Differences in the Corticospinal Projection from Primary Motor Cortex and Supplementary Motor Area to Macaque Upper Limb Motoneurons: An Anatomical and Electrophysiological Study

To further our understanding of the functional roles of different motor cortical areas, we made a quantitative comparison of the density of corticospinal projections from primary motor cortex (M1) and supplementary motor area (SMA) to spinal motor nuclei supplying hand and finger muscles in four macaque monkeys. We also compared the action of corticospinal outputs excited by electrical stimulation of these two areas on upper limb motoneurons recorded in three anaesthetized macaques. The hand representations of SMA and M1 were first identified using structural magnetic resonance imaging scans and intracortical microstimulation. In the anatomical study we then made focal injections of wheatgerm agglutinin–horseradish peroxidase into these representations, which were subsequently confirmed by analysis of retrograde cortical labelling. Densitometric analysis showed that corticospinal projections from M1 were denser and occupied a greater proportion of the hand muscle motor nuclei than did projections from SMA. In caudal Th1 the densest projections from M1 occupied 81% of this motoneuronal area, compared with only 6% from SMA. In the electrophysiological study, bipolar intracortical stimulation of the hand representation of M1 and SMA evoked direct (D) and indirect (I) corticospinal volleys. Volleys elicited by M1 stimulation had larger amplitudes and faster conduction velocities than those evoked from the SMA. Intracellular recordings were made from 84 contralateral upper limb motoneurons. M1 and SMA stimulation evoked markedly different responses in tested motoneurons: EPSPs were larger and more common from M1 (88% of motoneurons) than from SMA (48%). Some motoneurons (16/84) showed evidence of excitatory post-synaptic potentials mediated by monosynaptic action of the D-wave evoked from M1; these early effects were not observed from the SMA. In most motoneurons (74/84) EPSPs had segmental latencies indicating that they were due to monosynaptic action of the I-wave. The results are consistent with corticomo-motoneuronal (CM) connections originating from both SMA and M1 converging upon single motoneurons, but those from M1 are far more numerous and exert stronger excitatory effects than from the SMA. Thus although they may function in parallel, the two CM projections probably make different contributions to upper limb motor control.

Introduction

It is well-established that many different areas of the cerebral cortex contribute to the corticospinal projection. In the frontal lobe, projections arise from primary motor cortex (M1), dorsal and ventral premotor areas, from a number of cingulate motor areas and from the supplementary motor area (SMA) (Dum and Strick, 1991, 1996; He et al., 1993; Galea and Darian-Smith, 1994; Picard and Strick, 1996; Wise, 1996). The function of these different projections is the subject of an ongoing debate: are specific functions carried out by individual structures that together go to make up the motor network, or are they distributed throughout the network (Fetz, 1992; Tanji, 1994; Dum and Strick, 1996)? Single neuron recordings in monkeys and brain-imaging studies in humans have stressed that both M1 and SMA are co-activated during many motor tasks (Alexander and Crutcher, 1990; Crutcher and Alexander, 1990; Dettmers et al., 1995; Fink et al., 1997; Kazennikov et al., 1999) and the ‘executive’ functions of both areas have been emphasized (Matsumura et al., 1991; Boecker et al., 1998; Jenkins et al., 2000; Grammond and Kalaska, 2000). However, characteristic functional differences between the two areas have also been identified in relation to bimanual activity, to external vs. internal guidance of movement, and to the planning of complex movement sequences (Tanji 1994; Jahanshahi et al., 1995; Picard and Strick, 1996; Sadato et al., 1997).

Insight into the functional specificity of the different corticospinal projections can be gained by comparing their pattern of spinal connections, since these connections ultimately determine the influence a cortical motor area can exert on the spinal machinery for movement. Of particular interest is whether each motor cortical area gives rise to direct cortico-motoneuronal (CM) connections. CM connections are a distinctive feature of the primate motor system and are known to be important for the capacity to perform independent finger movements (Porter and Lemon, 1993; Bortoff and Strick, 1993; Lemon, 1993; Maier et al., 1997b; Nakajima et al., 2000).

Dum and Strick (Dum and Strick, 1996) made a systematic study of corticospinal projections and showed that there was a high degree of similarity between the corticospinal projections from the hand/arm regions of M1, SMA and the different cingulate motor areas. They emphasized that all of these regions gave rise to corticospinal projections to lamina IX throughout the cervical enlargement, and they suggested that these different corticospinal outputs may function in a parallel fashion. Rouiller et al. (Rouiller et al., 1996) also demonstrated terminals from SMA and from M1 close to retrogradely labelled motoneurons supplying hand muscles. However, in neither study was it possible to make a quantitative comparison between the density of projections from M1 vs. those from SMA. Without this information it is difficult to gauge to what extent the M1 and SMA have similar potential to generate and control movements.

While there is extensive electrophysiological evidence of direct CM connections from M1: motoneuron recording (Landgren et al., 1962; Shapovalov, 1975; Maier et al., 1998); spike-triggered averaging of EMG (Fetz and Cheney, 1980; Lemon et al., 1986; Cheney et al., 1991; McKiernan et al., 1998), there is far less information about CM connections from SMA. Early work demonstrated that single pulse intracortical microstimulation (ICMS) in SMA could evoke short-latency EMG responses (Hummelsheim et al., 1986), but there has been no direct demonstration of CM connections from SMA, nor has a systematic comparison with CM effects from M1 been made.

The objectives of the current study were twofold. First, we made a quantitative comparison of the density of anterogradely labelled corticospinal projections from the hand representations of M1 and SMA to the hand muscle motor nuclei in the lower
cervical cord. Second, to assess the strength of functional CM connections from M1 and SMA, we made intracellular recordings from motoneurons innervating hand and arm muscles while stimulating these cortical areas. The results establish that both areas give rise to CM connections which converge on single motoneurons. They also demonstrate that M1 exerts larger and more widespread CM excitation than does SMA, which has important implications for the respective roles of the two structures in movement control.

Preliminary accounts have been published previously (Maier et al., 1997a).

Materials and Methods
The study comprised parallel anatomical and electrophysiological experiments in seven adult macaque monkeys (see Table 1). Anatomical data were obtained from four animals (one Macaca mulatta and three M. fascicularis); some results from one monkey (case D3) have been reported previously (Armand et al., 1997). The electrophysiological study was based on three monkeys (one M. mulatta and two M. fascicularis). All animals were captive bred for research purposes. Animal care and use was in accordance with the UK Animals (Scientific Procedures) Act 1986.

Identification of the SMA and M1 Hand/Arm Representations
Two approaches were used to locate accurately these representations: magnetic resonance imaging (MRI) and intracortical microstimulation (ICMS).

MRI
MRI was used to determine the precise sulcal geometry of the relevant cortical regions and allowed accurate placement of recording chambers, the angle of microelectrode penetrations for ICMS mapping, bipolar stimulating electrodes and of needle tracks for wheatgerm agglutinin–horseradish peroxidase (WGA–HRP) injection. Scans were carried out under deep general anaesthesia [for detailed methods see Baker et al. (Baker et al., 1999)].

