps of epsps recorded at stimulus strengths of 1.0 mA (a1), 0.7 mA (b1) and 0.4 mA (c1) showing unvarying latency at different stimulus strengths.

a2, b2, c2. Averaged records of epsps at the different stimulus strengths.

d. Averaged record of epsps evoked with stimulus trains of 150 Hz at 0.4 mA stimulus strength showing unvarying latency of each epsp.

Vertical calibration bar represents 10 mV for a1, a2, b1, b2, c1, c2 and 2.5 mV for d.
Labelled neurones in lamina III were located in the precentral gyrus, in the anterior lip of the central sulcus, and in the upper third of the anterior bank of that sulcus. Labelled neurones in lamina V were located only in the precentral gyrus and anterior lip of the central sulcus. Labelled pyramidal cells in both laminae were located in the primary motor area behind the arcuate spur in the "forelimb" area of the precentral gyrus.

Some pyramidal neurones located in lamina V and excited at short latency by cortical stimulation were also characterized as PTNs by antidromic activation from the medullary pyramids or cerebral peduncles (Fig. 3). Antidromic thresholds varied from 50 μA to 500 μA and were usually greater for activation from the peduncles. In the large sample of neurones that was encountered in the superficial cortical laminae (some of which were penetrated and shown to be pyramidal neurones of lamina III) only 4 neurones could be antidromically activated from the premotor or the somatosensory areas with bipolar surface stimulation at the strengths used. None of these neurones was penetrated satisfactorily for long enough for a full search for reliable synaptic responses to be made.

Nature of synaptic excitation and inhibition

Pyramidal neurones located in lamina III and lamina V were excited at short latency following stimulation of the premotor and somatosensory areas. These short latency epsps were found to have secure latencies. At any given stimulus strength individual trials showed practically no variation of latency (< 0.1 msec) of the compound epsp. With varying stimulus strengths the latencies of excitation remained similar even though the amplitude of the compound epsp changed, and epsps followed trains of stimuli at 150 Hz at unvarying latency (Fig. 4). Stimulus
Fig. 5
Corticocortical epsps followed by ipsps evoked by somatosensory cortical stimulation in a pyramidal neurone located in lamina III A. Camera lucida reconstruction of the labelled neurone
a, b, c. Superimposed sweeps (a, b) and averaged record (c) of the response at a stimulus strength of 0.5 mA
d, e, f. Superimposed sweeps (d, e) and averaged record (f) of the response at a stimulus strength of 0.4 mA
Fig. 6

Corticocortical ipsp evoked by somatosensory cortical stimulation in a pyramidal neurone located in lamina V
A. Camera lucida reconstruction of the labeled neurone
a, b. averaged records of ipsp. Horizontal calibration bar represents 30 msec for a and 10 msec for b
Fig. 7

Corticocortical epsps and ipsps recorded from a pyramidal neurone located in lamina III
A. Camera lucida reconstruction of the neurone
a1, a2. Averaged records of epsps evoked at a stimulus of 0.3 mA strength
b1, b2. Averaged records of ipsps evoked in the same neurone at a stimulus of 0.6 mA strength
strengths required to evoke such compound epsps were usually less than 500 $\mu$A, although larger currents were often needed to produce epsps of larger amplitude and to evoke orthodromic spikes. In all cases, stimulus currents were kept below 3 mA.

Short latency corticocortical excitation in pyramidal neurones was usually followed by a long inhibitory response (lasting for 50 msec to 200 msec). The inhibitory response was then followed by rebound excitation and a burst of spikes (Fig. 5). The amplitude and duration of the inhibitory response could be altered by altering the stimulus current. The amplitude of the inhibitory response could also be increased by depolarizing the membrane by passing current through the microelectrode. In rare instances, only the inhibitory response was seen and was not preceded by an epsp (Fig. 6). In one case the excitatory response evoked from the premotor area in a lamina III pyramidal neurone could not be seen when the stimulus current was increased and now only a pure inhibitory response was recorded (Fig. 7).

**Latencies of synaptic excitation and inhibition**

Of the fifty three pyramidal neurones located in lamina III, eleven responded to both premotor and somatosensory cortical stimulation. The response in all these neurones was a short latency epsp followed by a long ipsp. This group also includes the neurone which showed a reversal of response to premotor cortical stimulation when the stimulus intensity was altered (illustrated in Fig. 7). Thirty six neurones responded only to stimulation of the somatic sensory cortex. In 31 of them the response was a short latency epsp followed by an ipsp; in the other 5 a pure ipsp was seen. Six neurones responded only to premotor cortical stimulation; all responses were the common epsp/ipsp sequence (Table 1).
Table 1. Numbers of pyramidal neurones responding to stimulation of corticocortical afferents.

<table>
<thead>
<tr>
<th>Type of response and afferent source</th>
<th>Lamina III pyramidal cell</th>
<th>Lamina V pyramidal cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epsp from SI/PMA</td>
<td>fPTN</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Epsp from SI only</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Ipsp from SI only</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Epsp from PMA only</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Totals</td>
<td>53</td>
</tr>
</tbody>
</table>

Abbreviations: fPTN, sPTN, nPTN = fast, slow and non pyramidal tract neurones; SI = somatosensory cortex; PMA = premotor cortex
* Pyramidal cells not antidromically characterized.
Latencies of epsps in lamina III pyramidal neurones to somatosensory cortical stimulation (n = 42) ranged from 1.4 msec to 6.5 msec (mean = 2.96 msec, SD = 1.21 msec); latencies of ipsps in these neurones (n = 5) ranged from 2.5 msec to 7.9 msec (mean = 5.57 msec, SD = 2.51 msec). Latencies of epsps from premotor cortical stimulation (n = 17) ranged from 1.2 msec to 4.0 msec (mean 2.46 msec, SD = 0.85 msec); the latency of the ipsp (n = 1) was 7.3 msec.

Eight lamina V pyramidal neurones responded to cortical stimulation at both sites (premotor and somatosensory). A short latency epsp was followed by an ipsp in all cases. This sample also included 2 PTNs (both fast). Twenty eight neurones responded only to somatosensory cortical stimulation; in 22 cases the response was an epsp/ips sequence while in 6 cases a pure ipsp was seen. This sample included 6 PTNs (5 slow PTNs, 1 fast PTN). Three neurones in this lamina responded only to premotor cortical stimulation (all epsp/ips sequence, no PTNs) (Table 1).

Latencies of epsps in lamina V pyramidal cells to somatosensory cortical stimulation (n = 30) ranged from 1.1 msec to 5.7 msec (mean = 3.12 msec, SD = 1.2 msec); 3 fast PTNs (latencies 2.1, 4.3 and 5.7) and 3 slow PTNs (latencies 1.2, 2.4 and 4.1 msec) were included in this sample. Latencies of ipsps in these neurones (n = 6) ranged from 2.7 msec to 7.9 msec (mean = 4.88 msec, SD = 1.79 msec); 2 slow PTNs (latencies 4.4 and 5.9 msec) were included in the sample. Latencies of epsps from premotor cortical stimulation (n = 11) ranged from 1.1 msec to 3.4 msec (mean = 2.36 msec, SD = 0.72 msec); 2 fast PTNs (latencies 1.7 and 2.4 msec) were included in this sample. No ipsps without preceding epsps were seen in these pyramidal cells in response to premotor cortical
Fig. 8

Histograms of the distribution of latencies of corticocortical epsps evoked in lamina III and lamina V pyramidal cells following stimulation of the premotor and somatosensory cortices.
stimulation.

The distribution of latencies of epsps in lamina III and lamina V pyramidal neurones to cortically evoked epsps from both the premotor and somatosensory cortices is shown in Fig. 8. Statistical analysis of variance (F test) of these results showed that there were no significant differences in the latency distributions for corticocortical epsps evoked from the premotor cortex and from the somatosensory cortex in pyramidal neurones of the motor cortex. Such analysis also failed to show any significant differences in the distribution of latencies of corticocortical epsps evoked from either the premotor cortex or the somatosensory cortex in lamina III pyramidal neurones as compared to lamina V pyramidal neurones. Some pyramidal neurones in lamina V were identified as PTNs. PTNs were also excited at short latency by corticocortical afferents from the premotor cortex and somatosensory cortex. The sample size of PTNs in individual groups was too small to warrant statistical tests.

Excitatory and inhibitory responses to stimulation of the somatosensory cortex were seen more frequently than those to stimulation of the premotor area in the impaled neurones. In general these responses to stimulation of the premotor area were more difficult to evoke and smaller in amplitude than those responses produced by stimulation of the somatosensory cortex.
DISCUSSION

As has already been stated, intracellular sampling is biased towards penetration of neurones with bigger somata. Therefore the finding that almost all neurones, that were penetrated satisfactorily, shown to be influenced by the stimuli used, and subsequently labelled successfully, were pyramidal neurones is most likely to be a result of this bias. Pyramidal neurones, apart from being the larger of the cortical neurones, also account for nearly two-thirds of all cortical neurones (Sloper, 1973a). The finding that pyramidal neurones are excited at short latency by corticocortical afferents is also not surprising, since these afferents synapse predominantly on dendritic spines (Sloper, 1973b) and pyramidal neurones constitute the great majority of spiny neurones in the cortex.

Bifocal stimulation of the cortical surface was used to study corticocortical synaptic effects from both the postarcuate premotor area and the somatic sensory areas. Phillips and Porter (1962) studied the efficacy of surface anodal, surface cathodal and bifocal cortical stimulation in activating pyramidal tract cells of the precentral gyrus. Using short duration stimuli (0.2 msec) they found that threshold currents for such activation rose steeply in a hyperbolic manner as the focal stimulating electrode was moved away from the best point in all three types of stimulation. Surface anodal stimulation was found to require the smallest currents to directly activate PTNs (due to the formation of a virtual cathode in the depth of the cortex close to the low threshold axonal initial segments of PTNs). Bifocal stimulation was found to excite a much smaller proportion of PTNs directly; however a larger proportion of such neurones were synaptically activated than by surface cathodal or anodal cortical stimuli. This could have
occurred as a result of direct activation of neurones located in the superficial cortical layers which exerted synaptic effects on PTNs. Corticocortical neurones are also more superficially situated in lamina III of the cerebral cortex and this could have favoured activation of these neurones by bifocal stimulation of the cortical surface, which was found to be the best method for evoking corticocortical epsps in these experiments.

