Corticoreticular Pathways in the Cat. I. Projection Patterns and Collaterization

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Kably, Bouchra and Trevor Drew. Corticoreticular pathways in the cat. I. Projection patterns and collaterization. J. Neurophysiol. 80: 389–405, 1998. This paper summarizes and compares the projection patterns and the receptive fields of cortical neurons in areas 4 and 6 that project to the pontomedullary reticular formation (PMRF). A total of 326 neurons were recorded in area 4 and 129 in area 6 in four awake, unrestrained cats that were chronically implanted with arrays of electrodes in the PMRF and the pyramidal tract (PT). In area 4, 47% of the neurons projected to the caudal PT but not to the PMRF (PTNs); 19% were activated only from the PMRF (PMRFNs), whereas 27% were activated from both the PT and the PMRF (PTN/CRNs). More PTN/CRNs conducted at velocities >20 m/s (82%) than did CRNs (23%). In area 6, only 19% of the neurons were identified as PTNs, 12% were PTN/CRNs and 31% were CRNs; a further 38% could not be activated from either structure. Collateral branches within the PMRF conducted at maximum velocities of 20 m/s (average = 6.5 m/s). No significant differences in the conduction velocities of the collateral branches were found either between fast and slow PTNs or between area 4 and area 6 neurons. A large proportion of neurons in area 4 (85/173, 49%) were activated by passive manipulation of the more distal, contralateral forelimb, with approximately equal numbers being classed as PTNs, PTN/CRNs and CRNs. Most neurons in area 6 for which a receptive field could be found were excited by lightly touching or tapping the face and neck; a receptive field could not be determined for 39% of the area 6 neurons compared with only 5% of those in area 4. Finally, there was evidence that neurons in quite widespread areas of the pericruciate cortex, including both areas 4 and 6 projected onto similar, restricted regions of the PMRF. The fact that the cortical projection from area 4 to the PMRF includes a high percentage of fast PTNs with a receptive field on the distal forelimb is consistent with the view that this projection may serve to integrate movement and the dynamic postural adjustments that accompany them. The fact that the cortical projection from area 6 to the PMRF is primarily from slow PTNs with receptive fields on the face, neck and back is consistent with a role for this cortical area in adjusting the general posture of the animal on which movements are superimposed.

INTRODUCTION

Movements that displace the center of gravity are normally accompanied by postural adjustments that provide the support on which the movements are superimposed. The biomechanical and electromyographic correlates of such changes in posture have been well described in a variety of tasks in human subjects (Bouisset and Zattara 1981, 1986; Brown and Frank 1987; Cordo and Nashner 1982; Mouchonino et al. 1992; Rogers and Pai 1990), as well as in cats trained to make conditioned movements of a forelimb (Alstermark and Wessberg 1985; Dufossé et al. 1982). Modification of postural activity is also important before (Jian et al. 1993) and during (MacKinnon and Winter 1993; Yang et al. 1990) locomotion, especially in situations in which the gait has to be modified to step over obstacles (Lavoie et al. 1995; Patla et al. 1991).

The neural mechanisms underlying the integration of the voluntary and postural components of a movement are less well understood. Of importance, however, is the finding of Gahery and Nieoullon (1978) that stimulation of the motor cortex in standing cats induced both a flexion movement of the contralateral forelimb and anticipatory changes in posture (MacKinnon and Winter 1993; Yang et al. 1990). The corticoreticular pathway may play a role in the production of these feed-forward postural responses is provided by reports that injection of the cholinergic agonist, bethanecol, into the pontine reticular formation in standing cats disrupts the postural responses that accompany both stimulation of the motor cortex (Luccarini et al. 1990) and voluntary movements of the forelimb (Sakamoto et al. 1991).

That the corticoreticular pathway may play a role in integrating movement and posture is certainly supported by the strong projections that have been demonstrated from the pericruciate cortex to the PMRF (Berrevoets and Kuypers 1975; Canedo and Lamas 1993; He and Wu 1985; Jinnai 1984; Keizer and Kuypers 1984; Kuypers 1958; Lamas et al. 1994; Magni and Willis 1964; Matsuyama and Drew 1997; Newman et al. 1989; Pilyavsky and Gokin 1978; Rho et al. 1997), including a significant projection from the relatively more lateral regions of area 4 that control movements of the limbs (Matsuyama and Drew 1997; Rho et al. 1997). However, there is little direct evidence of the extent of this projection, or of its properties, especially concerning the receptive fields of the projection neurons, the extent of divergence and convergence at the single cell level, or of the conduction velocity of the collateral branches. In addition, there is no information at all concerning the discharge characteristics of these neurons that would provide evidence for or against the hypothesis that they play a role in the integration of posture and movement.
We have therefore carried out a series of studies designed to reply to these questions with the dual aims of determining which neurons are most likely to play a role in the integration of posture and movement and what their projection patterns are. The first of these two reports examines the characteristics of the corticoreticular projection with a special emphasis on the similarities and differences in the projections from different parts of areas 4 and 6. The second paper in this series addresses the question of the signal that is carried by corticoreticular neurons in area 4 during voluntary gait modifications.

Two abstracts of this work have been published (Kably and Drew 1992, 1994).

Methods

These experiments were carried out in four cats that were trained to step over obstacles attached to a moving treadmill belt (see Drew 1993).

Surgical procedures

After training, all animals were chronically implanted with a recording chamber that gave access to the pericruciate cortex (areas 4 and 6) and with arrays of stimulating electrodes in the PMRF to permit the antidromic identification of corticoreticular neurons. The methods for the implantation of the electromyographic recording electrodes and for the motor cortical chamber are detailed elsewhere (Drew 1993; Drew et al. 1986) and will be described only briefly here. All procedures were carried out under the guidelines published by the Canadian Medical Research Council and were approved by the local animal ethics committee at the University of Montréal.

