Striking Differences in Transmission of Corticospinal Excitation to Upper Limb Motoneurons in Two Primate Species

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Received 8 December 1999; accepted in final form 12 April 2000

Nakajima, K., M. A. Maier, P. A. Kirkwood, and R. N. Lemon. Striking differences in transmission of corticospinal excitation to upper limb motoneurons in two primate species. J Neurophysiol 84: 698–709, 2000. There is considerable debate as to the relative importance, for cortical control of upper limb movements, of direct cortico-motoneuronal (CM) versus indirect, propriospinal transmission of corticospinal excitation to cervical motoneurons. In the cat, which has no CM connections, a significant proportion of corticospinal excitation reaches forelimb motoneurons via a system of C3–C4 propriospinal neurons (PN). In contrast, in the macaque monkey most motoneurons receive direct CM connections, and, under the same experimental conditions as in the cat, there is little evidence for PN transmission. We have investigated corticospinal transmission in the New World squirrel monkey (Saimiri sciureus) because its CM projections are weaker than in the macaque. Intracellular recordings were made from motoneurons identified from the ulnar, median, and deep radial (DR) nerves in four adult squirrel monkeys under chloralose anesthesia and neuromuscular paralysis. Responses to stimulation of the contralateral medullary pyramid were recorded before and after a lesion to the dorsolateral funiculus (DLF) at C5, designed to interrupt direct corticospinal inputs to the lower cervical segments and unmask PN-mediated effects. This lesion greatly reduced the proportion of motoneurons showing either CM EPSPs or disynaptic IPSPs, but the proportion showing late EPSPs with segmental latencies beyond the monosynaptic range, evoked by repetitive but not single PT stimuli, was unaffected: 23 of 29 motoneurons (79%) before and 32 of 37 (86%) after the lesion; 41% of these late EPSPs had strictly disynaptic latencies after the lesion, only 14% before. These results are in striking contrast to the macaque (late EPSPs in only 18% of motoneurons before a C5 lesion, 19% after it). Transmission of the late EPSPs via C3–C4 PN s in the squirrel monkey was indicated by their absence after an additional C5 DLF lesion. Nearly all tested motoneurons also responded with short latency EPSPs to stimulation in the ipsilateral lateral reticular nucleus. By analogy with the cat, these EPSPs probably reflect antidromic activation of ascending collaterals of C3–C4 PNs with monosynaptic connections to motoneurons; the EPSPs were significantly smaller than in the cat but larger than in the macaque. These results suggest that the positive correlation across species between more advanced hand function and the strength of the CM system is accompanied by a negative correlation between hand function and the strength of the PN system. We hypothesize that in primates with more advanced hand function, the CM system effectively replaces PN-mediated control. This would include a contribution to the control of reaching movements, which are said to be specifically under the control of the PN system in the cat, and we speculate that these differences may be related to the degree of dexterity exhibited by the different species. This interpretation of the results predicts that in man, where the CM system is highly developed, the PN system is unlikely to be responsible for significant transmission of cortical commands to upper limb motoneurons.

INTRODUCTION

Cortical commands associated with voluntary movements can be transmitted to alpha motoneurons over a number of different parallel pathways. The largest descending pathway from the motor areas of the cerebral cortex is the corticospinal tract. This tract exists in all mammals, but there are important species differences in its areas of origin, patterns of termination, and functions (Bortoff and Strick 1993; Kuypers 1981; Nudo and Masterton 1990a,b; Porter and Lemon 1993). In primates, some corticospinal fibers make direct corticomo- neuronal (CM) connections to spinal motoneurons, and this CM system is particularly well-developed in Old World monkeys, apes and humans (Heffner and Masterton 1975, 1983; Kuypers 1981; Palmer and Ashby 1992). A large amount of evidence suggests that the normal development and integrity of the CM system is essential for the capacity to perform skilled hand and finger movements (Armand et al. 1997; Galea and Darian-Smith 1995; Lawrence and Hopkins 1976; Olivier et al. 1997).