It was hoped that the short duration of the stimulus would reduce current spread. Hern, Landgren, Phillips and Porter (1964) found that cortical surface stimulation with 5 ms pulses was capable of directly activating PTNs several millimetres away from the best point; this capacity could be reduced drastically when stimuli of shorter duration were used. In our study, none of the neurones studied in the precentral gyrus were directly activated by brief stimuli of up to 3 mA delivered to the surface of the premotor cortex, about 5 mm away. Most corticocortical synaptic effects were studied with much smaller stimulating currents (500 mA or less). Therefore it is likely that these corticocortical effects were due to the relatively simultaneous synaptic actions of large numbers of tightly packed neurones in the posterior bank and posterior lip of the arcuate spur that project to the primary motor area (Ghosh et al., 1987). The stimulating electrodes over the somatosensory cortex were further away from the recording site and separated from this site by the central sulcus. The maximum density of corticocortical neurones projecting to the motor cortex from the somatosensory areas is seen in area 2 and fewer neurones project from the adjacent areas 1 and 5 (Ghosh et al., 1987). However, spread of the stimulating current to these areas cannot be excluded.

Compound corticocortical epsps that were studied were found to be of short latency; this latency was not
significantly affected by changing stimulus strength or by delivering trains of stimuli at 150 Hz. Therefore it is likely that these epsps were monosynaptically transmitted by the corticocortical afferents which had been activated. This conclusion is strengthened by comparing the distribution of latencies of these epsps with the latencies at which corticocortical neurones in the stimulated regions are antidromically activated from the precentral cortex. Neurones in the postarcuate premotor area that project to the precentral motor cortex are antidromically activated from area 4 at latencies between 0.6 and 2.1 msec (mean 1.2 msec) (Godschalk, Lemon, Kuypers and Ronday, 1984). Antidromic latencies of neurones in area 2 projecting to area 4 have not been determined in the primate but antidromic latencies of corticocortical neurones projecting from area 5 (in the anterior bank of the intraparietal sulcus) to the precentral motor area range from 0.9 msec to 4.4 msec (mean = 1.8 msec) (Zarzecki, Strick and Asanuma, 1978). Comparison with the latencies of corticocortical epsps in this study indicates that, in general, the epsp latencies were longer by 0.6 msec or more than the shortest antidromic latencies.

Fewer pyramidal neurones were synaptically excited and inhibited from the premotor cortex than from the somatic sensory cortex. In a quantitative study of neurones projecting to the precentral motor cortex Ghosh et al. (1987) found that a much greater number of neurones projected to area 4 from postarcuate area 6 than from areas 1, 2 and 5. On the other hand degeneration studies have found that fewer degenerating synapses are seen in the precentral cortex following ablation of the premotor area than the somatic sensory areas (Sloper, 1973b). Therefore it is possible that although many more neurones project from the premotor area to the primary motor area the synaptic contribution of individual fibres is small
when compared to other extrinsic afferent fibres, perhaps because the number of synaptic contacts delivered to this region from each cell in the premotor area is small. This would be consistent with the finding of fewer premotor epsps in these cells.

Due to the reciprocity of corticocortical connections (Jones et al., 1978), some of the corticocortical synaptic effects studied could have been due to antidromic activation of the collaterals of corticocortical axons originating from area 4. Such collaterals could have been synapsing on the dendrites of the impaled neurone. However in this study antidromic activation of pyramidal neurones in the superficial layers of the precentral cortex from cortical stimulation was extremely rare. This was possibly due to the fact that the stimuli of short duration were ineffective in activating the fine branches and terminals of corticocortical fibres in the premotor and somatic sensory cortices. Therefore it is likely that most of the synaptic effects studied have been mediated by corticocortical afferents and not the recurrent collaterals of corticocortical pyramidal neurones projecting from the precentral area.

There was no statistically significant difference between the latencies of corticocortical epsps evoked in lamina III pyramidal neurones and those evoked in cells located in lamina V. Pyramidal neurones located in lamina III are likely to be corticocortical neurones, whereas those located in lamina V usually project to subcortical sites (Jones and Wise, 1977). Thus pyramidal neurones with different projection zones are synaptically influenced by these afferents at similar latencies. Similarly, these projection neurones can be excited by corticocortical afferents from two widely different sources (premotor and somatosensory cortex) at similar latencies. Many other
factors need to be considered in examining the
significance of this connectivity for influencing the
temporal relationships between the activities of
different pyramidal neurones when they are discharging
naturally. These include the convergence or divergence of
single corticocortical fibres on to different pyramidal
neurones with similar or dissimilar projections, and the
synaptic interactions that must be occurring in
individual receiving neurones from a vast number of other
inputs not examined in this study.

Short latency epsps from corticocortical afferents were
often followed by ipsps. One explanation for this is that
at least one inhibitory interneurone is also synaptically
excited by these afferents. It then mediates
oligosynaptic corticocortical inhibition to pyramidal
neurones. In some cases this inhibition was the only
response. This pattern of response i.e. excitation
followed by inhibition has been recorded from other areas
of the cortex (Toyama, Matsunami, Ohno and Takahashi,
1974) and within the motor cortex following antidromic
volleys in the cerebral peduncles (Stefanis and Jasper,
'64a). This implies that when a number of afferent input
fibres are simultaneously activated mixed excitatory and
inhibitory responses will be obtained. Separate
excitatory and inhibitory synaptic effects could be
produced on different target neurones by the direct and
indirect actions of an individual fibre.

In this study the amplitude of both the excitatory and
inhibitory responses obtained in individual neurones
could be varied by varying the stimulus intensities of
cortical stimulation at the premotor area and the
somatosensory areas. This is probably due to convergence
of many corticocortical afferent fibres on to individual
neurones. It is also likely that individual afferent
fibres influence a large number of neurones through their
terminal branches. The excitatory drives from individual afferent fibres on to different neurones are unlikely to be equal. It is possible that the excitatory drive from these afferents on to a population of neurones is sharpened by the inhibitory influences which are also initiated and which prevent activation of neurones receiving the weakest excitation. Such a mechanism, and the as yet undefined spatial distribution of excitation and inhibition from a given corticocortical afferent may be involved in topographic sculpturing of cortical cellular activity.

Several pyramidal neurones were found to be excited at short latency by corticocortical afferents both from the premotor cortex and the somatosensory cortex. Thus pyramidal neurones are synaptically influenced by a number of afferent fibres from a single afferent source as well as from different afferent sources. Along with the many other afferent influences not studied, these synaptic inputs provide the substrate for integration by single neurones of responses from a large number of extrinsic afferent inputs, depending on the temporal and spatial patterns of activity in these afferent fibres. If one also includes synaptic actions on these pyramidal neurones from nonpyramidal neurones and from the recurrent collaterals of other pyramidal cells the capacity for interactions between multiple sources of afferent input by cortical cells is immense.

In summary, this study provides evidence that corticocortical afferents from the postarcuate premotor area and the somatic sensory area to the precentral motor cortex excite pyramidal neurones located in lamina III and in lamina V (including PTNs) monosynaptically. These neurones are also inhibited by corticocortical afferents at slightly longer latencies. Recruitment of more fibres by increasing the stimulus intensity increases the
amplitude of both the excitatory and inhibitory response implying convergence of many afferents on to single neurones. Convergence of afferent fibres also came from different cortical areas on to individual pyramidal neurones in the motor cortex.
Pyramidal Neurons in the Motor Cortex of the Cat: Detailed Morphology and Synaptic Inputs

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Pyramidal neurons in laminae II, III, V and VI of area 4γ of the cat motor cortex were studied by intracellular techniques. Antidromic and orthodromic responses from stimulation of the cerebral peduncles and of the ventrolateral nucleus of the thalamus were investigated in these neurons. Then horseradish peroxidase was iontophoresed into the same neurons to allow examination of their detailed morphology.

The morphology of pyramidal neurons whose somata were located in a particular lamina was similar, but differed from that of pyramidal neurons in other laminae. None of the pyramidal neurons of lamina II had a typical apical dendrite; they were spiny multipolar neurons. All pyramidal neurons, except some in lamina VI, had apical dendrites which terminated in lamina I, subpially. No intracortical collaterals were seen originating from the axons of lamina II or of lamina VI pyramidal neurons. All somata of pyramidal tract neurons (PTNs) labeled in the study were located in lamina V except for one which was located in lamina VI.

Recurrent collaterals of pyramidal tract neurons were activated by peduncular stimulation. Recurrent epsps could be evoked in fast PTNs, slow PTNs, other pyramidal neurons of lamina V and pyramidal neurons of lamina VI at latencies between 1.3 and 6.25 msec; in some slow PTNs a recurrent ipsp of long duration was the predominant response. Epsps were evoked in pyramidal neurons of lamina III, V and VI from stimulation of the ventrolateral nucleus of the thalamus at latencies between 1.0 and 5.0 msec.
Pyramidal neurons, with rare exceptions, are the only neurons of the cerebral cortex whose axons project out of the cortex. They are found in all laminae except lamina I. They have characteristic dendritic arbors consisting of apical dendrites, which ascend to arborize in lamina I; basal dendrites, which extend outward from the lower portion of the soma; and lateral and oblique dendrites, which extend as branches from the shafts of apical dendrites. All dendrites are profusely spiny. Their axons emerge from the base of the soma and project downward into the white matter. Pyramidal neurons of the motor cortex project to other areas of the cortex in the same hemisphere and to the contralateral hemisphere; subcortical projections include those to the striatum, thalamus, brain stem and spinal cord. The majority of corticocortical neurons are located in lamina II and III, whereas cells of origin of subcortical projections are located mainly in lamina V and VI (Berrevoets and Kuypers, '75; Jones and Wise, '77). Callosal neurons on the other hand have been found in all laminae of the cortex except lamina I (Jacobson and Trojanowski, '74, Miller, '75) as have corticostriatal neurons (Royce, '82). Corticothalamic neuronal somata are located in lamina V and VI (Catsman-Berrevoets and Kuypers, '78). The detailed morphology of these neurons has been studied using the Golgi technique (reviewed by Feldman, '84) and more recently by intracellular injections of horseradish peroxidase (HRP).