ICMS Mapping
After an initial injection of ketamine (10 mg/kg i.m.), the monkey was intubated and anaesthetized with isoflurane (2–2.5% in a 1:1 O2/N2O mixture). A craniotomy was made and stainless steel chambers (i.d. 18 mm) were mounted over the SMA and, for monkeys CS5 and CS8, over M1. The final location and orientation of the chambers was guided by MRI scans. Following the surgery, an antibiotic (tetracycin LA 20 mg/kg; Pfizer, Sandwich, Kent, UK) and an analgesic (buprenorphine hydrochloride 5–10 µg/kg i.m. Vetgesic, Reckitt & Colman, York, UK) were administered. Over the next 2–3 weeks, repetitive-pulse ICMS (rICMS) was used to map the motor representation of the SMA and M1. For this, the monkey was lightly sedated with ketamine (initial dose 10 mg/kg, subsequent doses of 10 mg/kg/h), so that there was clear movements were noted. Mapping included the full extent of the SMA motor representation, including face, hand/arm, trunk, leg and tail areas (see Fig. 1). ICMS tracks were reconstructed from histological sections.

Anatomical Study
Injection of WGA–HRP
In three of the monkeys our aim was to make a large injection of WGA–HRP to include the hand representation of either M1 (D3) or SMA (CS5 and CS6). In the fourth (CS8), the objective was to make a smaller, focused injection centred on the hand representation of M1 in one hemisphere, and of the SMA in the opposite hemisphere. All injections were carried out under deep general anaesthesia [for details see Armand et al. (Armand et al., 1997)]. 0.1 µl of 10% WGA–HRP (Sigma) in 0.15 M saline was pressure injected via a 29 gauge stainless steel needle at 1 mm intervals along each track. One minute was allowed to elapse between injections at successive depths. The volume injected in each case is given in Table 1. The survival period was 72–90 h.

Histochemical Processing
At the end of the survival period, the animal was deeply anaesthetized with Nembutal (30 mg/kg i.v.) and perfused through the heart with a vascular rinse (0.9% NaCl, 10 mM NaNO2 5% polyvinyl pyrrolidone (PVP40), 5000 IU heparin at 36°C) followed by fixative (1% paraformaldehyde, 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.3 at 4°C). After 30 min, the perfusion with fixative was stopped and continued with 0.1 M phosphate buffer (at 4°C) containing 10% sucrose for a further 15 min, and 20% sucrose for a final 15 min. After perfusion, blocks of tissue containing the injection sites were cut in the stereotaxic plane. After laminectomy, each spinal segment was carefully identified and the cord cut into blocks. Frozen sections were cut at 50 µm. The cortical sections were collected in two series; the first to visualize HRP using the conventional nitroprusside–tetramethyl benzidine (TMB) method (Mesulam, 1982) and the second for cytoarchitectonic analysis (stained with cresyl violet). Alternate spinal cord sections were processed with nitroprusside–TMB and paratungstate–TMB (Weinberg and Van Eyck, 1991) respectively; the latter sections were counterstained with neutral red to identify the motor nuclei.

Reconstruction of the Cortical Injection Site
Drawings of nitroprusside–TMBreacted sections and cresyl violet stained sections were superimposed and the boundary of the injection site determined from the zones of reaction product density (Armand et al., 1997). Cytoarchitectonic areas in the region of the M1 and SMA injection sites were delineated according to previously established criteria (Jones et al., 1978; Luppino et al., 1991; Dum and Strick, 1991, 1996).

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*MF, Macaca fascicularis; MM, Macaca mulatta.
Figure 1. Intracortical mapping and WGA–HRP injection sites. Intracortical microstimulation (ICMS) map and injection site of M1 (A; left hemisphere) and SMA (B; right hemisphere) in a single monkey (case CS8) and location of SMA injection site in case CS3 (C). (A) Three ICMS tracks are shown on sections through M1, whose location is shown on the inset diagram above (black area: injection site). Movements of the hand/digits were observed at different depths in the anterior bank of the central sulcus (I, II, III, IV, V refer to digits I–V; w, wrist; bold type: threshold < 15 μA). The M1 injection site consisted of three needle tracks at A9.75, A9 and A8.25 (entry points indicated by arrowheads). WGA–HRP was deposited along these tracks between 2.5 and 6.5 mm from the surface. The series of coronal sections from A10.5–A7.5 (750 μm separation) show the extent of the injection site; cross-hatched area shows the focus of the injection where the reaction product is as dense in axons and perikarya as in the extracellular space, whereas the area enclosed by the discontinuous line is the surrounding zone of diffusion and/or transport of WGA–HRP (based on TMB–nitroprusside-reacted sections). The boundaries between the grey and the white matter as well as between the cytoarchitectonic areas are represented by a thin solid line (based on cresyl violet-stained sections). CS, central sulcus; SPC, superior precentral sulcus; numbers refer to cytoarchitectonic areas; F2, dorsal premotor cortex. (B) Eight ICMS tracks are shown on a parasagittal plane of the superior frontal gyrus, whose location is shown by the black frame on the inset diagram (ArSg, level of the genu of the arcuate sulcus; ArSs, superior limb of arcuate sulcus; CiS, cingulated sulcus; black area, injection site). Movements of the arm, hand and digits are indicated on each track (e, elbow; s, shoulder; t, trunk). The SMA injection site consisted of four needle tracts from A12 to A15 (arrowheads). The series of coronal sections from A16 to A11 (1 mm separation) show the injection site. WGA–HRP was deposited at depths 2, 3 and 4 mm from the pial surface. In this case (CS8), the same restricted volume of WGA–HRP (1.2 μl) was injected in M1 and SMA. (C) Injection site of SMA (F3) in case CS3, represented with the same symbols as in (A) and (B). A total of eight needle tracks were made, four in a row 0.5 mm lateral from the surface of the interhemispheric cortex and four in a second row 1 mm lateral. Tracks in these two rows were made at 1 mm apart, and to depths of 6 and 5.5 mm respectively. Total volume injected 4 μl. To obtain the flattened reconstruction of the hemisphere (black frame in the inset diagram), a representative 50 μm coronal section was taken for each millimetre of tissue and projected such that the medial wall of the hemisphere was plotted above the midline and the lateral surface below it (Dum and Strick, 1991). The depths of the superior and inferior limb of the arcuate sulcus (ArSs; ArSi), of the principal sulcus (PS), the anterior bank of the central sulcus (CS), as well as the depths of the cingulate sulcus (CS) are unfolded. A heavy discontinuous line indicates the fundus of the central and cingulate sulci. CC, corpus callosum; IPS, intraparietal sulcus; LatS, lateral sulcus. Cytocarchitectonic areas are labelled according to Luppino et al. (Luppino et al., 1991) and delineated by dotted lines.
Densoptic Analysis of Corticospinal Projections

Our aim was to obtain a quantitative analysis of the relative density and distribution of reaction product in different parts of the spinal grey matter and at different segmental levels, so that we could carry out a detailed comparison of the corticospinal projection labelled from M1 and from SMA. The reaction product in the nitroprusside–TMB-reacted sections was photographed with polarizing filters and a dark-field effect; all sections were digitized with the same standard level of illumination (Armand et al., 1997). We chose this photographic method to enhance the resolution of the images. We carefully controlled for possible sources of error: quality of film, development, illumination, exposure and saturation effects (for details see Armand et al. (Armand et al., 1997)). Densitometric analysis was carried out on sections from five half segments: caudal C7 to caudal Th1; 6–12 sections per half-segment were analysed. A ‘window of termination’ was selected which excluded any false labelling due to background or other artefacts (Armand et al., 1997).

This range of labelling within the spinal grey matter was divided into five equal ranges (i.e. 1–20%, 21–40%, etc).

Density of Corticospinal Projection to Hand Muscle Motor Nuclei

Using an xy-plotter the locations of all motoneurons were determined (i) in each nitroprusside–TMB-reacted section with phase contrast and (ii) in the consecutive paratungstate–TMB-reacted section (counterstained with neutral red) with bright field. This allowed direct and precise delineation of the motor nuclei supplying the hand and finger muscles (Jenny and Inui, 1983; Armand et al., 1997). On each nitroprusside–reacted section from caudal C7 to caudal Th1, the region of lamina IX containing these motor nuclei was selected and its area measured. The proportion of this area that was occupied by labelled projections in the different density ranges was determined (Armand et al., 1997).