After presurgical treatment (see Drew 1993 for details), the animals were anesthetized (pentobarbital sodium, Somnotol, 25–30 mg/kg iv), and a rectangular craniotomy (∼8 × 2 mm) was made in the occipital bone on the right side of the skull adjacent to the midline. A small window was then made in the dura and pia mater at the most rostral aspect of this craniotomy, and a bundle of six microwires (25- or 50-μm-diameter stainless steel wire insulated with TriML: Cooner Wire), each vertically separated from the other by 1 mm, was inserted stereotaxically into the rostral regions of the PMRF at L1.5 using a variation on the method originally described by Palmer (1978; see also Drew 1993). The most ventrally located wire was identified as wire 1. Two other bundles were subsequently inserted 2 and 4 mm, respectively, caudal to the first and at the same laterality. A fourth bundle was placed more caudally and directed at the pyramidal tract (PT) at L1.0. In one animal (MC18) this bundle of electrodes was positioned at P9.3, whereas in the other three animals, it was placed more caudally (P13–P15), at the level of the pyramidal decussation. In one cat (MC21), electrodes also were placed in the cerebral peduncle at A4.0, L3.5. The craniotomy was closed with dental acrylic and a similar procedure was followed on the left side. There were thus 18 microwires implanted in the PMRF on each side of the brain stem (see Figs. 1 and 2). The microwires were connected to a 51 pin D connector that was fixed to the skull of the animal with dental acrylic. The animal was left to recover, and analgesics (Buprenorphine 5 μg/kg) were administered for the 48 h after the surgery.

In a second surgery, 25 pairs of Teflon-insulated, stainless steel wires were implanted into selected fore- and hindlimb muscles. In the forelimb, electrodes were implanted, bilaterally, in the brachialis (Br: flexor of the elbow); cleidobrachialis (CIB: protractor of the shoulder and flexor of the elbow); extensor carpi radialis brevis (ECR: dorsiflexor of the wrist); extensor digitorum communis (EDC: dorsiflexor of the digits and wrist); palmaris longus (PaL: ventroflexor of the wrist and digits); teres major (TrM: retractor of the shoulder); and triceps brachii, lateral head (TriL). In the hindlimb, the electrodes were implanted bilaterally into tibialis anterior portion (St: flexor of the knee); semitendinosus (St: flexor of the knee) and vastus lateralis (VL: extensor of the knee). In addition, a craniotomy was made to provide access to the right pericruciate motor cortex (areas 4 and 6) and a stainless steel base-plate (10 × 6 mm internal diameter) was affixed. Dental acrylic was used to form a recording chamber by building four walls around this base-plate and to fashion a stream-lined head implant. Postsurgical care was as described earlier.

Experimental procedures

The results described in this report were obtained during a series of experiments in which the major goal was to determine the discharge characteristics of corticoreticular neurons during voluntary gait modifications (Kably and Drew 1998).

In each experiment, a microelectrode was advanced slowly into layer V of the cortex as determined on the basis of the short-latency negative potential evoked by stimulation through the pyramidal tract electrode and the presence of antidromically evoked action potentials. The electrode was withdrawn 0.5 mm and left to stabilize for 10 min. The electrode was then advanced slowly until a single unit was isolated. Each unit was initially tested to determine whether its axon projected through the PT. Cells that discharged at fixed latency to the stimulation of the pyramidal tract electrode (1 shock of 0.2-ms duration at 1 Hz) and that collided with spontaneous action potentials (see Lipski 1981) were classified as pyramidal tract axons (PTNs). All putative PTNs were tested rigorously by means of the collision test. The maximum current of 1.5 mA that was used for this testing never produced any signs of discomfort or distress. Subsequently, all cells, regardless of whether or not they were identified as PTNs, were tested to determine whether they discharged at fixed latency to any of the stimulating electrodes within the PMRF. All wires were initially stimulated at a fixed strength of 800 μA (single shock of 0.2 ms at 1 Hz), except in cat MC18, in which a few cells (n = 7) were tested at 1.5 mA. The value of 800 μA was chosen because theoretical calculations suggest that it should activate a sphere of tissue with a diameter of ∼1 mm assuming an index of excitability (k) of 859 μA/mm (Hentall et al. 1984). If a cell discharged at fixed latency to the stimulation, the current was normally decreased to obtain the threshold of the response. Collision tests were always applied to at least one of the effective PMRF wires. In no case in which a cell fired at fixed latency to the stimulation did any cell fail the collision test. In other words all cells that discharged at fixed latency to stimulation of an electrode in the PMRF, and that were tested with the collision test, successfully fulfilled the criteria for an antidromic discharge. In this paper, PTNs are classified as those neurons that could be identified from the most caudally located PT wires and that could not be antidromically activated from any of the microwires in the brain stem (excluding those which were in, or which bordered on, the PT: ● in Fig. 2). Cells that discharged only to wires within the PMRF were classified as corticoreticular neurons (CRNs), whereas cells that discharged to both were classified as PTN/CRNs.

As all neurons that project to the spinal cord and to the PMRF are located in layer V (Groos et al. 1978; Rho et al. 1997), the recordings were restricted to this layer as determined by the presence of the antidromic action potentials produced by stimulation of the PT or the PMRF. To ensure that neurons from outside this layer were not included in the statistics, neurons that did not discharge to stimulation of either structure were included in the...
database only if they were located between neurons that were positively identified as being in layer V.

Each cell was also tested to determine its discharge characteristics during locomotion; the data from these experiments for neurons in area 4 are reported in the companion paper (Kably and Drew 1998). After this period of recording, the cat was removed from the treadmill and the receptive field of the unit carefully evaluated with the cat sitting quietly on the experimenter’s lap. At the end of the recording session, the cat was again removed from the treadmill and held gently while microstimulation (11 pulses at 330 Hz, each pulse of 0.2-ms duration) was applied through the recording electrode in layer V in the areas in which identified neurons were recorded. Stimulus currents were normally ±25 μA; in penetrations in which these currents had no effect, the intensity was increased to a maximum of 35 μA. The responses to this stimulation were evaluated both by palpation and examination of the body and by recording the evoked twitch responses from the implanted muscles. In some penetrations small lesions (10 μA) were made either just above or below layer V to aid in histological reconstruction of the recording sites.