There are few complete descriptions of the morphology of pyramidal neurons in the motor cortex. Using the Golgi technique Sholl ('53), in the cat and Kemper et al. ('73), in the monkey have described the branching patterns, spine densities and lengths of dendrites of pyramidal neurons in different laminae of the motor
cortex. These studies do not provide a complete
description of the apical dendritic terminals or the
intracortical axon collaterals of these neurons.
Intracellular labeling with horseradish peroxidase (HRP)
has several advantages. The dendritic and axonal
processes of a single neuron can be followed
unequivocally for long distances across several serial
sections, myelinated axons are labeled, and the labeled
neuron can be characterised electrophysiologically. Using
this technique, the morphology of pyramidal tract neurons
(PTNs) in the motor cortex of the cat (Deschenes et al.,
'79; Landry et al., '80) and in the sensorimotor cortex
in the rat (Landry et al., '84) have been described.
Descriptions of pyramidal neurons with other axonal
destinations are not available for the motor cortex.

A number of studies show that pyramidal neurons in
different cortical areas and in different species may
differ in the branching and arborization of their
dendritic processes and intracortical collaterals. Some
pyramidal tract neurons (PTNs) in the rat motor cortex
(Landry et al., '84) and lamina V pyramidal neurons in
the cat visual cortex (Gilbert and Wiesel, '79) have
collaterals that arborize in the superficial cortical
layers (I, II and III) as well as in the deeper layers (V
and VI), while collaterals of PTNs in the cat motor
cortex arborize only in the deeper cortical laminae (V
and VI) (Landry et al., '80).

Morphological studies have shown that extrinsic afferents
(both thalamocortical and corticocortical) to the motor
cortex make synaptic contacts predominantly on dendritic
spines (Sloper, '73; Strick and Sterling, '74). Since
pyramidal neurons make up the majority of spiny neurons
in the cortex, it is likely that the postsynaptic targets
of most of these synapses are pyramidal cells. There is
only one study which has attempted to combine
electrophysiological studies of these projections with morphological identification of pyramidal neurons in the motor cortex (Kosar et al., '85). In that study two superficially located pyramidal neurons (and several spiny multipolar neurons) were found to be excited at short latency by thalamocortical afferents.

In this study, the morphology of pyramidal neurons in different laminae of the motor cortex have been studied by intracellular labeling with HRP. In addition, the responses of these neurons to stimulation of the cerebral peduncles and the ventrolateral nucleus of the thalamus were examined. A knowledge of the morphology and connectivity of these neurons would help in the understanding of the anatomical substrate for cortical motor control.
METHODS

Thirty three adult cats of either sex, weighing between 1.6 and 2.8 kg, were used for the experiments. They were anaesthetized with Nembutal (induction 40mg/kg, maintenance 2mg/kg/hour). The trachea and femoral artery and vein were cannulated. Normal saline was administered intravenously throughout the experiment. Arterial pressure and pulse rate were monitored continuously and the systolic pressure kept above 100 mm of Hg by administration of Dextran solution (MW 70,000) whenever necessary. The rectal temperature was maintained between 36 °C and 38 °C by using an electric blanket. Following initial surgery the animals were paralysed using Flaxedil (10 - 15mg/kg/hour) and ventilated with a positive pressure pump to maintain an expiratory CO₂ of 3% to 4%. Bilateral pneumothorax was performed and end expiratory pressure kept at 2 cm of water.

The pericruciate cortex on both sides was exposed by a frontal craniotomy. The dura was reflected and a drawing of the surface features (blood vessels and sulcal patterns) of the cortex was made. The exposed surface of the brain was kept moist at all times. A closed recording chamber was glued on to the surface of the skull over the craniotomy and sealed with silastic paste. The chamber was then filled with oil and all air bubbles expelled. A cervical laminectomy was performed at vertebrae C1 to C4. The dura was reflected over the roof of the fourth ventricle to allow free drainage of CSF and over segments C2 and C3 to allow positioning of stimulating surface electrodes over the dorsolateral funiculus.

Monofocal tungsten stimulating electrodes (Clark Electromedical Instruments, impedance of 5MΩ at 1KHz) were implanted stereotaxically into the cerebral peduncles (Horsley-Clarke coordinates A 8.0, L 6.0, and V 96)
3.0) and into the ventrolateral nucleus of the thalamus (Horsley-Clarke coordinates A 11.0, L 5.0, and D 2.0) on the left side (Berman and Jones, '82). The indifferent electrode was a silver wire implanted in the left temporalis muscle. Bipolar silver ball electrodes were positioned over the dorsolateral funiculus of the spinal cord at C2 level. Rectangular pulses, 0.04 msec in duration, were delivered from an isolated stimulator (Digitimer DS2) at all stimulation sites.

All neurons studied were in the motor cortex of the left hemisphere. The technique for intracellular penetration, recording of data, and injection of HRP was identical to that described in the monkey (Chapter IV).

Antidromic responses to stimuli at the peduncles, thalamus or spinal cord, if present, were confirmed by the following criteria: a) their latencies were unvarying even at threshold stimulation, b) the spike was an all or nothing response without underlying epsps at threshold stimulus, and c) they followed stimulus trains at 150 Hz. Orthodromic responses (epsps and ipsps) from stimulation of the cerebral peduncles and ventrolateral thalamus were investigated at various stimulus strengths and recorded when present. Stable intracellular penetration usually could be maintained for between 5 and 10 minutes, rarely for more than 30 minutes. Following iontophoresis of HRP the electrode was withdrawn and extracellular fields were recorded using the same parameters of stimulation.

After the experiment the animals were perfused and the brains processed for microscopic examination of the labeled cells. The region of the thalamus and cerebral peduncles was also blocked and cut parasagittally on a freezing microtome at a thickness of 120 μm. The sections were mounted on slides, stained with thionin and coverslipped. The electrode tracts, thalamic nuclei and
Fig. 1

Projection drawings from relevant parasagittal sections of the thalamus in one of the experiments showing tracks left by the stereotaxically implanted electrodes in the ventrolateral thalamus (A) and cerebral peduncles (B).
fiber tracts were identified and drawn on a projection microscope at a magnification of 17 (Fig. 1). The distance between the cerebral peduncles and the motor cortex was estimated to be 25 mm on average.

Satisfactory labeling with HRP was determined by the following criteria. The neuronal soma and dendrites were darkly stained up to the farthest dendritic tips and spines could be seen clearly along even the finest dendritic branches. The apical dendrites could be followed clearly up to lamina I where their terminal subpial branches were well filled. The axon could be traced into the white matter. Only those pyramidal neurons which satisfied these criteria were used for morphological description and analysis. There were two exceptions to this rule. The axons of lamina II pyramidal neurons were never traced as far as the white matter but only till the upper border of lamina V (although traced for about 500 μm of their intracortical course). Some pyramidal neurons in lamina VI had apical dendrites which clearly terminated in lamina III and did not ascend any further. These apical terminals were always darkly stained up to their ends and the rest of the neuron including the axon was well stained too. Therefore we felt that the shorter apical dendrites of these neurons were not an artifact of inadequate labeling.

Apart from those neurons which were adequately labeled and whose morphology have been described, there were other injected neurons, clearly identifiable as pyramidal cells, which were not completely filled with HRP. Usually their soma and basal dendrites were well stained but their apical dendrites became fainter as they ascended towards the pia with poorly defined or absent subpial terminals. Their axons also became fainter as they descended and could not be followed into the white matter. These neurons were not included for description.
of morphology, but electrophysiological responses in them have been described and attributed to pyramidal neurons of that layer.

Electrophysiological data on tape were redisplayed on the oscilloscope and photographed using a polaroid camera (Tectronix C5 oscilloscope camera). Data of interest were transferred to a computer (Pyramid). Epson and ipsps were averaged and extracellular fields subtracted from the averaged records. Individual sweeps and averaged records were plotted on an XY plotter (Hewlett Packard 7470A) and analysed.
ABBREVIATIONS FOR FIGURES

a = axon
C2 = spinal cord, second cervical segment
epsp = excitatory post synaptic potential
IC = internal capsule
ipsp = inhibitory post synaptic potential
LV = lateral ventricle
OT = optic tract
PP = pes pedunculi
SNR = substantia nigra, reticular division
I, II, III, V and VI = cortical laminae

Thalamic nuclei:
CM = centre median nucleus
LD = lateral dorsal nucleus
LP = lateral posterior complex
Pul = pulvinar nucleus
VA = ventroanterior nucleus
VBa = ventrobasal complex, arcuate nucleus
VBx = ventrobasal complex, external nucleus
VL = ventrolateral nucleus
Fig. 2

Top row: Photomicrographs of pyramidal neurons in lamina II (A), lamina III (B), lamina V (C) and lamina VI (D, two neurons) stained by intracellular iontophoresis of HRP. Bottom row: Photomicrographs of the same neurons after counterstaining the sections with thionin. The calibration bar below panel B applies to panels A and B and that below D applies to panels C and D; both bars equal 100 μm.
RESULTS

Intracellular penetration of a neuron was accompanied by an abrupt change in membrane potential, spikes more than 30 mV in amplitude, and firing of the neuron to injection of positive current. Only those penetrations where a stable membrane potential of at least -40 mV was maintained for more than 5 minutes were used for analysis. The membrane potential in different penetrations varied from -40 mV to -60 mV with a mean of -48 mV (n = 122).

All stained pyramidal neurons were confirmed to be in cytoarchitectonic area 4γ (Hassler and Muh-Clements, '64) and their somata were accurately localized in lamina II, III, V or VI (Fig. 2). All PTNs which were stained (poorly or well) were found to be pyramidal neurons of lamina V, except for one well stained pyramidal neuron of lamina VI.

Antidromic activation of neurons

Pyramidal tract neurons (PTNs) were identified by antidromic activation from the cerebral peduncles. Antidromic thresholds varied from 100 μA to 500 μA. In all cases strengths of stimuli applied to the electrodes in the cerebral peduncles and ventrolateral thalamus, to investigate antidromic and orthodromic effects, were kept below 500 μA. Larger currents (upto 2 mA) were used to investigate antidromic responses to spinal cord stimulation. Only 4 PTNs (3 slow and 1 fast) were also antidromically activated from the spinal cord. One neuron in the study was antidromically activated from the ventrolateral thalamus (but on subsequent histological examination was found not to have stained at all).