In case C8 we were able to make a direct comparison of corticospinal projections to the hand motor nuclei from M1 hand area (injected on the left side; Fig. 1A) and SMA (injected on the right side; Fig. 1B) because there were few, if any, ipsilateral projections from either SMA or M1 to the dorsolateral hand motor nuclei (Kuypers, 1981; Armand, 1982; Dum and Strick, 1996; Armand et al., 1997). Therefore any labelling within lamina IX must have arisen from the contralateral injection site. This approach avoided any possible errors due to variations in survival time, anterograde transport, strength of the histochemical reaction, etc.

Electrophysiological Study

Terminal experiment

When the ICMS mapping of SMA and M1 was complete, a terminal electrophysiological experiment was carried out. All preparatory surgery was carried out under isolurane anaesthesia [as detailed above and by Maier et al. (Maier et al., 1998)]. Cuff electrodes were mounted on the median, ulnar and radial nerves at the axilla (Ma. Ua and Ra respectively), median and ulnar nerves at the wrist (Mw and Uw) and the deep radial nerve (DR). A laminectomy over spinal segments C3 to Th1 and an intraventricular overdose of barbiturate, and perfused through the heart with 100% oxygen.

Intracellular Recordings from Motoneurons

These recordings were made with glass microelectrodes filled with 3 M potassium acetate and having a DC resistance of 2–5 MΩ. A small pressure foot was used to reduce movement of the spinal cord. All motoneurons were identified antidromically from the forelimb nerves. Intracellular and cord surface recordings were digitized directly at 10 kHz using a 1401plus interface. Densitometric Analysis of Corticospinal Projections

Results

Identification of the M1 and SMA Hand Representations

Of fundamental importance to this study was the accurate identification of the hand representation in M1 and SMA. Detailed ICMS mapping was carried out to guide both the WGA–HRP injections in the anatomical study, and the location of cortical stimulating electrodes in the electrophysiological experiments. Figure 1 shows reconstructions from case C8 of ICMS tracks. In M1 (Fig. 1A) and SMA (Fig. 1B) hand/digit movements were evoked at low threshold (5–15 µA in M1, 12–15 µA in SMA). As noted by previous investigators, we found that effects from SMA had higher thresholds than those from M1, and that whereas tracks made in the centre of the M1 hand representation tended to evoke only hand or digit movements, the representation in SMA was far more intermingled, with the lowest threshold hand/digit loci being close to points from which elbow or shoulder movements could be evoked (Fig. 1). (Brinkman and Porter, 1979; Macpherson et al., 1982; Hummelsheim et al., 1986; Mitz and Wise, 1987; Luppino et al., 1991).

Anatomical Study

Injection Sites

M1. In case D3 a large injection (8.7 µl) of WGA–HRP was made in M1 hand region. Details of the injection site are given in Armand et al. (Armand et al., 1997) (Fig. 5, adult case 3). In case
CS8 we made a direct comparison of corticospinal projections to the hand motor nuclei from M1 hand area and SMA (see Materials and Methods). The M1 injection (Fig. 1A; total volume injected 1.2 µl) was made in the immediate vicinity of the ICMS tracks that yielded low threshold digit movements (see inset). The injection site was restricted to the portion of area 4 located on the lip and the rostral bank of the central sulcus, and extended rostro-caudally ~3.5 mm.

SMA. In cases CS3 and CS6, large injections were made in order to label the great majority of corticospinal projections arising from SMA and terminating in the cervical enlargement. A total of 4 µl was deposited at 40 different sites. In these two cases, the injection site extended outside the cortical area from which hand and forearm movements were elicited by ICMS. Thus in CS3 (Fig.1C), for example, the injection site occupied a cortical surface area of ~95 mm², and was centred on the ICMS tracks which yielded hand and arm movements at the lowest threshold, whereas the cortical surface area covered by these ICMS tracks was ~35 mm². This injection site involved the rostral three-quarters of area F3 (SMA proper) located in the medial wall of the hemisphere, as well as the adjoining cortex extending on to the convexity of the hemisphere. It also involved the region of SMA in the dorsal bank of the cingulate sulcus, an area known to give rise to a dense corticospinal projection (Dum and Strick, 1996). This injection slightly encroached the F3/F2 (SMA/PMd) border, on the convexity of the hemisphere, and the F3/24d (SMA/CMAd) border in the depth of the cingulate sulcus. The caudal boundary of the injection site was 2 mm rostral to the SMA/M1 border: the M1 leg area was not injected. A very similar injection was made in case CS6.

In case CS8 the SMA injection was made in the right hemisphere (Fig. 1B); the total volume injected (1.2 µl) was the same as for the M1 site. The SMA injection site was restricted to the portion of area F3 (Luppino et al., 1991), located on the medial wall of the hemisphere, and extending dorsally on the adjoining convexity, and ventrally to the dorsal bank of the cingulate sulcus (Fig. 1B). The injection site included the sites at which low-threshold digit movement was evoked by ICMS (Fig. 1B, inset).

**Cortico-cortical and Callosal Labelling**

Confirmation that the injection site in different cases did include the SMA hand area was obtained by plotting the distribution of neurons retrogradely labelled from the injection site. In the ipsilateral hemisphere, there was extensive labelling in M1 (area

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**Figure 2.** (A) Photomicrograph under dark-field illumination and polarized light of a representative spinal cord section at rostral Th1, after a restricted injection of WGA–HRP in the hand area of the primary motor cortex (M1) on one side and the supplementary motor area (SMA) on the other, in the same animal (CS8). Within the hand motor nuclei, the density of labelled fibres arising from SMA was much lower than the density of labelled fibres arising from M1. Note that this comparison in a single monkey is valid because neither M1 nor SMA gives rise to any significant ipsilateral projections to lamina IX at C8–Th1. (B) Density of corticospinal projections from the hand area of the primary motor cortex (M1, case D3) and of the supplementary motor area (SMA, case CS3) to C8 and Th1. For each monkey, four sections have been selected, one from each half segment (R, rostral; C, caudal); these sections had a distribution that most closely matched the mean value on the 6–12 sections taken from that half segment (see Fig. 3). The colour scale corresponds to the five equal-density ranges of the ‘window of termination’ (see Materials and Methods). The region of lamina IX occupied by the hand and finger motor nuclei is delineated by a white line. Note that the labelling of the dorsal horn in case D3 is most probably due to spread of the injection site to the postcentral gyrus; see case 3 of Armand et al. (Armand et al., 1997).
A pattern (Dum and Strick, 1996). This was particularly clear in M1 injection, although the overall distribution followed a similar matter was relatively light and uniform compared to that after an SMA injection. The overall labelling was much stronger in the hemi-corticospinal projections was focused in the C8–Th1 grey matter. The overall labelling was mainly distributed contra-laterally in the base of the dorsal horn (laminae V–VI) and the dorsolateral part of the intermediate zone (lamina VII). Lighter labelling was seen bilaterally in its ventromedial part (lamina VIII and adjoining part of VII). Note the much lighter labelling of the dorsolateral motor nuclei from SMA compared with M1. Similar labelling from SMA was obtained in CS6. There was a clear rostro-caudal increase in lamina IX labelling from both SMA and M1.