**Histological reconstruction**

At the end of the recording sessions, each cat was deeply anaesthetized with pentobarbitol sodium (40 mg/kg ip) and electrolytic marking lesions (30- to 75-μA DC current) were applied through selected brain stem stimulating electrodes. Electrolytic lesions (50 μA) also were made in three to four locations within the motor cortex to further aid reconstruction of the recording sites. The animals then were perfused per aortum with saline and formaldehyde (10%) and the cortex and brain stem removed, sectioned (40 μm) in the parasagittal plane and stained with cresyl violet.

Each cortical and brain stem section was traced, and the location of each of the recording penetrations and the stimulating wires, respectively, were marked onto these sections using the electrolytic lesions as a guide. In the case of the brain stem sections, each section containing a stimulating electrode was magnified to match, as closely as possible, a standardized parasagittal section, at the appropriate laterality, from the atlas of Berman (1968: see Drew et al. 1986). The location of each stimulating electrode was drawn onto these standardized sections to permit calculation of the stereotaxic coordinates. The position of electrodes that were not visible in the sections was interpolated.

The location of each recording penetration was entered accurately on an unfolded map of the pericruciate cortex using previously described methods (see Jiang and Drew 1996; Rho et al. 1997). In the representations illustrated in Figs. 3, 5, and 6, the x coordinate is the distance from the fundus of the cruciate sulcus and the y coordinate indicates the laterality of the section. Identical methods were used to transfer the borders between cytoarchitectonic areas, determined according to the criteria of Hassler and Muhs-Clement (1964), Hassler (1966), and Avendano et al. (1992: see also Rho et al. 1997) onto these unfolded maps (see Fig. 3 D).
FIG. 2. Location of the effective stimulating wires in the brain stems of the 4 cats used in this study. Each circle indicates the theoretical physical spread of a current of 800 μA passed through the respective microwire (see Hentall et al. 1984). Completely filled circles illustrate microwires that were either in or stimulation of which excited the pyramidal tract. The letters (A-F) on the sections identify the different arrays of electrodes in each cat. Locations of the wires are plotted on standard sagittal sections of the brain stem taken from the atlas of Berman (1968). DMV, dorsal motor nucleus of the vagus; INT, nucleus intercalatus; IOP, principal nucleus of the inferior olive; PH, nucleus praepositus hypoglossi.
evoked antidromic activity in cortical neurons and/or through which it was possible to pass a current of 800 μA as determined by measuring the voltage drop across a resistance. Stimulation (11 pulses of 0.2-ms duration at 330 Hz, current 35 μA) through many of these wires evoked characteristic motor effects, including ipsilateral head movement, flexion of the ipsilateral limb and extension of the contralateral limb (Drew and Rossignol 1990a,b).

Similar regions of the brain stem, with respect to both the AP and the ML locations, were stimulated in each cat (Fig. 2). Considering all four cats together, the location of the stimulating electrodes was encompassed in a three-dimensional volume that extended from P11.9 to P1.5 and from lateral 1.9 mm on the right side of the brain stem (ipsilateral to the motor cortical recording site) to lateral 2.3 mm on the left (contralateral) side. This area included most of the magno- and gigantocellular regions of the PMRF, as well as some of the nucleus reticularis pontis caudalis.

Location of the motor cortical recording sites

Figure 3 shows the location and orientation of four electrode penetrations into the pericruciate cortex of cat MC19. The penetration shown in Fig. 3A traversed the hindlimb representations of area 3a and 4 before crossing the cruciate sulcus and entering area 6αβ on the rostral bank of the cruciate sulcus. The penetration illustrated in Fig. 3B was located in the rostral part of area 4 in the anterior sigmoid gyrus (ASG), as was one of the two illustrated in Fig. 3C (Track 13). The other track illustrated in Fig. 3C (Track 14) traversed layer V of the forelimb representation three times and identified neurons were recorded from both banks of the cruciate sulcus. The location of these four penetrations, together with all the other penetrations in this cat in which neuronal activity was recorded, are shown in Fig. 3D on a two-dimensional representation of the unfolded cortex.

Identification of different classes of neurons

An example of the identification of a PTN/CRN that was recorded from area 6αβ of cat MC21 is illustrated in Fig. 4. This neuron discharged antidromically to stimulation of the cerebral peduncle (latency of 0.8 ms), as well as to three wires located in the pyramid tract at progressively more caudal levels (wires B1, C1, and PT) at latencies of 1.05, 1.08, and 1.4 ms, respectively. In addition, this cell also discharged at fixed latency to suprathreshold stimulation of four wires within the PMRF (wires B4, C2, C3, and D2). In the case of wire B4, there were collisions between the spontaneously (*) and antidromically evoked activity. In all four cases (B4, C2, C3, and D2), the antidromic latency to stimulation of the electrodes in the PMRF was greater than that expected if the stimulation was simply activating the root axon within the PT. For example, the latency of the activation from wire B4 was 1.55 ms compared with a latency of activation of 1.05 ms from the most ventrally located wire in the bundle, B1, which was in the pyramid tract. Similarly, the antidromic latency from wires C2 and C3 was greater than that from the most ventrally located wire, C1. As a rule, when a cell was antidromically activated from more than one wire in a bundle, the latency was greater for the more dorsally located wires than for the more ventrally located ones (see also Fig. 8).