Electrophysiological responses
Penetration of neurons with the micropipettes used (tip diameters about 0.5 μm), was often accompanied by signs of injury. Spike durations were usually longer than those reported by investigators using finer electrodes (Phillips, '59) and occasionally stunted spikes were seen. Out of necessity, only a short time was spent on examining antidromic and orthodromic responses of the cells after intracellular penetration, after which the neuron was filled with HRP. Therefore orthodromic responses (epsps and ipsps) described in this study were the predominant response observed. Epsps were not minimal, they were compound i.e. due to activation of a population of afferent fibers, and sometimes followed by ipsps. Similarly very small epsps, which would have required averaging to reveal, were not detected. It is unlikely that any of the epsps described were actually reversed ipsps, since the neuron was usually in a depolarised state (mean RMP -48 mV) and not enough time was available for significant chloride leakage to occur. It is possible that many inhibitory responses were not revealed due to deep anaesthesia. For all the above reasons, in the small sample of pyramidal neurons in each lamina, where electrophysiological data could be correlated with morphological identity, only the latency and nature of the initial synaptic responses will be described. Excitatory responses (epsps), following stimulation of the thalamus or the cerebral peduncles, that were recorded and analysed in a particular neuron were found to have secure latencies (almost identical from trial to trial). These latencies were therefore measured in records of 5 to 10 superimposed sweeps. The latencies of early inhibitory responses (ipsps) were more variable and were measured in averaged records.

Lamina II pyramidal neurons
Fig. 3

A and B: Camera lucida reconstructions of the somata, dendrites and axons of 2 lamina II pyramidal neurons, both in the posterior bank of the cruciate sulcus (orientation: left = ventral, right = dorsal). The axon (labeled a), which could be traced down to the upper border of lamina V, was not seen to give off any collaterals.

C, D and E: Camera lucida reconstruction of the somata and dendrites only of 3 other pyramidal neurons of this lamina. The axons of all these cells could also be traced for about 500 μm (not illustrated) but were not found to give off any collaterals. All pyramidal neurons labeled in this lamina were spiny multipolar neurons.
Camera lucida reconstruction of a smooth multipolar neuron located in lamina II of the posterior bank of the cruciate sulcus (orientation: left = ventral, right = dorsal). The short axon, before terminating in lamina III, gave off 3 collaterals.
Table 1. Morphological dimensions of pyramidal neurons in the motor cortex of the cat.

<table>
<thead>
<tr>
<th>Location and Type</th>
<th>No. of Arbor (um)**</th>
<th>No. of Arbor (um)***</th>
<th>No. of Arbor (um)***</th>
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<tr>
<td></td>
<td>mean (range)</td>
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<td></td>
<td>AP</td>
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<td></td>
<td></td>
<td>(range)</td>
<td>(range)</td>
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<td>3.3</td>
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<td>Lamina 5 16-20</td>
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<td>4.0</td>
</tr>
<tr>
<td>Lamina III X 4-6</td>
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<td>3.3</td>
<td>6.2</td>
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<tr>
<td>Lamina V fast PIN</td>
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<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Lamina V slow PIN</td>
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<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Lamina V others</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Lamina VI 10-18</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
</tr>
</tbody>
</table>

*Since apical dendritic shafts branch as they ascend, their number changes. In this table the number was counted in the lamina above the location of the soma.

**Dendrite measurements refer to arbors in lamina 1.

***Lamina V pyramidal neurons not antidromically activated from the cerebral peduncles.

Abbreviations: coll. = collaterals, cond. vel. = conduction velocity, AP = anteroposterior, ML = mediolateral
Twelve lamina II pyramidal neurons were successfully penetrated and injected, out of which 8 were considered adequately filled for morphological study. They differed from all other pyramidal cells in that none of them possessed characteristic apical dendrites; in fact they looked like spiny multipolar cells. They had spiny dendrites and a long descending axon - therefore they were considered to be pyramidal neurons. It was difficult to define lateral and oblique dendrites, if any, in this neuron type. The ascending (apical) dendrites arborized in lamina I and most terminals were subpial. The descending (basal) dendrites arborized in lamina II and the superficial third of lamina III. A long thin axon arose from the lower pole of the soma and descended towards the white matter. These axons could only be traced up to the upper border of lamina V and therefore their course in lamina V and VI could not be examined. Where they could be followed, the axons of all these neurons were carefully examined under the microscope at high power and no collaterals could be seen originating from any of them. Five lamina II pyramidal neurons are illustrated in figure 3. A nonpyramidal neuron, successfully impaled and stained in lamina II, is shown in figure 4 for comparison. It was also multipolar but non spiny, possessing smooth beaded dendrites and a short axon which, before terminating in a string of boutons in the superficial part of lamina III, gave off 3 locally arborizing collaterals. On average, the size of the somata of lamina II pyramidal neurons was the smallest of all the pyramidal neurons studied, but the tangential span of their apical dendrites often exceeded that of lamina III and lamina VI pyramidal cells (Table 1). No obvious orthodromic responses (epsps or ipsp) were seen in any of these neurons following stimulation of the cerebral peduncles or the ventrolateral thalamus.

Lamina III pyramidal neurons
Camera lucida reconstructions of the soma, dendrites and axon (left panels) and of only the soma, axon and axon collaterals (right panels) of 2 lamina III pyramidal neurons, one in the posterior bank of the cruciate sulcus (orientation: left = ventral, right = dorsal) (A), and the other in the posterior sigmoid gyrus (orientation: left = anterior, right = posterior) (B). The soma of the neuron illustrated in B had burst.
Twenty seven pyramidal neurons located in lamina III were injected with HRP out of which 5 were considered suitable for morphological analysis. Two of these neurons are illustrated in figure 5. A single apical dendritic trunk branched as it ascended. The branches arborized in lamina I. The apical shafts gave off from 2 to 5 lateral and oblique branches in laminae II and III. Three to six basal dendrites arborised within lamina III. The axon, which was usually thicker than that of lamina II pyramidal cells, emerged from the lower pole of the soma or the proximal part of a basal dendrite and could be traced into the white matter. Along its course in the cortex, the axon gave off 6 to 13 collaterals which arborized in and contributed boutons to all the laminae of the cortex. The initial collaterals were usually ascending, while deeper collaterals were more horizontal. Only a few collaterals arborized within the territory of the dendrites. The collaterals were of different lengths; some would terminate in a small spray or a single terminal bouton close to their origins while others could be traced as far as 1.5 mm away from the soma or tangential axis of the cell. The collaterals emerged from the axon and coursed along different tangential planes. Short collaterals had several en passant and terminal boutons along their course, whereas long collaterals usually could be traced for several hundred microns without any branches or boutons until their termination in a small number of clustered terminal and en passant boutons. In all the neurons analysed there were one or two long collaterals that were not completely filled. Spines were common along the course of the axon of some cells and looked like short collaterals, 1 to 3 μm in length, terminating in a single bouton. We did not find any collaterals crossing the borders of area 47.

In 3 lamina III pyramidal neurons, stimulation of the
Camera lucida reconstruction of the soma and dendrites of a lamina III pyramidal neuron in the posterior sygmond gyrus (orientation: left = anterior, right = posterior). This neuron was incompletely filled. The soma and dendrites were well stained, but the apical dendrites became fainter as they ascended and their subpial terminals were not seen. The axon could be traced only for a short distance (100 μm). Stimulating the ventrolateral thalamus at different stimulus intensities evoked suprathreshold epsps and spikes (a) and subthreshold epsps (b and c). All records: 10 superimposed sweeps. Vertical calibration bar equals 40 mV for a and b, and 5 mV for c.
Fig. 7

Camera lucida reconstructions of the soma, dendrites and axon (left panels) and of only the soma, axon and axon collaterals (right panels) of 2 slow PTNs, both in the anterior sigmoid gyrus (orientation: left = anterior, right = posterior). The antidromic responses (10 superimposed sweeps) from the cerebral peduncles are shown alongside each reconstructed neuron.
Fig. 8

Photomicrograph of a slow PTN (the neuron illustrated in figure 7A) intracellularly labelled with HRP (A). The terminal portion of the apical dendrites (B), lateral and oblique dendrites (C), apical dendritic shafts (D), basal dendrites (E), axon collaterals and terminal boutons (F), and soma (G) have been photographed at higher magnification from the same section illustrated in A. Calibration bar equals 120 μm for A and 10 μm for B, C, D, E, F and G.
ventrolateral thalamus evoked suprathreshold epsps and the discharge of the neuron. The latencies of the epsps were 2.5 msec, 3.0 msec and 3.7 msec. Reducing the stimulus intensity evoked subthreshold epsps but the latencies were not altered. None of these three neurons was completely filled but all were clearly pyramidal neurons. The results from one such neuron are illustrated in figure 6. In all other pyramidal neurons in this lamina no obvious psps were seen following peduncular or thalamic stimulation.

**Lamina V pyramidal neurons**

A total of 69 pyramidal cells were stained in this lamina. This included 32 slow PTNs (conduction velocities 5.9 m/sec to 20.0 m/sec) and 11 fast PTNs (conduction velocities 27.5 m/sec to 62.5 m/sec) of which 7 slow and 3 fast PTNs were satisfactorily filled. Twenty six pyramidal neurons, which were not antidromically driven from the peduncles, were also stained in lamina V, out of which 8 neurons were considered adequately stained.