Figure 3 summarizes the densitometric results. The first measure illustrated is the fraction of the area occupied by the hand muscle motor nuclei that was labelled (i.e. all labelling within the area, irrespective of density). After M1 injections (left panel) this measure saturated in most of the half-segments analysed i.e. the entire region occupied by the hand muscle nuclei was labelled. In the SMA cases (right), between 17.7 ± 5.1% and 65.1 ± 11.4% of this region was covered by label, with a clear rostro-caudal gradient from rostral C8 to caudal Th1. Similar results were obtained for both large and small M1 (D3 and CS8, respectively) and SMA injections (CS3 and CS8 respectively).

A more sensitive measure is given by the area of the hand muscle motor nuclei occupied by the densest labelling (i.e. in the range from 61–100% density; yellow and red pixels in Fig. 2B). Using this measure, it is clear that at all levels the labelling was much more intense from M1 than from SMA (Fig. 3, hatched columns). In caudal Th1, for example, the area covered from the SMA projections in case CS5 was only 6.4 ± 2.4% compared to 81.0 ± 1.4% from M1 (case D3), a ratio of 12.7. This M1/SMA area ratio for these two cases showed even larger differences in Th1 rostral (26.9) and in caudal and rostral C8. Once again there was a good correspondence between the analysis for large and small M1 injections (D3 and CS8, respectively) and large and small SMA injections (CS3 and CS8, respectively). For case CS8 the M1/SMA ratio based on the 40% densest projections varied from 13.4 in caudal Th1 to 31.2 in caudal C8.

Electrophysiological Study

Cortical Stimulation Sites

The cortical stimulation sites for the terminal experiment were positioned within the hand representation of SMA and M1 identified by previous ICMS mapping. In Figure 4A–C, penetrations yielding low-threshold arm or hand responses are marked by diamonds (♦). The entry points of the final anode (+) and cathode (–) tracks in each of the three cases are shown in Figure 4A–C; several other exploratory tracks were made (♦). Examples of electrode tracks in M1 are shown in Figure 4D,E (anterior bank of central sulcus) and in SMA (Fig. 4F). Electrode orientation was guided by previous ICMS tracking and MRI. Their final position and depth were adjusted to obtain the maximal corticospinal direct volley (see below). In the M1 case, the anode lay amongst the large layer V pyramidal neurons (Fig. 4D), and the cathode lay deeper (Fig. 4E). In the SMA case (Fig. 4F) the anode lay in laminar V, and the cathode in the white matter deep to it.

Corticospinal Volleys Recorded from the Contralateral DLF

Volley from PT and from M1 and SMA cortex. A single 200 µA shock to the PT (Fig. 5A) evoked a simple, biphasic volley followed by a longer-lasting positivitv, which probably reflects postsynaptic activation (Maier et al., 1998). Unlike PT stimulation, single bipolar stimulation of M1 (400 µA) elicited a complex series of volleys (Fig 5B). These volleys were identified...
as corticospinal by collision from the PT (not shown) (Edgley et al., 1990). The short latency of the first volley indicated that it was a D-wave, produced by direct excitation of corticospinal neurons (Patton and Amassian, 1954; Edgley et al., 1990; Baker et al., 1995). When recorded at the C3 level, this D-wave had a latency about double that of the volley evoked from the medullary PT (compare Fig. 5A with B), consistent with the longer conduction distance from cortex compared with PT. The later volleys from M1 stimulation were I-waves, resulting from indirect stimulation of corticospinal neurons (Patton and Amassian, 1954; Edgley et al., 1990, 1997). The interval between the D-wave and the first I-wave (labelled I1 in Fig. 5B) was typically 1.0–1.4 ms, and a similar interval was observed between I1 and I2. Stimulation of the SMA also elicited D- and I-waves (Fig. 5C); they had longer latencies and smaller amplitudes than those elicited from M1.

In case CS9, the conduction velocity of the fastest volleys recorded between C3 and Th1 was highest for the PT volley (86 m/s); the M1 D-volley was a little slower at 79 m/s, and that from SMA slower still at 61 m/s (Fig. 5A–C). For a given cortical stimulation site, the I1 wave had a similar conduction velocity to the D-wave: for M1 this was 79 m/s (D-wave) and 81 m/s (I1-wave). The D–I1 interval was similar for both the faster M1 and the slower SMA volleys (Fig. 5B,C). Comparable results were obtained in the other monkeys.

The amplitudes of the volleys evoked from the cortex were much smaller than those evoked from the PT (note the size of the calibration bars in Fig. 5A vs. 5B,C) and the I-waves were usually larger than the D-wave. Comparing the effects of a 200 µA PT shock (at which intensity it was close to maximal) and a 400 µA cortical shock revealed that the amplitude of the D-wave from M1 ranged from 3.7 to 3.9% of the PT volley; the larger I-waves were typically 3.3–9.3%. For SMA stimulation the corresponding ranges were 1.1–2.8% and 2.4–4.5%.

Volleys Elicited by Different Intensities and Locations in M1 and SMA. In CS9 the threshold for eliciting cortical volleys was ~40 µA for both M1 and SMA (Fig. 5D,E). D- and I-wave amplitude grew approximately in parallel with increasing intensity. Only at the stronger intensities (200–400 µA) could the volleys be clearly identified in single traces (Fig. 5D,E).

Evidence for Independent Activation of M1 and SMA. That corticospinal neurons in SMA and M1 were stimulated independently of each other was demonstrated by combined stimulation. If different populations of corticospinal neurons were activated
Responses in Motoneurons

Intracellular recordings were made from a total of 84 antidromically identified motoneurons (35 from CS2, 37 from CS5 and 14 from CS9) recorded in spinal segments C7, C8 and Th1. Most of these motoneurons innervated forearm finger or wrist flexors (U: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Uw: 15, Mw: 1); 17 motoneurons were identified from flexors (Ua: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Ua: 25, Ma: 12). Most of these motoneurons innervated forearm finger or wrist flexors (U: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Uw: 15, Mw: 1); 17 motoneurons were identified from flexors (Ua: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Ua: 25, Ma: 12). Most of these motoneurons innervated forearm finger or wrist flexors (U: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Uw: 15, Mw: 1); 17 motoneurons were identified from flexors (Ua: 25, Ma: 12) or extensors (DR: 14) or intrinsic hand muscles (Ua: 25, Ma: 12).

Excitatory Postsynaptic Potentials (EPSPs). Two examples are shown in Figure 7, both from radial motoneurons. In the first case (A–D), PT stimulation evoked an early EPSP, followed by an IPSP. The segmental latency of the EPSP (calculated as shown in Fig. 7B as the time from the positive peak of the corticospinal volley recorded from the same segment to the onset of the EPSP) was 1.0 ms, within the monosynaptic range (Jankowska et al., 1975b; Maier et al., 1998). Responses to cortical stimulation were always more complex than from the PT. A single 400 µA shock to M1 (Fig. 7C) evoked two EPSPs, the second following shortly after the first, and both having a size similar to the PT-evoked EPSP. The segmental latency of the first EPSP from the D-wave was 1.1 ms (see left-hand set of dotted lines in Fig. 7C), i.e. a value similar to that from the PT. These EPSPs were therefore termed direct-wave EPSPs because their segmental latencies from the D-wave were consistent with monosynaptic action of corticospinal impulses in that wave. Note that the absolute latency of the EPSP measured from the shock artefact was 2.3 ms, which is longer than that from the PT (1.4 ms), as would be expected from the longer conduction time. Direct-wave EPSPs were only seen in a minority of motoneurons, and were never seen after SMA stimulation.