Characterization of different classes of cortical neurons

Altogether 455 neurons were recorded from areas 4 (n = 326) and 6 (n = 129) of the pericruciate cortex of the four cats from a total of 83 electrode penetrations (Fig. 5A). Figure 5, B and C, illustrates the relative percentage of the different types of identified cells recorded. Most cells recorded from area 4 (152/326; 47%) were identified as PTNs. A further 62/326 (19%) were identified only from wires in the PMRF (CRNs), and 87/326 (27%) were identified from both sets of stimulating wires (PTN/CRNs); only 25/326 (8%) of the neurons were not identified from any wires (Other). In contrast, of the neurons in area 6, only 24/129 (19%) were identified as PTNs and 16/129 (12%) as PTN/CRNs. However, 40/129 (31%) of the neurons discharged only to stimulation of the PMRF (CRNs) and fully 49/129 (38%) could not be activated by either stimulation of the PT or the PMRF (Other). Thus although the total proportion of the projection from area 4 and area 6 to the PMRF was almost equal (46% from area 4 and 43% from area 6), most of the area 4 projection (87/149) was from collaterals of PT axons, whereas most of the area 6 projection (40/56) was direct.

The locations of the penetrations in which each of these classes of neurons was recorded within the pericruciate cortex are illustrated in Fig. 5, D–F. Whereas penetrations in which PTNs were recorded were scattered widely throughout area 4, those in which PTN/CRNs, and particularly CRNs, were recorded were largely restricted to the rostral regions of the motor cortex. In area 6, no obvious differences in the locations of the recorded cells were evident. Cells that were not identified from any electrode were widely scattered among the identified neurons (not illustrated).

Receptive fields

Peripheral receptive fields could be tested for 173 corticofugal neurons recorded in area 4 and 40 corticofugal neurons in area 6. The majority of the neurons recorded in area 4 (106/173, 61%) had a receptive field that included parts of the forelimb, whereas 34/173 (20%) had a receptive field that included the hindlimb, 37/173 (21%) had one that included the face and neck (head), and 7/173 (4%) had one that included the trunk; very few cells were tested for which no receptive field could be determined (8/173, 5%). In all except four neurons, the receptive field was contralateral to the recording site. In area 6, few neurons were recorded that included a receptive field on the fore- or hindlimbs (7/40, 18%) and, as indicated in Fig. 6E, the majority of cells, of all classes, for which a receptive field was determined were activated from the face and neck (26/40, 65%). In addition, proportionally more of the neurons in area 6 could not be activated by passive manipulation of any part of the body (13%), and a substantial proportion discharged to passive manipulation of the body but no specific receptive field could be determined (Other: 26%).

Figure 6, A–C, illustrates the locations of penetrations in which neurons with receptive fields that included different...
FIG. 3. A–C: tracing of 3 histological sections from cat MC19 showing the localization of 4 electrode penetrations. Each tracing illustrates the surface of the cortex and layer V; the dots in layer V give a representation of the density of cells in this layer. *Figurines* (identified alphabetically) *outside* the contours of the cortex illustrate the receptive fields of cells recorded in each penetration. Filled regions in these figurines indicate cutaneous receptive fields, whereas the curved arrows indicate that the neurons were activated by passive movement of the indicated joint or region. *Figurines inside* the contour indicate the result of microstimulation in layer V (*mstim*); curved arrows on these figurines indicate the joint movement evoked by the stimulation. D: layer V of the cortex has been unfolded and the pericruciate cortex represented as a 2-dimensional sheet. Cytoarchitectonic areas are separated by dashed lines, whereas solid lines indicate the fundus of the cruciate sulcus (CRU) and the rostral and caudal margins of the cruciate sulcus. Location of all electrode penetrations made in this cat, including those illustrated in A–C, are represented on this surface with filled circles representing penetrations in area 4, open circles those in area 6αβ and 6αγ, and open squares those in the sensory areas of the pericruciate cortex. 4FL, forelimb representation of area 4; 4HL, hindlimb representation of area 4; Orb, cortex orbitales; Pro, proreus cortex; Spre, sensory cortex rostral to the cruciate sulcus; Spo, sensory cortex caudal to the cruciate sulcus.
parts of the body were recorded. In agreement with previous reports (Armstrong and Drew 1984b; Nieoullon and Rispal-Padel 1976), there was a basic somatotopic organization in area 4 with cells with receptive fields on the forelimbs and the hindlimbs being spatially segregated (Fig. 6A). Further, those few cells in area 4 with a receptive field on the trunk were mostly located relatively more medially in the sulcus (Fig. 6B), and those neurons with a receptive field that included the face and/or neck were mostly more rostral and medial (Fig. 6C). In most cases, the receptive field was restricted to one of these divisions. However, in 24 neurons there was some overlap: 5 of the forelimb neurons had a proximal receptive field that extended onto the neck, 5 had a proximal forelimb receptive field that extended onto the trunk, and 14 others had a receptive field that included the forelimb as well as the face and vibrissae. The penetrations...
including these neurons are represented on each of the relevant maps. Eighty five (85/106) of the neurons of the neurons responsive to manipulation of the forelimb were activated by manipulation of the forelimb distal to, and including, the elbow. As described in previous studies (Armstrong and Drew 1984b; Nieoullon and Rispal-Padel 1976) neurons with receptive fields on the more distal parts of the forelimb were located relatively more lateral than those with more proximal receptive fields (not illustrated). No clear somatotopy was observed in area 6.

The proportion of neurons with similar receptive fields in areas 4 and 6 was approximately equal regardless of the classification of the neuron (Fig. 6, D and E). Nevertheless, slightly fewer CRNs in area 4 had a receptive field that included the fore- or hindlimb than did the PTNs and PTN/CRNs, and relatively more CRNs than PTNs and PTN/CRNs had a receptive field that included the face and neck. It also should be noted that those PTN/CRNs and CRNs with a receptive field on the forelimb included a high percentage that could be activated from areas of the skin distal to the elbow (37/41 and 13/17, respectively).