The conduction velocities of slow PTNs whose morphology has been described ranged from 6.7 m/sec to 19.2 m/sec. Two of these neurons are illustrated in figure 7. Photomicrographs of the neuron in figure 7A are shown in figure 8. In all slow PTNs a single apical dendritic trunk branched close to its origin to form 5 to 9 shafts which ascended to arborize in lamina I. From 3 to 10 lateral and oblique dendrites branched from the apical shafts in lamina V (Fig. 7A) or in lamina V and the deeper parts of lamina III (Fig. 7B). Along with the lateral and oblique dendrites the basal dendrites formed a sphere of dendritic arbor around the soma, whose tangential dimensions were similar to those of the subpial arborization of the apical dendrites. Some slow PTNs had a long antiapical dendrite. The axons, before
Fig. 9

Camera lucida reconstructions of the soma, dendrites and axon (left panels) and of only the axon and its collaterals (right panels) of 2 fast PTNs, both in the anterior sygmoid gyrus (orientation: left = anterior, right = posterior). The antidromic responses (5 to 10 superimposed sweeps) from the cerebral peduncles and spinal cord (neuron B only) are shown alongside each reconstructed neuron (intracellular responses for neuron A and extracellular responses for neuron B). The axons are marked a.
Camera lucida reconstructions of the soma, dendrites and axon (left panels) and of only the soma, axon and axon collaterals (right panels) of two lamina V pyramidal neurons, both in the anterior sigmoid gyrus (orientation: left = anterior, right = posterior). These neurons could not be activated antidromically from the cerebral peduncles. The neuron illustrated in B has an atypical morphology: the apical dendrites terminate in lamina III and all the intracortical collaterals have an ascending trajectory and arborize in lamina V and the lower half of lamina III.
The illustrations in this study resemble those in all ways except one. The neurons of spinal ganglia in lamina II were, in our study, automatically injected with 

* Glenon aly, Gl.0, * to which only the ventral spines were connected. This was considered to be a large pyramidal neuron. On the other hand, there was another group of neurons which probably served as the sensory ones. These could be demonstrated in the same sections and could be traced into the white matter. * 200 µm
entering the white matter, gave off 2 to 5 collaterals which branched and terminated in laminae V and VI. Rarely some collateral branches were seen to cross the lamina III/V border for a short distance. The initial collaterals were usually ascending and most of them arborized and terminated in and around the region of the basal and lateral dendrites (Fig. 8E, F). The deeper collaterals were more horizontal and arborized in the deeper parts of lamina V and in lamina VI. Some of these collaterals coursed for longer distances within lamina V and could be traced as far as 1 mm or more away from the soma before being lost. In all cases, one or two long collaterals were incompletely filled. Spines from the axon were very rare.

Conduction velocities of the three well filled fast PTNs were 27.5, 30.9 and 33.3 m/sec. Two of them are illustrated in figure 9. The morphology of fast PTNs, in our study, resembled slow PTNs in all ways except one. The number of apical shafts in lamina III was, on average, larger in fast than in slow PTN.

Eight well stained pyramidal neurons in lamina V were not antidromically driven from the cerebral peduncles. Seven of them resembled PTNs morphologically and one of these is illustrated in figure 10A. One labeled neuron was different from all other pyramidal neurons in this lamina (Fig. 10B). This neuron had a single apical dendrite which terminated in lamina III. The collaterals of its axon were all ascending and they arborized mainly in lamina V and the deep half of lamina III. Since the axon could be followed into the white matter, it was considered unlikely to be a nonpyramidal neuron. On the other hand, spiny stellate and star pyramid neurons, which project their axons into the white matter, have been described in the sensori-motor and visual cortex (Jones, '75; Lund, '84). Also the dendritic and axon
Fig. 11

Camera lucida reconstructions (of neuron s1 only) and intracellular potentials from two slow PTNs, s1 and s2, located in the anterior sigmoid gyrus.

A, B: Camera lucida reconstructions of the soma, dendrites and axon (A) and of only the axon and collaterals (B) of a slow PTN (s1).

a, b and c: Antidromic responses followed by recurrent epsps (a and b) on stimulation of the cerebral peduncles (in b all antidromic spikes are A spikes), and epsps following stimulation of the ventrolateral thalamus (c) recorded in neuron s1. a, b and c show 3, 5 and 4 superimposed sweeps respectively.

d and e: Antidromic responses (d, 5 superimposed sweeps) and recurrent ipsp (e, average of 11 sweeps) recorded in another slow PTN, s2.

Calibration bars between b and c apply to b and c.
Fig. 12

Camera lucida reconstruction of a lamina V pyramidal neuron in the anterior sigmoid gyrus. This neuron could not be antidromically activated from the peduncles. Its axon gave off 3 intracortical collaterals (not illustrated) which arborized in laminae V and VI. Stimulation of the peduncles evoked epsps (some suprathreshold) (a, 7 superimposed sweeps). Subthreshold epsps (b, 5 superimposed sweeps) were averaged (c, average of 5 sweeps). Stimulation of the ventrolateral thalamus also evoked suprathreshold epsps at high stimulus intensities (d, 4 superimposed sweeps). Subthreshold epsps at low stimulus intensities (e, 5 superimposed sweeps) were averaged (f, average of 14 sweeps).
Fig. 13

Histogram of latencies of pspS evoked from the cerebral peduncles and the ventrolateral thalamus in lamina V pyramidal neurons in the motor cortex of the cat. Open rectangles represent fast PTN, filled rectangles represent slow PTN and the striped rectangles represent those lamina V pyramidal neurons that were not antidromically identified. Epsps are shown above the baseline while ipsps are shown below the baseline.
collateral morphology of this neuron resembles that of a spiny stellate cell with an asymmetrical dendrite, or a star pyramid cell. Therefore this neuron has not been included in our analysis of pyramidal neurons.

Antidromic responses in fast PTN were usually followed by epsps. These epsps could in some cases be obtained in isolation by reducing the stimulus below the antidromic threshold. This was achieved in two cases and the latencies of the epsps were 1.3 msec and 2.3 msec. Stimulation of the ventrolateral thalamus, usually at low stimulus strengths (≤100 µA), evoked suprathreshold epsps in three fast PTN, all at latencies of 1.3 msec.

Epsps were evoked in slow PTNs by stimulating the cerebral peduncles, either in isolation or along with antidromic responses (Fig. 11, neuron s1). Latencies of recurrent epsps were measured in 4 slow PTNs to be 2.5, 3.6, 5.6 and 6.25 msec. In three other slow PTN, reduction of the stimulus intensity below antidromic threshold, evoked an ipsp of long duration (>100 msec) at latencies of 1.9, 3.6 and 6.4 msec (Fig. 11, neuron s2). In 10 slow PTNs, stimulation of the ventrolateral thalamus evoked epsps, some suprathreshold, at latencies ranging from 1.4 msec to 5.0 msec.

Epsps were also evoked, following stimulation of the cerebral peduncles and the ventrolateral thalamus, in non PTN pyramidal neurons in lamina V (Fig. 12). In 4 such neurons epsps were evoked from peduncular stimuli at latencies of 1.9, 3.2, 3.4 and 4.0 msec. In 5 of these neurons epsps were evoked by thalamic stimulation at latencies of 1.0, 1.6, 3.3, 3.6 and 4.9 msec. Figure 13 summarizes the latencies of epsps evoked in all lamina V pyramidal neurons to stimuli in the ventrolateral thalamus and cerebral peduncles.
Fig. 14

Photomicrograph (A), camera lucida reconstructions (B and C), and antidromic responses (D) of a fast PTN and a slow PTN adjacent to each other, stained by intracellular iontophoresis of HRP.

A. Photomicrograph of the soma, dendrites and axon of the fast PTN (soma more superficial) and slow PTN (soma deeper).

B. Camera lucida reconstructions of the soma, dendrites and axon of the slow PTN (continuous lines) and of the fast PTN (interrupted lines).

C. Camera lucida reconstructions of the soma, axon and collaterals of the slow PTN (continuous lines) and of the fast PTN (interrupted lines).

D. Antidromic responses recorded from the fast PTN (a) and slow PTN (b) following stimulation of the cerebral peduncles.
Light microscopic evidence of a possible synaptic contact between the axon collaterals of a slow PTN and the basal dendrites of a fast PTN. The two neurons were located adjacent to each other in the anterior sigmoid gyrus (shown in figure 15).

A. Camera lucida reconstruction of the soma and basal dendrites of the fast PTN and the axon and collaterals of the slow PTN. The rectangle marks off the region shown at greater magnification in B and C.

B. Reconstruction at high power (oil immersion objective) of the synaptic contact between a terminal bouton of the slow PTN and the shaft of a basal dendrite of the fast PTN (marked by arrow).

C. Photomicrograph of the area reconstructed in B.
In one experiment, two PTNs, one fast (conduction velocity 27.5 m/sec) and one slow (conduction velocity 19.2 m/sec), located adjacent to each other, were impaled and injected with HRP. The slow PTN was impaled first and filled with HRP. On withdrawing the microelectrode about 100 μm, a fast PTN was encountered which was also penetrated and labeled. Both the PTNs were found to be very well stained and their somata were located about 100 μm apart (Fig. 14A). These two neurons were carefully reconstructed at a magnification of 800. Care was taken to ensure that dendritic and collateral processes followed through subsequent sections were accurately apposed to those from the previous section so that dendrites and collaterals belonging to one neuron were never connected to those of the other. There was considerable intermingling of the dendritic and axon collateral arbors of the two neurons (Fig. 14B,C). Further, there were close crossings of apical dendritic shafts, apical terminals, basal dendrites and lateral dendrites of the two neurons. These crossings were carefully examined under the oil immersion objective (X100) and no visible separations could be observed. Such close crossings were never observed between dendrites of the same neuron. Similar close crossings were also observed at several points between axon collaterals of one neuron and basal dendrites of the other. In one such crossing a synaptic bouton was involved. A terminal bouton arising from a collateral of the slow PTN was found closely apposed to the shaft of a basal dendrite of the fast PTN (Fig. 15). No visible gap separated them even when studied at the highest power of the light microscope (see Brown and Fyffe, '81).

**Lamina VI pyramidal neurons**

Eight pyramidal neurons were injected in this lamina of which 5 were chosen for morphological study. One of them
Fig. 16

Camera lucida reconstructions of 3 lamina VI pyramidal neurons in the anterior sigmoid gyrus (orientation: left = anterior, right = posterior). The apical dendrites of the neurons illustrated in A and B terminated in lamina III. Neuron B was antidromically activated from the peduncles (inset, 10 superimposed sweeps). The apical dendrite of neuron C ascended to lamina I. The axons of none of the pyramidal neurons of this lamina were seen to give off any intracortical collaterals.
Fig. 17

Camera lucida reconstruction of and intracellular potentials from a lamina VI pyramidal neuron in the anterior sigmoid gyrus. Stimulating the ventrolateral thalamus evoked suprathreshold epsps (a, 5 superimposed sweeps). Reducing the stimulus intensity evoked subthreshold epsps (b, 5 superimposed sweeps) which were averaged (c, average of 8 sweeps). Stimulating the cerebral peduncles also evoked epsps (d, 3 superimposed sweeps) which were averaged (e, average of 4 sweeps).
was antidromically activated from the peduncles (conduction velocity 35.7 m/sec) (Fig. 16B). Two other pyramidal neurons of this laminae are also illustrated in figure 16 (A and C). Of the 5 pyramidal neurons described in this layer, three were bitufted neurons with almost symmetrical ascending and descending dendrites (Fig. 16 A,B). The ascending dendrites of the bitufted neurons terminated in lamina III, while the descending dendrites arborized in lamina VI, sometimes crossing into the white matter. The two other pyramidal neurons of this layer were more typical. They had long thin apical dendrites which ascended to arborize in lamina I and along their course gave off several lateral and oblique dendrites in laminae III, V and VI. Their basal dendrites arborized in lamina VI (Fig. 16C). Of all the pyramidal neurons studied, the tangential dimensions of the apical terminals and basal dendrites of pyramidal neurons of this lamina were the smallest (Table 1), while on average these neurons had the largest number of lateral and oblique dendrites. The axons of these neurons, which could always be traced into the white matter, were not found to give off any intracortical collaterals.