The second, later EPSP in Figure 7C had a segmental latency from the D-wave of 2.3 ms, which places it beyond the monosynaptic range of the D-wave. The absence of any late excitatory effects from the single corticospinal volley generated by PT stimulation (Fig. 7B) makes it unlikely that it was due to non-monosynaptic effects of the D-wave (Maier et al., 1998). Rather, it was probably due to monosynaptic action of impulses in the I1-wave; the segmental delay after this wave was 1.1 ms (see right-hand set of dotted lines in Fig. 7C), identical to the value for monosynaptic action from the D-wave. Accordingly, EPSPs with segmental latencies from the first I-wave that were within the monosynaptic range are referred to as indirect-wave EPSPs. In this motoneuron no effects were observed from the SMA electrodes (Fig. 7D).

In the second motoneuron (Fig. 7E–H), PT stimulation also evoked a monosynaptic EPSP (segmental delay 0.9 ms), again followed by an IPSP (Fig. 7F). M1 stimulation (400 µA) produced a clear EPSP (Fig. 7G) with a segmental latency of 2.3 ms after the D-wave, but only 1.1 ms after the I1 wave. This EPSP was therefore also classified as an indirect-wave EPSP. Note that the form and size of the EPSP is practically identical to that from the PT, and that there was very little jitter in EPSP onset latency. In this motoneuron, SMA stimulation evoked an EPSP that was
smaller than that from M1, but with a similar form; it had a segmental latency of 1.2 ms from the I1-wave. This demonstrates convergence of excitation from both cortical areas upon the same motoneuron.

**Effect of Stimulus Intensity.** In many motoneurons, increasing the intensity of cortical stimulation evoked successive EPSPs associated with the augmented D- and I-waves; temporal summation between these effects often led to motoneuron discharge. In most cases I-wave EPSPs had lower thresholds than D-wave EPSPs. The intrinsic hand muscle motoneuron shown in Figure 8 showed a monosynaptic EPSP after PT stimulation (50 μA) and a segmental latency of 0.7 ms from the I1-wave. This demonstrates that EPSPs had an absolute latency of 3.0 ms and a segmental latency of 0.7 ms from the D- or I1-volley. Note presence in (C) of an early EPSP from M1 with a monosynaptic segmental delay after the D-volley, followed by a later EPSP with a similar delay after the I1-volley; only this later EPSP was seen in (G) and (H).

**Inhibitory Postsynaptic Potentials (IPSPs).** In some motoneurons cortical stimulation produced ‘pure’ IPSPs uncontaminated by a preceding EPSP. Figure 9 shows a Ma motoneuron with a monosynaptic EPSP followed by a large IPSP from the PT (Fig. 9B). The IPSP had a segmental latency of 1.5 ms (dotted line in Fig. 9B), within the disynaptic range of 1.3–1.9 ms (Maier et al., 1998). Stimulation of M1 evoked only IPSPs: a small IPSP after the D-volley and a much larger one after the I1-volley (Fig. 9C). In both cases, the segmental delay (1.6 and 1.7 ms) was within the disynaptic range. A disynaptic IPSP after the I1-volley was also seen after SMA stimulation (Fig. 9D).

**Segmental Latency of Motoneuron Responses**

In Figure 10A–D, segmental latencies of EPSPs and IPSPs have been plotted with respect to the arrival of the D-volley from the appropriate area (M1 or SMA) at the appropriate segment, as shown by the arrow at far left of Figure. 10. This normalization procedure took account of the different corticospinal conduction velocities evoked from M1 and SMA (Fig. 5).

**M1 EPSPs.** EPSPs evoked from M1 fell into three clear groups. The first group (16 motoneurons) had latencies of 0.6–1.3 ms after the D-wave (Fig. 10A, hatched columns) and were categorized as *direct-wave EPSPs*. For 14 of these motoneurons, the range of delays corresponded exactly with that of monosynaptic EPSPs evoked from the PT (0.5–1.1 ms, horizontal arrow in Fig. 10A) and the mean delay for this group of EPSPs, 0.95 ± 0.2 ms (n = 16), was only slightly longer than the segmental latency of the monosynaptic EPSP to PT stimulation (0.8 ± 0.1 ms, n = 78). A second, larger (n = 7) group of EPSPs had segmental latencies of 1.6–3.0 ms from the D-volley (filled columns in Fig. 10A). These EPSPs were categorized as *indirect-wave EPSPs* due to monosynaptic action from the I-wave. If this classification is correct, one could predict that the latencies might be rather dispersed, because of the differences in D–I1 interval in the three different monkeys (see arrows above Fig. 10A). Indeed, when the segmental latencies of these EPSPs are replotted, as in...
Figure 10, to normalize the D-1 interval to 1.1 ms (arrow in Fig. 10E), the distribution of latencies became considerably narrower. The segmental delays of these EPSPs relative to the I volley ranged from 0.5 to 1.4 ms (mean 0.86 ± 0.24 ms, n = 74).

A final group of late EPSPs had longer latencies (>2.7 ms after the D-wave); these late effects occurred just after the second I-wave.

**SMA EPSPs.** No direct-wave EPSPs were observed and EPSPs with segmental latencies of 2.0–3.0 ms were considered to be indirect-wave EPSPs from the I volley. The segmental delays of these EPSPs, relative to the normalized I volley (Fig. 10E), ranged from 0.6 to 1.5 ms (mean 1.08 ± 0.21 ms, n = 32).

**IPSPs.** Eight motoneurons showed IPSPs in response to the D-wave (hatched columns in Fig. 10B), with segmental latencies of 1.8–2.0 ms from the D-volley (mean 1.9 ± 0.1 ms). These delays were similar to those of disynaptic EPSPs evoked from the PT (1.4–2.2 ms, horizontal arrow in Fig. 10B). No such early IPSPs were seen from SMA (Fig. 10D). M1 and SMA stimulation also evoked IPSPs with longer latencies (filled columns in Fig. 10B,D) beginning at least 2.5 ms after the D-volley; many of these effects had segmental latencies with respect to the I volley that were within the disynaptic range [for M1, range 1.4–2.3 ms, mean 2.0 ± 0.2 ms (n = 34; Fig. 10F) and for SMA range 1.7–2.3 ms, mean 2.06 ± 0.16 ms (n = 18; Fig. 10H)].

**Summary of Responses Evoked from PT, M1 and SMA**

Table 2 lists the number of motoneurons in each type of response evoked by stimulation of the PT, M1 and SMA with a single shock, while Table 3 lists the number of response types observed.

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**Figure 9.** Inhibition of a motoneuron by M1 stimulation. IPSPs in a median motoneuron from CS5. (A) Antidromic identification. (B) Response to PT stimulation at 200 µA. (C) Response to M1 stimulation at 400 µA: a small early IPSP was present at a disynaptic latency after the D-volley (left dotted line), with a later larger IPSP after the I volley (right dotted line). (D) Response to SMA stimulation at 400 µA; only a later IPSP was present.

**Figure 10.** Distribution of segmental latencies. Segmental latencies of EPSPs and IPSPs recorded in the sampled motoneurons in response to PT, M1 and SMA stimulation. (A–D) Latencies normalized to arrival of D-volley (vertical arrow at time zero) in the three monkeys. Arrival time of I volley in each monkey is indicated by vertical arrows. (E–H) Latencies of direct effects as in (A–D) but latencies of later, non-direct effects normalized to arrival of I volley (vertical arrow at time 1.1 in E,F and at 1.3 in G,H). Hatched bars: early responses to D-volley (monosynaptic EPSPs and disynaptic IPSPs). Filled bars: later responses to the I volley (monosynaptic EPSPs in and disynaptic IPSPs after I volley). Open bars: late responses (di- and oligosynaptic EPSPs and oligosynaptic IPSPs after I volley). Horizontal arrows indicate range of latencies of monosynaptic EPSPs evoked from the PT (A,E) or disynaptic IPSPs from the PT (B,F). All measurements were for 200 µA shocks to the PT and 400 µA to M1 and SMA.
PT stimulation (200 µA) produced a monosynaptic EPSP followed by a disynaptic IPSP (61/84 motoneurons; 75%). ‘Pure’ monosynaptic EPSPs (uncontaminated by later IPSPs) were found in 15 (18%) motoneurons, while IPSPs with no preceding EPSP were seen in five (6%) motoneurons. Longer latency EPSPs were seen in only three cases (Maier et al., 1998).