Microstimulation

In area 4, there was a general correspondence between the receptive fields of a neuron in a given area and the effects of microstimulation in that region. This was as true for neurons with receptive fields on the limb (e.g., Fig. 3) as for those with more axial receptive fields, and the results were essentially the same as those reported previously (Armstrong and Drew 1984b; Asanuma et al. 1968; Nieoullon and Rispal-Padel 1976; Sakata and Miyamoto 1968). In all cases, the evoked responses were contralateral to the stimulated cortex. Microstimulation in those penetrations in which cells had receptive fields on both the limbs and the vibrissae produced shoulder retraction and/or elbow flexion as a threshold effect. There was little difference in the effects evoked by microstimulation in areas from which different classes of neurons having a receptive field restricted to the forelimb were recorded (Table 1). Microstimulation in loci from which PTN/CRNs and CRNs were recorded evoked responses around the elbow and wrist with only slightly less frequency than...
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Fig. 6. A–C: location of the penetrations in which cells having receptive fields that included the parts of the body indicated by the key (above each figure) were recorded. These plots include only those neurons that could be identified as corticofugal. Forelimb includes all cells activated from anywhere on the forelimb and/or scapula, head those with a receptive field including the vibrissae, face, and/or neck, ● and ■, neurons in area 4; ○, those neurons in area 6. D and E: histograms illustrating the proportion of each class of neurons that had a receptive field that included each of the indicated parts of the body. The totals are >100% as some neurons had a receptive field that included >1 category. Other, neurons that discharged to exploration of the body surface but for which a receptive field could not be precisely defined.

Did loci in which PTNs were recorded. Thus in both terms of their input and their output, a proportion of the neurons projecting to the PMRF were recorded from areas representing the more distal parts of the limb.

In area 6, microstimulation at up to a maximum of 35 μA was normally ineffective in evoking movements. In only 3/25 loci in which microstimulation was applied was any movement at all evoked. In two of these loci, a weak movement of the head was evoked, in the other weak elbow extension and shoulder abduction.

Latencies and conduction velocities

Root axons. The conduction velocity of each type of cell is illustrated in Fig. 7. For PTNs and PTN/CRNs, the conduction velocity was calculated on the basis of the estimated distance from the cortex as in our previous studies (Armstrong and Drew 1984a). Although, by definition, the axon of CRNs did not project as far caudally as the PT stimulating electrodes, most of them (96%) were also activated from wires situated rostrally in the PT (e.g., wire B1 from cat MC21, Fig. 4), and therefore the conduction velocity could also be calculated for these neurons. The conduction velocities of the PTNs within area 4 ranged from <10 up to 55 m/s, with the majority (90/152, 59%) classed as slow PTNs (conduction velocity <20 m/s) (see Takahashi 1965). In contrast, most PTN/CRNs (71/87; 82%) were classified as fast PTNs, whereas 48/62 (77%) of the CRNs had conduction velocities <20 m/s. In area 6, as for area 4, most PTNs (19/24, 79%) and CRNs (26/40, 65%) had mostly slowly conducting axons, whereas more than one-half (11/16; 69%) of the PTN/CRNs were identified as fast PTNs.

Collaterals

In many cases, the latency of the antidromic responses was greater for the more dorsally located electrodes than for those more ventrally situated (see Fig. 4). This can be seen
TABLE 1. **Effect of microstimulation in the forelimb representation of area 4**

<table>
<thead>
<tr>
<th>Class</th>
<th>Shoulder</th>
<th>Elbow</th>
<th>Wrist and Digits</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTN</td>
<td>8/24 (33)</td>
<td>12/24 (50)</td>
<td>6/24 (25)</td>
</tr>
<tr>
<td>PTN/CRN</td>
<td>9/23 (39)</td>
<td>10/23 (43)</td>
<td>5/23 (15)</td>
</tr>
<tr>
<td>CRN</td>
<td>4/11 (36)</td>
<td>4/11 (36)</td>
<td>2/11 (19)</td>
</tr>
</tbody>
</table>

Proportion of sites from which movement around a joint was evoked as a threshold effect. Data are plotted only for loci in which neurons having a receptive field that included the forelimb were recorded. Percentages (in parentheses) add up to >100% as threshold microstimulation sometimes evoked movements around more than one joint (see, e.g., Fig. 3). Movements at the shoulder were invariably a retraction of the arm. Effects around the elbow were normally flexion (all except 3 loci), while movements at the wrist involved both dorso- and ventroflexion. PTN, pyramidal tract neurons; CRN, corticoreticular neurons.

more clearly in Fig. 8, which illustrates two neurons from cat MC19 that were activated antidromically from a series of electrodes in one bundle. The top trace in each series illustrates the antidromic activation of the neuron from an electrode that activated the PT (B2 and C1, respectively), whereas the other traces illustrate the longer latency activation from successively more dorsally located wires. Figure 8C shows that there was a linear relationship between the change in the antidromic latency of the activation and the relative distance of the electrode from the PT. The slope of the regression yielded a value of 4.8 m/s for the conduction velocity of the collateral branch of the neuron illustrated in Fig. 8A and 5.6 m/s for that illustrated in Fig. 8B. Because the conduction velocity of these collateral branches was linear, similar values were obtained using simply the difference in latency between the most dorsally located wire from which a cell was activated and the latency of activation from the PT. As many of the other cells that we recorded were only activated from one wire in an array, we used this latter method to calculate the conduction velocity of the collateral branches of the population of neurons that projected to the PMRF. The distribution of the conduction velocities of the 93 collateral branches for which this was possible is shown in the form of a histogram in Fig. 9B. The distribution of the conduction velocity of the root axons from which these collaterals diverged is illustrated in Fig. 9A. Figure 9C plots the conduction velocity of the collaterals against the conduction velocity of the corresponding root axon and illustrates that the two values were independent (r = 0.15, P = 0.16). In addition, inspection of Fig. 9C shows that there were no significant differences (t = 1.43, df = 91, P > 0.1) in the conduction velocities of collateral branches from area 4 [mean = 6.0 ± 4.96 (SD) m/s] compared with those from area 6 (mean = 7.6 ± 5.26 m/s).