In two pyramidal neurons of this lamina stimulation of the cerebral peduncles evoked epsps at latencies of 2.7 and 3.4 msec (Fig. 17). In two others, stimulation of the ventrolateral thalamus evoked epsps at latencies of 2.5 and 3.1 msec (Fig. 17).
DISCUSSION

The principal findings of the study may be summarized as follows:

i. Pyramidal neurons, whose somata were located in a particular lamina, had similar dendritic arbors and intracortical collateral distribution, but differed from pyramidal neurons in other laminae in that regard.

ii. All pyramidal neurons of laminae II, III and V and some pyramidal neurons of lamina VI possessed apical dendrites that arborised in lamina I under the pia.

iii. Pyramidal neurons of lamina II did not possess a typical apical dendrite; all were spiny multipolar neurons.

iv. The axons of pyramidal neurons of lamina II and lamina VI were not seen to give off any intracortical collaterals.

v. The intracortical collaterals of lamina III pyramidal neurons arborized in all cortical laminae, whereas those of lamina V pyramidal neurons were restricted to laminae V and VI.

vi. Pyramidal neurons of lamina III, V and VI were orthodromically excited at short latencies by thalamocortical afferents.

vii. Pyramidal neurons of lamina V and VI were excited by recurrent collaterals of PTNs; a few (all slow PTNs) were recurrently inhibited.

In intracellular studies there is a bias towards sampling of neurons with larger somata. This was evident in this study. In lamina V, where pyramidal neuron somata are the largest, the number of identified neurons successfully impaled and injected was the highest. Fewer pyramidal neurons were impaled successfully in lamina III and the smallest number in laminae II and VI. On the other hand, of the pyramidal neurons that were injected and identified, the highest proportion of well filled neurons
was seen in laminae II and VI (presumably a smaller amount of HRP was needed to fully stain the smaller neurons). The sampling bias is also affected by the density of packing of pyramidal neurons and the width of different laminae. Nevertheless, a reasonable sample of pyramidal neurons was obtained in each lamina for the study. It is likely that these represent the larger neurons in that lamina.

In all pyramidal neurons which gave off intracortical collaterals, a few long collaterals were incompletely filled (since they were not seen to terminate in boutons). In most cases, these incompletely filled collaterals could be traced for a mm or more away from the soma before they were lost. Apart from these long collaterals, the complete intracortical arborization of the processes of pyramidal neurons have been described.

Pyramidal tract neurons were identified by antidromic activation from the cerebral peduncles. The nomenclature is therefore not strictly accurate since not all descending fibers from the motor cortex contained in the peduncles reach the medullary pyramids. Some labeled pyramidal neurons in lamina V were not antidromically driven from the peduncles. It is possible that some of these were PTNs but were not activated by the testing stimuli. This could be due to insufficient strength of the stimulus current or anodal block of axons situated at a distance from the electrode tip (Phillips, '59). Only one neuron was antidromically activated from the thalamus although many pyramidal neurons, some possibly corticothalamic, were labeled in laminae V and VI. The short duration stimuli used in this study (to ensure acceptably short artifacts for studying short latency psp's) may not have been effective in activating the fine terminals and branches of corticothalamic neurons (Lipski, '81). Stronger stimuli (>500 μA) were not used.
because of the possibility of stimulus spread to the internal capsule.

Orthodromic responses studied in pyramidal neurons following stimulation of the cerebral peduncles are most likely to be responses to activation of recurrent collaterals of PTN. Afferent pathways to the motor cortex (in the medial lemniscus and internal capsule) were located more than 2 mm away from the stimulus site and stimulating currents were always lower than 500 μA. Similarly, low intensities of stimulus (<500 μA) were used to study thalamocortical projections to pyramidal neurons to prevent stimulus spread to the internal capsule. Due to the restricted nature of the stimuli at these two sites, absence of any orthodromic (or antidromic) responses in the impaled neurons, cannot be interpreted to imply absence of any such input. Epsps that were recorded in pyramidal neurons were found to have secure latencies. Therefore the excitatory responses described, probably involving monosynaptic pathways, will be related to anatomical evidence, where available, of synaptic contacts made by thalamocortical afferents and recurrent collaterals on pyramidal neurons.

All previous descriptions of the morphology of lamina II pyramidal neurons, in the visual cortex of the infant monkey (Tigges and Tigges, '82), in the somatic sensory cortex of the rat (Kirsch et al. '73), in the visual cortex of the adult cat (Gilbert and Weisel, '83) and in the visual cortex of the rat (Parnevelas et al., '83), have stated that many intracortical collaterals arise from their axons in laminae II and III. Similarly, all previous descriptions of lamina VI pyramidal neurons (Tombol, '84; Scheibel and Scheibel, '70; Martin and Whitteridge, '84; Gilbert and Wiesel, '83) have found intracortical collaterals originating from the axon in lamina VI. Therefore our findings that pyramidal neurons
of laminae II and VI in the motor cortex lacked such collaterals requires careful examination. It is possible that intracortical collaterals exist but were not labeled in this study. This seems unlikely, since their axons were traced for long distances (>500 µm in most cases), without any sign of the origin of intracortical collaterals. At the same time a number of pyramidal neurons in lamina III (and one nonpyramidal neuron in lamina II) of similar size and similar density of staining were found to have well stained collaterals. Motoneurons without recurrent collaterals have been described before (Lipski et. al., '85, Lipski and Martin-Body, '87), but not pyramidal neurons of the motor cortex. This finding in the motor cortex may have important implications regarding the efferent output from these two laminae. Indirect evidence supports our result. Following large injections of HRP in the sensori-motor cortex of monkeys, pyramidal neurons which were labeled in the region surrounding the injection site at some distance, were seen only in laminae III and V and not in laminae II and VI, where the lack of axon collaterals would preclude labeling of such neurons (Jones et. al., '78).

The morphology of PTNs has been studied previously by the same technique in the cat motor cortex (Deschenes et al., '79b; Landry et al., '80). The morphological dimensions and descriptions of slow PTNs in this study compare very well with theirs. However, our descriptions of fast PTNs differ in several respects. The sizes of somata of fast PTNs in their study (range of transverse diameters 28 to 53 µm, range of longitudinal diameters 68 to 85 µm) was much greater than in our study (Table 1). Similarly the conduction velocity of the fast PTNs studied by them ranged from 25.0 to 62.5 m/sec while those stained by us had velocities that were at the lower end of this range. These investigators found that apical dendritic shafts of
fast PTNs were almost devoid of spines whereas in our study they were found to be profusely spiny. Therefore, it is likely that our small sample of fast PTN are representative of only the smaller cells of the fast PTN population.

Pyramidal neurons, whose axons and collaterals have an exclusively intracortical distribution, have been described in the visual cortex (Gilbert and Wiesel, '79; Martin and Whitteridge, '84). Such neurons were not observed in our study.

Pyramidal neurons, whose somata were located in lamina III, had many intracortical collaterals which arborised extensively in and contributed boutons to all the laminae of the motor cortex. The postsynaptic targets of these boutons are not known. In one study (Winfield et al., '81) the postsynaptic targets of the proximal axon and recurrent collaterals of a Golgi impregnated pyramidal neuron in lamina III of the monkey somatosensory cortex were studied electron microscopically. Of the 62 synapses studied, 49 were formed by the axon and 13 by the collaterals. Sixty percent of these synapses were on the shafts of dendrites, about half of which were on dendrites of large smooth stellate cells (basket cells). Forty percent of the synapses were axospinous. It is likely that these collaterals mediate recurrent excitation and inhibition on other pyramidal neurons. These investigators also found a large number of spines emerging from the axon of the neuron studied.

The distribution of intracortical collaterals of lamina V pyramidal neurons was found to be restricted to laminae V and VI. A number of studies show that recurrent collaterals of PTNs excite and inhibit other PTNs (Phillips, '59; Armstrong, '65; Takahashi et al., '67; Stefanis and Jasper, '64a; Deschenes et al., '79a) and
non PTNs (Tsukahara et al., '68; Landry et al., '84). These findings were confirmed in the present study. In addition it was found that lamina VI pyramidal neurons were also post synaptic targets of PTNs. The physiological significance of recurrent excitation and inhibition mediated by PTNs is not fully understood. Many questions remain to be answered, including those that relate to the interactions between specific PTNs through their collaterals. We had a chance to examine a few of these questions by complete reconstruction of two labeled PTNs located adjacent to each other. With reservations regarding the small sample size and the absence of electron microscopic evidence, this result shows that monosynaptic recurrent collateral excitation from one PTN on to another is capable of linking adjacent neurons. The synapse involved the basal dendrite of the target PTN and was represented by a single bouton. The excitation produced by such a synapse from one individual PTN on another is therefore likely to be weak. There is some evidence to suggest that inhibitory interneurons mediating recurrent inhibition from PTN collaterals synapse on the axon initial segments of the target neurons (Stefanis and Jasper, '64b). Hence the other postsynaptic targets of PTN collaterals could include basket cells or chandelier cells (Somogyi et al., '83; Somogyi et al., '82).