M1 stimulation (400 µA) produced a response in all 84 tested motoneurons. Direct-wave EPSPs were found in 16 cases (19%; see Fig. 11A), all 16 also showed later I-wave related effects. Indirect-wave EPSPs were found in 74 motoneurons (16 + 58; 88%); the remainder (12%) had only IPSPs. In any given motoneuron, there was a close parallel between excitatory effects from PT and M1: thus 88% of the 16 motoneurons with a ‘pure’ EPSP from the PT showed a similar response from M1. However, of the 63 motoneurons with an EPSP/IPSP sequence from the PT, only 24 (38%) showed an EPSP/IPSP from M1, while 34 (54%) showed a pure EPSP, and the remainder (8%), a pure IPSP. This suggests that cortical stimulation activates a rather different balance of corticospinal inputs to a given motoneuron than does PT stimulation.

SMA stimulation (400 µA) did not produce any direct-wave EPSPs (Figs 10C, 11A). Indirect-wave EPSPs were recorded in 36 motoneurons (48%) and IPSPs in 10 (13%). Thus all of these received convergent effects from both SMA and M1. Of the 16 motoneurons with a pure EPSP from the PT, 11 (78%) also showed a pure EPSP from the SMA; of the 63 that showed an EPSP/IPSP sequence, only seven (21%) showed the same sequence from the SMA, while 21 (62%) showed a pure EPSP.

Amplitudes of EPSPs
The distribution of EPSP amplitudes is shown in Figure 11B–D. PT stimulation (200 µA) evoked monosynaptic EPSPs with a broad range of amplitudes, the mean being 2.0 ± 1.1 mV (n = 53; range 0.5–5.0 mV; Fig. 11B). A similarly broad distribution was seen after M1 stimulation (400 µA), with a mean of 1.5 ± 1.1 mV (n = 104; range 0.2–4.4 mV). Direct-wave EPSPs tended to be rather small (1.2 ± 0.7 mV, n = 16) and indirect-wave EPSPs were larger (mean 1.5 ± 1.0 mV, n = 59, range 0.2–4.4 mV; Fig. 11C). This difference in amplitude was not significant (Mann–Whitney test, P > 0.3). SMA stimulation (400 µA) evoked a narrower distribution with a strong skew towards very small effects (Fig. 11D). The average amplitude of only 0.5 ± 0.5 mV (n = 50) was significantly smaller than for M1 evoked EPSPs (Mann–Whitney test, P < 0.0001).

Figure 12 plots the relationship between the amplitude of EPSPs observed in a given motoneuron from PT, M1 and SMA. Motoneurons that showed a large CM EPSP in response to stimulation of the entire PT tended to show large direct-wave EPSPs after stimulation of M1 (Fig. 12A, P < 0.06, slope = 0.27, r = 0.45, n = 16). This correlation was also seen for M1 indirect-wave EPSPs (Fig. 12B, P < 0.05, slope = 0.44, r = 0.47, n = 42) and SMA indirect-wave EPSPs (Fig. 12D; P < 0.01, slope = 0.12, r = 0.54, n = 28). There was also a significant difference between the amplitude of indirect and late EPSPs evoked from M1 and those from SMA (Mann–Whitney test, P < 0.0001).

It was notable that even the direct-wave EPSP from M1 could amount to a substantial proportion of the total EPSP from the pyramidal tract (mean 47%, range 12–100%), suggesting that a large proportion of the fibres giving rise to effects from the PT originated in M1. Many of the indirect-wave EPSPs were even larger (Fig. 12B, C). There was a clear correlation between the indirect

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**Table 2**

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<th>PT</th>
<th>Cortex</th>
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<tbody>
<tr>
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<td>81</td>
<td>direct-wave EPSP</td>
</tr>
<tr>
<td>‘Pure’ mono EPSP</td>
<td>15</td>
<td>indirect-wave EPSP</td>
</tr>
<tr>
<td>Mono EPSP/late EPSP</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>‘Pure’ IPSP</td>
<td>5</td>
<td>direct-wave IPSP</td>
</tr>
<tr>
<td>No effect</td>
<td>0</td>
<td>no effect</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>84</td>
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</table>

-Mono EPSP: monosynaptic EPSP; mono EPSP/late EPSP: monosynaptic response to D-wave; indirect-wave EPSP: monosynaptic response to I1-wave; direct-wave IPSP: IPSP at disynaptic latency after D-wave.

A) Followed by later EPSPs and/or IPSPs.

**Table 3**

<table>
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<th>Cortex</th>
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<tr>
<td>Mono EPSP/late EPSP</td>
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<td>Disynaptic IPSP</td>
<td>68</td>
<td>late EPSP</td>
</tr>
<tr>
<td>Late IPSP</td>
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-PT stimulation: 200 µA (PT), 400 µA (M1, SMA).

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**Figure 11.** Occurrence and amplitudes of EPSPs. (A) Frequency of occurrence of direct- and indirect-wave EPSPs from stimulation of M1 (84 tested motoneurons) and SMA (75). (B–D) EPSP amplitudes in response to PT (B), M1 (C) and SMA (D) stimulation. Hatched bars: monosynaptic responses to the D-volley; filled bars: monosynaptic responses to the I1-volley; open bars: late responses. All measurements were for 200 µA shocks to the PT and 400 µA to M1 and SMA.
and direct-wave EPSP amplitude (Fig. 12C, $P < 0.05$, slope = 0.78, $r = 0.61$, $n = 13$).

Discussion
An understanding of the relative importance of the corticospinal projections from macaque M1 and SMA to upper limb motoneurons is essential for our understanding of the contribution made by these motor areas to control of upper limb movements. Both the anatomical and electrophysiological results from this study indicate that corticospinal projections from both M1 and SMA can exert a direct excitatory influence on hand and arm muscles, and this includes convergence of CM connections upon single motoneurons. CM connections from M1 to the hand muscle motor nuclei are more numerous and stronger than those from SMA, and the amplitudes of EPSPs from M1 were consistently larger than those from SMA.

Quantitative Comparison of Corticospinal Projections Arising from M1 and SMA
Our conclusion regarding the much heavier corticospinal projection from M1 to the motor nuclei supplying hand and finger muscles depends upon a number of important assumptions. First, that the injection sites were appropriately targeted on the hand representations in both areas; second, that corticospinal projections from both areas were labelled to the same extent; and finally, that the quantitative densitometric analysis we have used provided a reliable comparison of the labelling.

Injection Sites
The injections described here were based on detailed ICMS mapping of the cortical areas, and the precise orientation and depth of the injection tracks were guided by MRI. In all cases the injection site would have included the low-threshold sites previously mapped with ICMS. The injection of the SMA hand representation resulted in a pattern of retrograde labelling in the ipsilateral and contralateral hemispheres that was very similar to that described by previous authors for this same representation (Luppino et al., 1993; Rouiller et al., 1994).