**Laterality**

The laterality of the projections to the PMRF was determined for 157 neurons. Forty-nine percent projected only to the ipsilateral side, whereas 35% projected bilaterally; only 16% of the neurons were activated solely from the contralateral side. There was little difference in these proportions between area 4 and area 6 neurons with, for example, approximately equal proportions of neurons in area 4 (36/110, 33%) and area 6 (17/47, 36%) being activated bilaterally. With respect to the different classes of projection neurons, 24/76 (32%) of PTN/CRNs in area 4 were activated bilaterally as were 13/24 (54%) of the CRNs. In area 6, most of the brain stem-projecting neurons were identified as CRNs (Fig. 5), and 10/24 (42%) of these neurons projected bilaterally.

**Divergence and convergence**

Although many of the neurons that discharged to stimulation of the PMRF could be activated antidromically from several wires (see Figs. 4 and 8), the majority of the
population of activated neurons were activated from only a few of the effective brain stem stimulating electrodes (see Fig. 2). As illustrated in Fig. 10, A and B, 69% of the neurons recorded from area 4 and 70% of those recorded from area 6 were activated from three or fewer brain stem electrodes. At the other extreme, several of the cells, in each cortical area, were activated from electrodes in widespread regions of the PMRF. Although not subjected to a strict statistical analysis, little correlation was observed between the receptive field of a cell and the extent of the divergence in the PMRF. For example, of 24 neurons in area 4 for which the receptive field was determined and that were activated from more than three electrodes, 12 neurons had a receptive field restricted to the fore- or hindlimbs and 9 cells had receptive fields restricted to the face, neck, or trunk. A similar comparison in area 6 was not made as nearly all of the neurons had face or axial receptive fields (see Fig. 6).

With respect to the convergence from the pericruciate cortex to any one wire in the PMRF, Fig. 10, C and D, shows that neurons in widespread areas of the cortex converged onto similar regions of the brain stem. For example, as illustrated in Fig. 10C, in the case of cat MC19, fully 27% (25/94) of all neurons that projected to the PMRF could be antidromically activated by stimulation of wire B4 (see Fig. 2B for the location of this electrode), whereas for cat MC21, 14% (12/86) of all the brain stem projection neurons were activated from wire A3 (not illustrated). The histogram in Fig. 10D shows the percentage of neurons in cat MC19 that were activated from each of the active electrodes in the PMRF. Inspection of this figure shows that several of these brain stem electrodes activated >10% of the 94 neurons that projected to the PMRF in this cat.

**DISCUSSION**

The results in this paper address the organization of the projections from areas 4 and 6 of the pericruciate cortex to the PMRF. These experiments, performed in intact animals with arrays of chronically implanted electrodes in the brain stem, allowed us to compare the divergence and convergence of the three types of projection neuron that were identified...
in these experiments to a greater extent than in most previous acute experiments and to obtain measurements of the conduction velocity of collateral branches of these fibers. In addition, the fact that these experiments were carried out in intact cats has allowed us to determine the receptive fields of the projection neurons, thus allowing correlation of the characteristics of the projection patterns of the neurons and their topographic localization.

Identification of corticoreticular neurons

An important consideration in the interpretation of our results concerns the weight that can be placed on the identification of each of the three classes of neurons. In the case of the PTN/CRNs, identification was based entirely on positive results, i.e., whether the cell discharged from the most caudally located PT electrode and whether it discharged from electrodes within the PMRF. As identification from the PT electrode was always based both on the presence of a fixed latency and the presence of collisions, we can be certain that the axon of these neurons projected at least as far as the PT and probably, in most cases, continued to the spinal cord. In the case of the identification from the brain stem, in most cells we also antidromically identified the neurons by latency and collision from at least one wire. However, because of the experimental constraints in these unrestrained animals, we did not use the collision test with all brain stem wires that produced antidromic activation of these cells. Nevertheless, the fact that we never observed a case where a cell that discharged with fixed latency did not also fulfill the criteria of the collision test when it was applied suggests that our identification criteria were adequate. Moreover, the possibility that some of the antidromic identifications that we observed might be simply explained by current spread to the PT is unlikely as the latencies from more dorsally located wires in the PMRF were invariably longer than those from the PT (see Figs. 4 and 8). As argued by Asif and Edgley (1992), this strongly implies that we were activating collateral branches and not the root axon.

Firm identification of the other two classes of neurons is less certain as, in each case, the identification relies on negative evidence, that is, that PTNs did not discharge to stimulation of any wires in the PMRF and that CRNs did not discharge to stimulation of the PT. Nevertheless, there are several factors that suggest that the number of neurons that were misclassified is probably small. Considering first the CRNs, the critical consideration is whether our stimulus parameters were sufficient to activate all axons in the PT. Although we cannot test this directly, cumulative histograms of the probability of activating a PTN as a function of threshold (not illustrated) showed that fully 90% of all PTNs that were identified were activated at current strengths of $\leq 400 \mu A$. As stimulus strength was always increased to a maximum of 1.5 mA, in all cats, the evidence suggests that the probability of not identifying an axon that passed through the PT is small. Accurate identification of cells that did not send collateral branches to the PMRF, (i.e., PTNs) is less certain as we have no means of determining whether collateral branches may have been given off at more rostral or more caudal levels than those examined in this study. However, the density of the stimulating electrodes in our arrays does imply that we should have at least identified most neurons with collateral branches within the examined region. Nevertheless, a consideration of the characteristics of these classes of neurons must take into account that only the PTN/CRNs can be unequivocally identified.