The distribution and arborization of the dendrites of pyramidal neurons of various laminae have been described and provide data regarding the spatial dimensions over which afferent input may be made to these neurons. This data can be related to the terminal arbors of extrinsic afferent fibers. Thalamocortical fibers from the ventrolateral thalamus have been found to terminate mainly in lamina III, the superficial part of lamina I and in lamina VI (Strick and Sterling, '74). Ninety one percent of VL synapses were found on dendritic spines, 8%
on stellate type dendritic shafts, and 1% on stellate neuronal somata. In a different study, Deschenes and Hammond ('80) stained individual thalamocortical axons by intraaxonal injections of HRP. Individual fibers were found to arborize widely in the motor cortex with tangential dimensions of more than 3 or 4 mm and to contribute synaptic boutons mainly to laminae III and VI. Electrophysiological studies show that both fast and slow PTNs are monosynaptically excited by thalamocortical afferents at latencies between 1 and 5 msec and that this latency is related to the conduction velocity of the target neuron (Deschenes et al., '82). The latencies of thalamocortical epsps in lamina V pyramidal neurons obtained in the present study fall within this range. We also found that thalamocortical fibers excite lamina III and lamina VI pyramidal neurons at latencies similar to those of activation of PTNs. Thalamocortical synapses have been demonstrated on the apical dendritic shafts of fast PTNs (Liu et al., '86) and on the apical dendritic spines of slow PTNs (Deschenes et al. '82), as well as on the spines and shafts of lamina III pyramidal neurons (Ishikawa et al., '85) in the motor cortex. Thalamocortical synapses have been demonstrated on retrogradely labeled corticothalamic neurons (in lamina VI) in the somatic sensory cortex (Hersch and White, '81) but not in the motor cortex.

Corticocortical afferents to the motor cortex from the somatic sensory cortex, premotor cortex and the contralateral motor cortex terminate mainly on dendritic spines (Sloper, '73). The terminations of somatic sensory cortical afferents are restricted mainly to the superficial cortical laminae (I, II and III), whereas callosal and premotor cortical afferent terminals are more evenly distributed in the superficial and deeper layers. Therefore all corticocortical afferents can potentially terminate on the dendrites of pyramidal
neurons of any lamina. This is supported by
electrophysiological evidence (Herman et al., '85).

The output neurons of the motor cortex, the pyramidal
neurons, project their axons to a large number of
cortical and subcortical targets. Pyramidal neurons are
segregated in the cortical laminae in relation to the
their distant projections. The morphology of these
neurons varies considerably in different laminae which
may be related to differences in synaptic influences on
the stratified output elements of the motor cortex.
Further studies are needed to understand how these
differences determine the different functional roles
played by pyramidal neurons in cortical control of
movement performance.
GENERAL DISCUSSION

A number of studies were carried out in the monkey and in the cat to investigate afferent inputs to the primary motor area of the cerebral cortex. The new findings revealed in these studies may be summarized as follows.

Although the locations of neurones projecting to the precentral motor area in monkeys have been investigated before using the retrograde transport of horseradish peroxidase (HRP), this is the first study (Chapter III) that has attempted to quantitate these results. This quantitative study made it possible to estimate the relative numbers of neurones in different cortical areas and thalamic nuclei that contribute extrinsic afferent input to the primary motor area and to emphasize the numerical importance of projections from premotor and supplementary motor areas.

Detailed descriptions of the morphology of pyramidal neurones in the motor cortex were provided. In the cat, the morphology of pyramidal neurones in laminae II, III, V and VI were described, while in the monkey, the morphology of pyramidal neurones in laminae III and V were detailed (Chapters IV and VI). Such detailed descriptions of morphology (made possible by revealing the nearly complete arborizations of processes of neurones through intracellular injections of HRP), have been restricted hitherto, in the motor cortex, to pyramidal tract neurones (PTNs). In the present studies, the descriptions revealed considerable differences in the dendritic and axon collateral arbors of pyramidal neurones in different laminae, not previously described. Of particular importance is the finding that lamina II pyramidal cells differ markedly in morphology from those in lamina III and have no intracortical collaterals. Lamina VI pyramidal cell axons also have no intracortical
collaterals.

The synaptic actions of the intracortical collaterals of PTNs were studied in the cat by antidromic volleys set up in the cerebral peduncles. In addition to the observations reported in previous studies, which have investigated these actions on other PTNs only, and which were confirmed in the present study, recurrent collaterals of PTNs were also shown to excite lamina VI pyramidal neurones as well as lamina V pyramidal cells that were not PTNs (which could have been corticostriatal cells).

Examination of the synaptic effects of recurrent collaterals of PTNs has not been achieved before in primates. The observations made in chapter IV were therefore, unique and made possible comparisons of observations made in the cat with those in the primate. One important finding in these observations was that recurrent collaterals of fast PTNs were capable of exciting other fast PTNs, an interaction not seen in the cat. Another important finding was an inhibitory influence initiated by axon collaterals of PTNs on pyramidal neurones of lamina III.

A number of degeneration studies in the cat and monkey have shown that extrinsic afferent fibres to the motor cortex, both thalamocortical and corticocortical, make synaptic contacts predominantly on dendritic spines. However, the synaptic actions of and the identity of neurones postsynaptic to these afferents were largely unknown. In the studies reported in the thesis, thalamocortical fibres (in the cat) and corticocortical fibres (in the monkey) were shown to monosynaptically excite pyramidal neurones located in a number of laminae of the motor cortex. Several afferent fibres were also shown to converge on individual pyramidal neurones and
also to initiate inhibition of pyramidal neurones through local interneurones. These findings may be important for inclusion in the broader framework of our knowledge of connections and functions for the motor areas of the cerebral cortex.

One of the characteristic features of primate evolution has been the increase in motor skills, especially with regard to the use of the forelimb. This is paralleled by an increase in size of the forebrain, associated with increased complexity and synaptic connectivity in the cerebral cortex. As Phillips and Porter state in their monograph (1977): "Primates, more than any other mammals, have depended for success in the struggle for existence on the behavioural adaptability conferred by big brains. Their behaviour is less stimulus bound than that of lower forms: more marked by learning, internal trial and error and prediction." The motor expression of this behaviour is therefore equally less stimulus bound and equally complex in relation to the internal and external cues that generate and modify this expression.

Not all parts of the forebrain have enlarged equally during evolution: areas in the frontal, parietal and temporal lobes (association areas) have achieved relatively greater increase in their size than others. The secondary motor areas are an example: in the monkey these areas are roughly equal in size to the primary motor area, whereas in humans they are six times larger (Jerison, 1973). These secondary motor areas have close links with the primary motor cortex and other motor centres in the brainstem, and have been found to play a significant role in the planning and programming of movement performance (Roland, 1984).

The findings from the quantitative study of corticocortical connectivity provide anatomical evidence
of the dominance of the secondary motor areas as the sources of afferent input to the primate motor cortex. These findings are in contrast to the anatomical study of Sloper (1973b) which found fewer degenerating synapses in area 4 following ablation of the postarcuate premotor area than after ablations of the somatosensory cortical areas or the ventrolateral thalamus. We must conclude that, although we have shown that a large number of neurones do project from area 6 to area 4, they individually make fewer synapses at their targets. This conclusion was supported by our electrophysiological studies; excitatory responses in pyramidal neurones of the motor cortex were more difficult to evoke from the premotor area and were smaller in amplitude than those from the somatosensory areas. Connections from the premotor area may be discrete and their functional importance may be found in the specificity of the connections that are established through this pathway.

An important finding of the quantitative study was the profuseness of connectivity demonstrated within the precentral motor cortical representation of the forelimb itself. It was suggested that all or a proportion of this connectivity may have been provided by the intracortical collaterals of the axons of the labelled pyramidal cells. However, most of the retrogradely labelled neurones were located in lamina III, and it may be argued that labelling through the axon collaterals would affect neurones in all laminae equally. The examination of the morphology of pyramidal neurones in different cortical laminae provided one possible explanation for this result. It was found that recurrent collaterals of pyramidal neurones of lamina V were restricted to the deeper cortical layers (V and VI), whereas those of lamina III pyramidal neurones could arborize more extensively in all the cortical laminae. Lamina II and lamina VI pyramidal neurones were found (in the cat) not
to have any intracortical collaterals at all. Therefore the injections of HRP made in the quantitative study, and which spread more in the middle layers of the cortex, were likely to label those pyramidal cells that had the most extensive axon collateral branching in those layers, i.e. pyramidal cells of lamina III. Thus, overall, pyramidal neurones in lamina III have a greater intracortical connectivity than other pyramidal cells. This may be recognized both by retrograde labelling from axon terminations of local corticocortical neurones in nearby regions of area 4, and by retrograde labelling through axon collaterals of corticocortical neurones with more distant terminal projections.

That the intracortical collaterals of pyramidal neurones, especially the long ones, make discrete connections, was suggested both by the retrograde tracer study and the morphological studies. The retrograde tracer study showed that connectivity within the precentral area was confined within the forelimb area even when large injections of HRP were made. In the description of the axon collaterals of pyramidal neurones, it was noted that the longer collaterals seldom branched, that they could be traced for long distances without providing any boutons, and that when they could be traced to their ends (rarely), they terminated in a small number of boutons. These collaterals are one source of horizontal connectivity in the predominantly vertical orientation of intracortical neuronal chains.

In his descriptions of the arborizations of the dendritic and axonal branches of neural elements and the terminal arbors of afferent fibres, Lorente de No (1949) pointed out the predominantly radial arrangements of these processes in the cerebral cortex. Since then, the radial architecture of the sensory and motor areas of the cerebral cortex has been studied extensively. In the
motor cortex, Asanuma and his colleagues (1975) used the method of intracortical microstimulation to produce evidence that the loci within the cortex at which stimulation with weak currents produces small movements of a distant joint are arrayed in segregated vertical columns 0.5 to 1.0 mm in diameter. By recording through the same electrode, they also found that these columns were recipients of short latency inputs from muscle, joint and cutaneous receptors closely involved with movements evoked by microstimulation. Anatomical evidence for a columnar organization also came from studies of corticocortical connectivity. Jones and Wise (1977) showed that the cells of origin of corticocortical and callosal projections, and their terminations, as well as the cells of origin of corticobulbar and corticospinal projections from the motor cortex occurred in clusters which formed mediolaterally oriented strips, 0.5 to 1.0 mm wide, separated by gaps of comparable size.

Other studies which sought to define the nature of the functional radial architecture of the motor cortex arrived at different conclusions. Andersen et al. (1975) investigated the spatial boundaries of the most direct spinal projections of the precentral motor area - the corticomotoneuronal colonies which engage a single motoneurone. A single corticomotoneuronal colony was found to be included in a zone of the precentral area several square millimetres in area. Lemon and Porter (1979) compared the natural activity of motor cortical neurones in conscious monkeys during specific movement tasks with the response of these neurones to passive manipulations of the involved joints and stroking of adjacent areas of the skin. Although they found a close relationship between these input and output parameters in a large sample of neurones, they also found neurones in close proximity to one another which could be naturally active during active or passive movements of joints (of
the forelimb) which were widely separated anatomically. So cells with similar functional associations were spread through large zones of cortex and were intermingled with those having different functional associations.