Comparable Labelling of the Corticospinal Projections from Both Areas.
A larger volume of WGA–HRP was injected into M1 in case D3 (8 µl) than in the SMA cases CS3 and CS6 (both 4 µl). However, in the SMA cases, the injection site was still very large and actually covered a greater amount of tissue than the arm/hand area determined by ICMS mapping. It seems unlikely that the differences in the labelling in the lower cervical cord from SMA and M1 in these cases can be explained by the amount of tracer injected, because in case CS8, where an identical and much smaller volume of tracer (1.2 µl) was injected into M1 and SMA in opposite hemispheres, the same contrast in labelling was seen, again with much denser labelling from M1 (Figs 2A and 3).

Reliability of Densitometric Analysis
The method, which we have used previously (Armand et al., 1997), relies on careful normalization of the densitometric data from different spinal cord sections from different cases, based upon a standard section and the use of an identical level of illumination for sampling each spinal cord section and avoidance of any saturation effects. That the method used provides reproducible results both within a single case and across cases is shown by the low coefficient of variation in the data presented in Figure 5.

Large Differences in the Corticospinal Projections from SMA and M1
In agreement with Dum and Strick (Dum and Strick, 1996) and Rouiller et al. (Rouiller et al., 1996) we found that the overall pattern of labelling from M1 and SMA observed in the lower cervical cord was rather similar: the densest projections from both cortical areas being found in the dorsolateral parts of laminae V–VII, the dorsomedial region of lamina VI and ventromedially in lamina VII and VIII. However, we observed large differences in the density of these projections, including those into the motor nuclei of lamina IX. The corticospinal projections from M1 occupy a larger area of lamina IX than do those from SMA and the projections are much denser from M1 than SMA, at least for the hand and finger motor nuclei (Figs 2 and 3). For
example in caudal Th1, which receives the heaviest projections from both areas, there was a 12- to 13-fold difference in the area of the hand muscle motor nuclei occupied by the densest labelling from M1 compared to that from SMA (see Fig. 3).

There was also a clear rostro-caudal gradient from rostral C8 segment to caudal Th1 in the density of both M1 and SMA projections into lamina IX; this gradient was particularly pronounced for M1, as shown previously (Fig. 2B, left) (Dum and Strick, 1996; Armand et al., 1997). This gradient probably reflects the preferential influence of CM connections over the intrinsic hand muscles, whose motoneurons are concentrated caudally in the cervical enlargement (Jenny and Inukai, 1983).

Activation of the Corticospinal System by Intracortical Stimulation

Our anatomical study indicated large quantitative differences in the number and density of corticospinal projections to lamina IX from SMA and M1. To understand the significance of these differences we compared the effects of activating these projections on upper limb motoneurons. Activation at monosynaptic delays after the D-wave is of particular interest, since these EPSPs can be unequivocally attributed to monosynaptic CM connections.

Unipolar intracortical microstimulation (ICMS), which is widely used in awake, behaving monkey studies, exerts mainly indirect effects on pyramidal tract neurons (Jankowska et al., 1975a; Lemon et al., 1987; Porter and Lemon, 1993). As an alternative, we used bipolar intracortical stimulation, which our pilot experiments indicated was the most effective approach for focal and direct activation of corticospinal neurons. We located the anode in the more superficial layers and the cathode in the deep layers or immediately adjacent to the white matter (Patton and Amassian, 1954; Hern et al., 1962). Single shocks evoked a typical pattern of D- and I-waves; the latter are thought to be due to transynaptic activation of corticospinal neurons (Patton and Amassian, 1954). It is probable there was substantial underestimation of the I-wave amplitude because of cancellation between I-wave potentials with slightly different latencies (Edgley et al., 1997; Deletis et al., 2001).

The focal nature of the bipolar stimuli used was confirmed by a number of observations: first, even with stimuli up to 400 μA in intensity the amplitude of the D-wave was never more than a few percent of the fast corticospinal volley evoked by a supra-maximal shock to the PT, which probably excites all fast axons (Maier et al., 1997b, 1998). Second, we never observed signs of occlusion between D- or I-waves evoked from SMA and M1 (Fig. 6), indicating that the outputs from these areas were activated independently. This was further confirmed by the clear differences in the conduction velocities of the volleys excited by stimulation in the two areas (Fig. 5). These observations confirm relatively small amounts of current spread with this type of electrode configuration (Asanuma and Sakata, 1967; Tokuno and Nambo, 2000).

Corticospinal Volleys from M1 and SMA

The amplitude of the volleys evoked from M1 were ~2-3 times larger than from SMA (see Figs 5, 6), and this can readily be explained by the fact that M1 provides ~49% of the frontal lobe fibres terminating in the cervical and upper thoracic segments, whereas SMA supplies ~19% (Dum and Strick, 1991). The density of corticospinal neurons within the SMA is slightly lower than within the M1 hand area (Dum and Strick, 1991; Wise, 1996). The fastest conduction velocity was seen for the PT volley (Fig. 5A); this probably reflects activation of the largest fibres originating from the M1 leg area (Murray and Coulter, 1981). Significantly slower volleys were evoked from the SMA compared with M1; this agrees with the smaller cell size in SMA, and longer antidromic latency of SMA PTNs (MacPherson et al., 1982; Dum and Strick, 1991). The presence of these slowly conducting corticospinal volleys further confirms that the stimulating electrodes were appropriately located in SMA, since a more rostral location in pre-SMA (F6), which lacks corticospinal neurons (Luppino et al., 1994) would have failed to activate a descending volley, while a more caudal location in M1 leg area would have activated the fastest corticospinal neurons.

Corticospinal Postsynaptic Potentials (PSPs) in Motoneurons

Categorization

We have argued above that the origin of the different PSPs can be deduced from their segmental delays in relation to the arrival of the D- and I-waves. Thus the earliest EPSPs from the PT are monosynaptic (CM) effects, with short segmental latencies (up to 1.1 ms) and the earliest IPSPs are disynaptic, with segmental latencies of 1.3–1.9 ms (Maier et al., 1998). Excitatory effects from M1 and SMA were categorized as direct-wave EPSPs, indirect-wave EPSPs or late EPSPs. These excitatory effects on hand and finger motoneurons are consistent with activation of M1 and SMA during distal limb movements (Porter and Lemon, 1993; Tanji, 1994; Fink et al., 1997).

Direct-wave EPSPs result from monosynaptic action of impulses making up the D-wave; our criterion for these EPSPs was a segmental latency of 1.3 ms or less. This criterion is slightly longer than for the PT-evoked EPSPs, but is consistent with the distribution of segmental latencies (shaded in Fig. 10A,E), and probably reflects the more dispersed nature of the cortical D-wave when compared with the PT volley (Figs 5,6). The mean segmental delays of these EPSPs (0.95 ± 0.2 ms) was only slightly longer than that from the PT (0.8 ± 0.1 ms).

Indirect-wave EPSPs had longer segmental delays (1.6–3.0 ms), relative to the D-wave, than did direct-wave EPSPs (Fig. 10) and could, in principle, have resulted from either oligosynaptic action of D-wave or monosynaptic effects of I-wave impulses (Kernell and Wu, 1967; Jankowska et al., 1975b). Although it is impossible completely to rule out a spinal oligosynaptic contribution, we favour the latter interpretation because single PT stimuli rarely evoked any non-monosynaptic EPSPs (only 4% of motoneurons showed such effects (Maier et al., 1998; Nakajima et al., 2000)). In addition, the mean segmental delay of these EPSPs was invariant and within the monosynaptic range of the first I-wave (0.86 ms). Finally, the segmental latencies of these EPSPs were better correlated with the I- than the D-wave: reploting the data relative to the arrival of the I-wave revealed a more compact distribution (Fig. 10E) than for the D-wave (Fig. 10A).