Collateralization

As might be expected from the fine diameter of corticoreticular fibers (see Matsuyama and Drew 1997), the conduc-
tion velocities of the collateral branches was uniformly slow with no fibers conducting faster than 20 m/s. These slow conduction velocities mean that there is often a delay of 1–2 ms between the activation of more ventral and more dorsal regions of the PMRF. This delay is compatible with the long latencies of some of the excitatory postsynaptic potentials that were recorded in the study of Canedo and Lamas (1993). From a functional point of view, it is interesting to speculate that this delay between the more ventral regions of the PMRF, which includes the major projection to the hindlimb muscles, and the more dorsal regions, which includes primarily the projections to the forelimb and neck musculature (Basbaum and Fields 1979; Drew and Rossignol 1990a,b; Hayes and Rustioni 1981; Kuppers and Maisky 1977; Zemlan and Pfaff 1979; Zemlan et al. 1984), may ensure the simultaneity of postural activities at the cervical and lumbar levels of the spinal cord.

An advantage of the chronic preparation is that the permanent arrangement of the stimulating electrodes, together with the systematic mapping of the cortex, provided information at the single cell level concerning the divergence of the projection from individual cortical neurons to the PMRF and the convergence of different areas of the cortex to small regions of the brain stem, which is not available from most anatomic studies. In particular, the data shown in Figs. 4 and 10 suggest that the terminal branches of some individual cortical neurons may innervate relatively widely separated regions of the brain stem. Although most cortical neurons could be activated from only a few brain stem electrodes, some (30%) could be identified from more than three electrodes in the brain stem, frequently from electrodes in different bundles (e.g., Fig. 4). Although such a divergence was expected on the basis of the widespread area of distribution of the corticoreticular projection (Matsuyama and Drew 1997; Newman et al. 1989; Schiebel and Schiebel 1958), the present findings extend these anatomic studies by showing that individual fibers may branch to innervate different regions of the PMRF and that many individual corticoreticular neurons branch to innervate both the ipsilateral and contralateral sides (see Matsuyama and Drew 1997). In addition, of course, it is likely both that some terminal branches were not activated by our stimulation parameters and that
others continued outside the area explored, suggesting that
the divergence of many corticoreticular fibers is undoubtedly
greater than that described here. The probability that some
of the bilaterally projecting neurons were misidentified is
low as only one bundle of wires (cat MC19, bundle D) was
close enough to the midline to theoretically identify fibers on
the other side of the brain stem and only 3/80 contralaterally
projecting neurons were identified only from wires of this
bundle.

With respect to the convergence of the projection, Magni
and Willis (1964) first showed that individual reticulospinal
neurons receive convergent input from both hemispheres
(see also He and Wu 1985). The present study extends these
findings by showing that a single electrode in the PMRF
frequently activated >10% of the neurons recorded in the
cortex in any one cat, and, in 12 cases, a single electrode
activated >20% of all neurons recorded in the pericruciate
cortex (Fig. 10, C and D). This suggests that neurons in
widely separate areas of the cortex converge onto relatively
small regions of the brain stem. Although we cannot be
certain that these fibers terminate in these regions, rather
than simply passing through, these results, again, are compat-
ible with the anatomy. For example, in the anterograde
study of Matsuyama and Drew (1997), it was found that
injections into four different regions of the pericruciate
cortex resulted in labeled terminal swellings throughout the
extent of the medial PMRF, with a substantial amount of
overlap. Similar results were obtained in the anterograde
study of Newman et al. (1989) and are implied by the results
of the retrograde study of Rho et al. (1997).

Receptive fields and microstimulation

Most studies on the corticoreticular projection have fol-
lowed Keizer and Kuypers (1984) in suggesting that most
of the projection from area 4 to the PMRF is primarily
from those regions that control the proximal musculature.
However, this suggestion was based entirely on the location
of the cells that they labeled in the pericruciate cortex as
compared with previously published maps of cortical soma-
topy (e.g., Nieoullon and Rispal-Padel 1976). In this re-
spect the results from the present study are particularly inter-
esting as they show that, both on the basis of receptive
fields and microstimulation, corticoreticular neurons are also
present in regions that control the more distal parts of the
limb, including the elbow and wrist (see Figs. 3 and 6 and
Table 1). Moreover, fully 58/106 (55%) of the neurons with
forelimb receptive fields projected to the PMRF, either
via a collateral branch or directly. This is in agreement with
our recent retrograde study (Rho et al. 1997) that showed
that injections of retrograde tracers into the more caudal
regions of the PMRF, and particularly into the nucleus reticu-
laris gigantocellularis, resulted in a substantial number of
labeled neurons in quite lateral regions of area 4, correspon-
ding to those from which the relatively distal receptive fields
were recorded in this study. Further, in the anterograde study
of Matsuyama and Drew (1997), injections localized in the
forelimb representation of area 4, in loci from which micro-
stimulation evoked brief twitches in shoulder and elbow
flexor muscles, also resulted in dense labeling within the
PMRF. Together, these results suggest that a substantial part
of the projection from area 4 of the cortex to the PMRF arises
from cortical areas that are actively involved in controlling
voluntary movements of the forelimb of the type studied by
Martin and Ghez (1985, 1993) or in producing voluntary
gait modifications (Drew 1993; see further text). These areas
of the cortex are also those that project preferentially to the
deeper layers of the spinal cord (Martin 1996) and are most
likely to be involved in the control of motor behavior. In
contrast, few neurons in the hindlimb representation of area
4 projected to the PMRF, an observation that is in accord
with anatomic studies that also show only a weak projection
from the hindlimb representation of the cat motor cortex
(Keizer and Kuypers 1984; Matsuyama and Drew 1997; Rho
et al. 1997). As we have discussed previously (Rho et al.
1997), this may reflect the fact that, in the cat, discrete
voluntary movements of the hindlimbs are rarely made in
isolation.