At present there seems to be no clear consensus regarding the nature of columns (if any) in the motor cortex, the spatial dimensions of such columns, and their relation to control of movement performance. Studies of the morphology of individual neurones in the motor cortex and their interconnections may provide clues in this regard. In the intracellular studies reported here an overwhelming bias was found towards impaling pyramidal cells and a large sample of pyramidal neurones was studied in different laminae of the motor cortex.

Individual pyramidal neurones had characteristic dendritic and axonal collateral arborizations which were found to be similar within a given lamina. However pyramidal neurones in different laminae differed in their morphology. The large pyramidal neurones located in lamina V included many PTNs. Their detailed morphology was examined in both the cat and the monkey. Their apical dendrites ascended to lamina I where the terminal branches arborized under the pia. The basal and lateral dendrites formed a sphere of dendritic branching whose radial dimensions were similar to those of the subpial arborization of the apical terminals. A cylindrical zone may then be envisaged through the cortical thickness which would enclose these arboris and be filled by them in lamina I and lamina V. The short axon collaterals would also be included in the volume of this cylinder and would be limited to its deeper parts, essentially in lamina V. However the long basal dendritic branches and the long axonal collaterals would cross the boundaries of this cylinder to ramify more extensively. This would allow the neuron to both receive synaptic inputs as well as exert
synaptic actions outside this envisaged cylindrical zone. In one exceptional case a PTN in the monkey was found to have axon collaterals that would be capable of exerting its synaptic influences almost exclusively outside this zone. A comparison of the morphology of PTNs in the cat with those in the monkey revealed that the dimensions of this cylindrical zone were smaller in the monkey, even though the monkey has a much larger volume of motor cortex. Each PTN in the monkey may then have a more limited zone in which it may receive and exert influence within the cortex and there may be more segregation of separate effects of individual PTNs. Collateral branches of the axons of pyramidal neurones of lamina V were found to be confined to the deeper cortical laminae (V and VI) in all cases.

Pyramidal neurones located in lamina III were also studied in both the cat and the monkey. These neurones are likely to be corticocortical neurones (Jones and Wise, 1977). Their dendritic arbors were similar to those of PTNs except that this arbor was now confined to the superficial layers. A cylindrical zone envisaged as above would now contain all the dendritic branches of corticocortical neurones and be filled by them in lamina III and lamina I. Since these neurones were not seen to have any long dendrites their receptive surface can be assumed to be restricted to this zone. If compared to lamina V pyramidal neurones, the dimensions of the zone are much smaller in the cat but of approximately equal size in the monkey (it must be remembered that the long dendrites of PTNs extend outside this cylindrical zone and do not determine its dimensions). The arborizations of the intracortical collateral branches of the axons of corticocortical neurones was found to be the most extensive of all the types of pyramidal neurones studied. They involved all the cortical laminae in most cases. In a few cases in the monkey these branches appeared to be
more restricted although this finding could be an artifact of inadequate labelling. The horizontal ramifications of these axonal collaterals were equal to or even greater than those recorded for PTNs. When the total arbors of these collaterals are considered, only a small proportion of the arbor contributed boutons to the zone containing the dendrites. Most of the sphere of influence of the intracortical collaterals must therefore be to regions outside the immediate vicinity of the lamina III pyramidal cells.

Lamina II pyramidal neurones were described only in the cat, where a small but well stained sample of these neurones was obtained. It has been mentioned that the morphology of these neurones departed in many ways from the classical descriptions of pyramidal neurones. They had no typical apical dendrite. Their dendritic arbor was contained in a spherical zone within lamina I and II, although such a zone may perhaps be considered to be a short truncated cylinder limited to the upper part of the cortical mantle. Their axons were not traced as well as the other pyramidal neurones examined. Where they could be seen they were not seen to give off any collaterals. Lamina II pyramidal neurones are also likely to be corticocortical neurones. It has been suggested from retrograde tracer studies that projection neurones in this lamina have cortical target zones that are closer than those of pyramidal neurones located in lamina III (Jones et al., '78). Since pyramidal neurones in these two laminae differ greatly in their morphology, especially with regard to the lack of axonal collaterals of lamina II cells, it seems likely that they form separate classes of corticocortical neurones with different functional associations.

Pyramidal neurones whose somata were located in lamina VI were also studied only in the cat. These neurons are
likely to be corticothalamic neurones (Jones and Wise, '77), although none of them were antidromically characterized (probably for technical reasons as mentioned earlier in the text). These neurones had the smallest dendritic arbors of all the neurones studied. In some cases the apical dendrites did not ascend to lamina I. In all cases the axon was seen not to give off any intracortical collaterals even though the stem was well filled and could be traced for considerable distances. These pyramidal neurones in lamina VI can exert no sphere of intracortical influence.

These descriptions of the morphology of pyramidal neurones provide additional support to the concept of laminar segregation of projection neurones in the motor cortex depending upon the target for projection of their axons. As has been indicated above it was found that pyramidal neurones in the different laminae have very different spatial distributions of their axonal collaterals (where such collaterals exist). Therefore the intracortical interactions initiated by these collaterals are likely to be significantly different for different classes of cells with different axonal destinations.

If functional columns exist in the motor cortex, then such columns may be envisaged as receiving cylindrical zones containing and filled by the dendritic arbors of pyramidal neurones with their somata, apical dendrites and axons located at the axis of the cylinder. Such a cylinder would also include other neurones with a variable portion of their processes within the same zone. The intracortical collaterals of the axons (if present) would extend a variable part of their influence outside such a hypothetical column, some populations of pyramidal neurones communicating with more distant territories, while others made no contacts with intracortical territories or had an influence limited within the
cylindrical volume. It was also noted that in PTNs some basal dendritic branches would provide a receptive surface which extended outside the column. Such a column was smaller in diameter for PTNs in the monkey than in the cat.

The synaptic effects of the recurrent collaterals of PTNs were studied in the cat as well as the monkey. These effects represent interactions between projection neurones. In the cat it was found that, apart from the interactions between PTNs described before by Stefanis and Jasper (1964a), collaterals of PTNs excited other pyramidal neurones of lamina V as well as pyramidal neurones located in lamina VI. Since intracortical axon collaterals of PTNs are restricted to the deeper cortical layers, their direct synaptic actions can be expected to be restricted to these layers. The basal dendrites of PTNs arborize extensively in lamina V. Therefore axon collaterals of PTNs are likely to synapse on the basal dendrites of other PTNs to produce the excitatory interactions which have been recorded. Such a predicted site of synaptic action was shown in one case by labelling two PTNs located adjacent to one another. In that case the synaptic contact that could be visualised was limited to only one bouton. Other possible targets of direct synaptic action of these collaterals are the dendrites of lamina VI pyramidal neurones; an excitatory action was confirmed electrophysiologically.

In the monkey, synaptic actions of the recurrent collaterals of PTNs were studied and provided comparisons with observations in the cat. It was found that, in addition to the interactions seen in the cat, fast PTNs were monosynaptically excited by the recurrent collaterals of other fast PTNs. The amplitude of synaptic excitation and inhibition was found to be affected by the intensity of stimulation of the pyramidal tract or
cerebral peduncles, indicating considerable convergence of axon collaterals on to individual neurones. Excitation was inevitably followed by inhibition.

Interactions between pyramidal neurones (including PTNs) through recurrent collaterals provide one avenue by which activity in individual projection neurones is linked obligatorily to activity in other, presumably related, neurones. Such interactions favour concurrent activity in a group of projection neurones while suppressing activity in another group. Some observations on the excitatory interactions reported earlier in the text provide additional data in this regard. It was observed (although in only one case) that recurrent collaterals of an individual PTN make a small number of contacts with the dendrites of an adjacent PTN, but may make such synaptic contacts with a number of PTNs (since convergence from several active collaterals was shown). Therefore though such actions may be weak, taken singly, the combined action of a number of PTN collaterals may increase this effect. This might help to determine the probability of different, selected combinations PTNs being active in different behavioural contexts.

The detailed excitatory and inhibitory synaptic effects of extrinsic afferent fibres on pyramidal neurones in the motor cortex were investigated. It was found that thalamocortical fibres and corticocortical fibres monosynaptically excite pyramidal neurones located in a number of laminae. This excitatory drive (at least from the thalamus and from the somatosensory cortex) was powerful and convergent. These extrinsic afferents are then capable of monosynaptically exciting pyramidal neurones, which though located in different laminae, may be part of a single functional unit, perhaps a column, at similar latencies. This would preserve the temporal pattern of activity in the afferent pathway for further
synaptic integration in all the associated target neurones. Synaptic excitation of pyramidal neurones by these afferents was followed by inhibition mediated through local interneurones.

The extent of arborization of individual extrinsic afferent fibres in the motor cortex is known for thalamocortical fibres. Single afferent fibres from the thalamus ramify in lamina III, V and VI of the motor cortex in a territory that may be several millimetres wide (Deschenes and Hammond, 1980). This territory must overlap with the territory of adjacent thalamocortical fibres considerably. Perhaps the overlapping and convergent inhibitory actions of corticocortical fibres (through interneurones) are capable of adjusting this territory of synaptic excitation of individual afferents in a more flexible manner even though their terminations are not restricted to a very limited zone.

Reciprocal corticocortical and thalamocortical connectivity has been observed during studies of connectivity. However it has not been known whether thalamocortical fibres can influence corticothalamic neurones, and whether corticocortical fibres can directly influence corticocortical neurones in the cortical target area. Both these relationships were shown to exist by the present studies in cats and monkeys. This opens up the possibility of reciprocal connectivity occurring at the level of individual corticocortical neurones, and between individual thalamocortical and corticothalamic neurones. This would need to be investigated at the level of single neurones by simultaneous examination of a pair of reciprocally connected neurones.

To conclude, this thesis has provided new observations concerning cell to cell connectivity, which underlies the generation and control of activity in the motor cortex.
The findings emphasize the immense variation in pyramidal cell morphology, and the very great complexity of the several forms of intracortical connectivity which exist in this area. If, as seems possible, different morphologies and different intracortical connectivities are characteristic of particular functional classes of pyramidal cells with particular axonal targets, these findings will have important implications for theories of operation of the synaptic machinery which determines activation of the multitude of cortical cells which change their firing in association with a simple movement performance.


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