Late EPSPs had segmental latencies beyond 3.0 ms; their origin is uncertain. Following the arguments detailed above, we suggest that they result from the monosynaptic action of later I-waves (Kernell and Wu, 1967; Day et al., 1989; Boniface et al., 1991; Olivier et al., 1995).

Differences in the Occurrence of Direct- and Indirect-wave EPSPs

Direct-wave EPSPs were relatively uncommon (19% of motoneurons tested from M1, none from SMA; Fig. 11A), confirming that of the relatively large population of fibres directly excited by the stimuli, relatively few made CM contacts with the sampled
motoneuron. This rather focused pattern confirms work done in awake monkeys, which has shown that a given CM cell exerts post-spike facilitation of a relatively small number of motoneuron pools (Fetz and Cheney, 1980; Buys et al., 1986; Porter and Lemon, 1993; McKiernan et al., 1998). In contrast, many more motoneurons showed EPSPs from indirect excitation (indirect-wave EPSPs 69% from M1, 48% from SMA).

These results could be most parsimoniously explained by bipolar stimulation activating more CM cells indirectly than directly; for reasons given above, the I-wave may actually have been much larger than the D-wave. Cortical stimulation may be particularly effective at evoking these indirect effects because it excites intracortical axonal systems which are presynaptic to CM cells and which are functionally organized to engage large numbers of output neurons. These outputs are known to be distributed over a relatively large cortical area (Andersen et al., 1975; Lemon et al., 1987; Lemon, 1988; Schieber and Hibbard, 1993), and thus there is the potential for a much larger number of outputs belonging to the same cortical ‘colony’ (the group of CM cells all terminating on the sampled motoneuron) being recruited in the I-wave than in the D-wave. The latter is restricted by the physical extent of current flow at the stimulation site. This might also explain why indirect-wave EPSPs had the lower threshold (e.g. Fig. 8) and could be as large as those from the whole PT (Figs 8D, 12B). Powerful augmenting responses of CM synapses to successive I-waves (Phillips and Porter, 1964; Kernell and Wu, 1967; Muir and Porter, 1973) explain why these compound EPSPs lead to motoneuronal discharge.

Thus although conduction velocity measures (Fig. 5B,C) suggest that a similar population of corticospinal neurons is activated in both D- and I-waves (Edgley et al., 1997), the EPSP data indicate that there are significant differences in the corticospinal neurons recruited in these waves.

Inhibition
The widespread corticospinal projection to the intermediate zone (Fig. 2) suggests a close involvement in the activity of spinal interneurons. Many of these neurons are last-order inhibitory interneurons (Perlmutter et al., 1998), receiving convergent peripheral afferent and corticospinal inputs (Jankowska et al., 1976). Interestingly, IPSPs were less frequently observed from M1 (50% of tested motoneurons; Table 3), or especially from SMA (24%) than from the whole PT (81%). The balance of excitation and inhibition from different motor areas must be of functional importance.

Differences in Motoneuron Responses to M1 vs. SMA
**Activation: Correlation with Anatomy**
The corticospinal tract terminates in every region of the spinal grey matter (Fig. 2), and this presumably reflects its widespread involvement in controlling many different functions (Bucy et al., 1964; Porter and Lemon, 1993). In this study we have focused only on the projection to lamina IX hand muscle motor nuclei and sought to correlate it with responses of motoneurons in these same nuclei to activation of corticospinal outputs from M1 and SMA. Our results establish that both M1 and SMA give rise to CM outputs, and that some motoneurons receive outputs from both areas. Excitatory effects from SMA were less common than from M1 (48% vs. 88%; Fig. 11A) and the amplitudes of SMA EPSPs were much smaller than M1 EPSPs (Fig. 11B–D). Weak facilitation of arm muscle EMG by single pulse ICMS in SMA has been reported (Hummelsheim et al., 1986; Wiesendanger et al., 1987), although we have not yet encountered any post-spike facilitation from PTNs recorded in SMA (K. Nakajima, J. N. Davis, M.A. Maier and R.N. Lemon, unpublished). These results are in keeping with the weaker termination of the corticospinal projection from SMA to upper limb motor nuclei.

It could be argued that indirect-wave EPSPs from SMA were actually more common that might be expected from our anatomical findings (Fig. 3). Although it seems likely that the effective I-wave activity did originate in the SMA, this remains uncertain. Part of this activity may have arisen from trans-synaptic activation of members of the CM ‘colony’ located in other cortical motor areas, of which M1 is a possible candidate. Against this is the lack of occlusion or facilitation of the I-wave activity when both areas were stimulated simultaneously (Fig. 6).

**Functional Interpretation**
Experiments in anaesthetized preparations cannot provide information on the activation of M1 and SMA corticospinal outputs during normal movement, but they can inform and constrain theories about how such activation might lead to movement. The muscle-like properties of M1 neurons in general, and of its CM neurons in particular, have reinforced the concept that it is a key part of the executive motor system, specially adapted for the selective activation of specific muscle synergies, especially those involved in skilled hand function (Lemon et al., 1986; Cheney et al., 1991; Matsumura et al., 1991; Maier et al., 1993; McKiernan et al., 1998; Crummond and Kalaska, 2000).

Many studies have stressed the apparent similarity in the functional involvement of M1 and SMA. These have included both single unit studies in monkeys (Alexander and Crutcher, 1990; Kazennikov et al., 1999) and brain imaging studies in humans (Dettmers et al., 1995; Fink et al., 1997; Toma et al., 1999). Others have emphasized the differences, stressing, for example, the activity of medial wall cortex in the selection of movement, performance of complex motor sequences and motor learning (Mushiake et al., 1991; Tanji 1994; Jahanshahi et al., 1995; Wise, 1996; Picard and Strick, 1996; Sadato et al., 1997). These differences are particularly striking when comparison is made between M1 and the more rostral parts of the medial wall cortex [the pre-SMA (Tanji, 1994) or F6 (Luppino et al., 1991)]. This region has been repeatedly implicated in these ‘higher’ aspects of movement performance, while the more caudal region (SMA-proper or F3) has been assigned a motor executive role (Tanji, 1994; Boecker et al., 1998; Jenkins et al., 2000). This suggests a role for SMA in which it acts ‘in parallel’ with M1, the other major motor executive structure.

In this context, it has been suggested on the basis of both neuroanatomy and clinical lesions that motor cortical areas have a capacity to act in parallel for the generation and control of distal limb movements (Fries et al., 1993; Dum and Strick, 1996; Wise, 1996). Fries et al. (Fries et al., 1995) speculated that the different motor areas ‘are able to substitute for each other functionally’. Although there are similarities in the corticospinal projections from the two motor areas, our studies highlight the special importance of the CM projection to upper limb motoneurons from M1, and make it doubtful that SMA, with a much weaker CM input, could substitute M1 in this respect. In support of this, it has been shown that the recovery of motor performance after a chronic M1 lesion was not dependent upon the integrity of SMA (Rouiller et al., 1998). The findings reported here are more in keeping with a role for the SMA which does not involve major CM actions. Rather, the SMA projections to the intermediate zone (especially medial parts of lamina VI and lamina VII) could reflect its contribution to the preparation and modulation of the excitability of intrinsic spinal circuitry.
Changes in motor set which are involved in the preparation and selection of movements are known to involve spinal interneurons (Prut and Fetz, 1999; Bizzi et al., 2000). Preparatory activity in SMA before particular sequences or types of movement could be mediated by descending control of such interneurons. Thus although corticospinal systems from different motor cortical areas may act in parallel, they may also have contrasting functions, as reflected in marked differences in their CM actions.

Notes
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