There was no clear relationship between the location of
the receptive field and the extent of the convergence and
divergence of the corticoreticular projection. Although most
neurons with a receptive field on the face frequently showed
largely divergent patterns of projections, the same was also
true of some neurons with receptive fields on the forelimb.
However, it must be emphasized that the stimulating elec-
trodes were located in a relatively restricted region of the
brain stem, and it is possible that some neurons may have
branched outside the region in which our electrodes were
placed (see Matsuyama and Drew 1997).

Comparison of areas 4 and 6

Whereas many of the neurons in area 4 had a receptive
field on either the forelimb and/or the hindlimb, as illustrated
in Figs. 3 and 6, most of the neurons in area 6 in the cat for
which a receptive field was found discharged in response to
passive manipulation of the face and neck (see Figs. 3, 4,
and 6). Although there is a possibility that this in part may
be due to some sampling bias, this result is in general agree-
ment with experiments that have shown that microstimula-
tion of area 6, and particularly those parts examined in this
study, results primarily in twitch responses of the neck and
trunk, together with eye movements (Guitton and Mandl
1978; Hassler 1966; Nieoullon and Rispal-Padel 1976;
Schlag and Schlag-Rey 1970). Two other differences are
related. First, whereas receptive fields were found for the
majority (95%) of neurons tested in area 4, the same was
not true for neurons in area 6 in which for a substantial
proportion (39%) a discrete receptive could not be deter-
mined even though some of these (10/39) discharged when
the body was manipulated. It is possible that in these latter
neurons the discharge might have been determined by the
attentive state of the animal, although our methods did not
allow us to critically test this possibility. Second, whereas
nearly all (92%) of the area 4 neurons recorded in layer V
could be antidromically activated from either the PTN or
the PMRF, many of the area 6 neurons (38%) could be dischargaed from neither. While this may simply reflect that
these recordings were from cortical layers other than V, both
the experimental methodology and the subsequent histology
suggested that all recorded neurons were localized to layer
V. This result more likely reflects the fact that fewer area 6
neurons project to the pyramidal tract and spinal cord and that perhaps more have direct connections to other subcortical structures. It is also worth emphasizing that fully 92% of the area 4 neurons that we recorded could be identified from either the PMRF and/or the PT. This suggests that many of these fibers must also have sent collateral branches to other subcortical structures as indeed has been suggested to be the case by Canedo (1997).

There was also a difference in the relative proportion of fast and slow fibers that projected to either or both of the PMRF and spinal cord (see Fig. 7). In area 4, a majority of PTNs had axons with fast conduction velocities while most of those in area 6 were more slowly conducting. More importantly, whereas the major projection to the PMRF from area 4 was in the form of collaterals from fast PTNs, in agreement with other studies (Lamas et al. 1994; Pilyavsky and Gokin 1978), the main projection from area 6 was direct (CRNs) and was from neurons with slowly conducting axons. This is in agreement with our anatomic anterograde studies in which a similar conclusion was reached from indirect evidence: namely that a large decrease in the number of labeled axons in the pyramid at progressively more caudal levels of the medulla was seen following the area 6 injections but not from the area 4 projections (Matsuyama and Drew 1997). Despite these differences, other characteristics of the projection, such as the conduction velocity of the collaterals, (Fig. 9) and the extent of the divergence or convergence from the two areas (Fig. 10) were similar.

Functional implications

The major findings are that a large proportion of neurons in area 4 and 6 project to the PMRF, either directly, or as collaterals, and that this projection is characterized by considerable convergence and divergence. Although with most previous studies on the corticoreticular projection there has always been the question of whether the distributions that are observed represent individual neurons branching profusely or different populations of neurons projecting less diffusely to different areas, the present electrophysiological results clearly show widespread divergence and convergence at the single cell level. These results demonstrate that single neurons within the pericruciate cortex may not branch to ipsilateral and contralateral sides of the brain stem as has been shown previously (He and Wu 1985; Magni and Willis 1964; Matsuyama and Drew 1997; Pilyavsky and Gokin 1978; Rossi and Brodal 1956), but on any one side, some of these fibers may branch to innervate widespread regions of the PMRF. Obviously such a branching pattern suggests that individual neurons may activate or modulate the activity of a large number of reticulospinal neurons, which themselves innervate different levels of the neuraxis (Drew and Rossignol 1990a,b; Matsuyama et al. 1988, 1997; Peterson et al. 1975). Such bilaterally divergent patterns are probably important in producing the complex, bilateral postural changes that are observed during gait modifications (Lavoie et al. 1995). At the same time, the extent of the convergence from neurons in different parts of the cortex suggests that neurons with quite different receptive fields and, probably, functions may activate the same groups of neurons in the PMRF. This latter organization would allow similar patterns of postural support to be produced in response to different movements.

In addition, the fact that a large part of the projection to the PMRF from area 4 is from collaterals of fast PTNs descending to the spinal cord suggests that the same descending command should be sent to the brain stem and to the spinal cord and that this signal may contain detailed information of the movement to be made (see companion paper). In contrast, the facts that much of the projection from area 6 is from slowly conducting fibers, that many of these axons do not project to the spinal cord, and that the receptive fields of area 6 neurons are primarily axial imply that these projections may serve to set the more tonic postural base on which the dynamic voluntary movements and postural adjustments are superimposed.

The authors thank N. de Sylva for help in the completion and analysis of these experiments. We also acknowledge the technical assistance of M. Bourdeau, P. Drapeau, G. Messier, and the late R. Bouchoux. D. Cyr, G. Filosi, and C. Gauthier are thanked for illustrations and photography and F. Cantin and J. Lavoie for histological assistance. We thank Drs. Chapman and Smith for helpful comments on this manuscript.

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Received 19 December 1997; accepted in final form 3 April 1998.

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