The particular importance of direct corticospinal projections to motoneurons is not self-evident from anatomical studies which show that the primate corticospinal tract makes widespread terminations throughout the spinal gray matter, not just in the motor nuclei (Bortoff and Strick 1993; Kuypers 1981; Ralston and Ralston 1985). For example, recent studies of the corticospinal projections from the macaque primary and other motor cortical areas have shown particularly dense terminations in Rexed laminae V–VII in the cervical spinal cord; these projections are generally stronger than those to the motor nuclei in lamina IX (Armand et al. 1997; Dunn and Strick 1996).

What is the function of indirect, non-monosynaptic projections from the cortex? One possibility is indicated by studies on the cat (Alstermark and Lundberg 1992; Illert et al. 1977, 1978). In this species, which completely lacks CM projections (Illert et al. 1977; Kuypers 1981), an important pathway for cortical control of the forelimb is a system of upper cervical

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Corticospinal effects on motoneurons at the segmental level are abolished by a lesion of the dorsolateral funiculus (DLF) at C2 and at more caudal levels (usually C4). These lesions sever the lateral corticospinal tract (LCST) and ablate any direct corticospinal monosynaptic input to MNs, which would be expected to contribute to the transmission of corticospinal volleys in forelimb MNs (segments C6–Th1). Stimulation of the lateral reticular nucleus (LRN) evokes disynaptic excitation in forelimb MNs via ascending axon collaterals of propriospinal neurons (PNs) which are activated monosynaptically by corticospinal fibers. The PN pathway contributes significantly to the transmission of corticospinal excitation to MNs via C3–C4, provides crucial new evidence in the debate about the roles of the CM and PN systems, and implies that direct CM connections to hand muscle motoneurons are significantly weaker in the squirrel monkey than in other monkeys, including the macaque (Bortoff and Strick 1993; Maier et al. 1997). Here we demonstrate that in the squirrel monkey, in parallel with the weak CM input to upper limb motoneurons, there is also clear evidence for corticospinal excitation of these motoneurons via a C3–C4 PN pathway, similar to that in the cat. We suggest that if PN transmission is strong in the cat, with no CM system, and moderately strong in the squirrel monkey, with a weak CM system, then in other species in which the CM system is well-developed, such as great apes and humans, the PN pathway is likely to play only a minor role in transmitting corticospinal excitation to upper limb motoneurons. We speculate therefore that these differences may be related to the different degrees of dexterity exhibited by the different species. This has important consequences for our understanding of cortical control of human upper limb function.

Methods

Four purpose-bred male adult Saimiri sciureus monkeys (0.65–0.77 kg) were used. Animal care and use were in accordance with the U. K. Animals (Scientific Procedures) Act. The methods used were identical to those described previously (Maier et al. 1998). Anesthesia was induced with 15 mg·kg⁻¹·h⁻¹ ketamine intramuscularly and maintained with 1.5–2.5% isoflurane in 1:1 O₂:N₂O. A tracheal cannula was inserted and one femoral artery and both femoral veins cannulated. Nerve cuff stimulating electrodes were mounted on the left median and ulnar nerves at the axilla and on the left deep radial (DR) nerve at the elbow. An occipital craniotomy and a C₂–Th1 laminectomy were performed. When surgery was complete, the isoflurane was discontinued and anesthesia was maintained with α-chloralose (50 mg·kg⁻¹·h⁻¹ iv). The animal was paralyzed with pancuronium bromide (Pavulon, Oregon-Technika, Cambridge, UK; 0.3 mg·kg⁻¹·h⁻¹ iv) and artificially ventilated at a rate of 45 cycles · min⁻¹. The adequacy of anesthesia was continuously assessed by reference to blood pressure, heart rate, and pupillary reflexes. Body temperature and general physiological condition were carefully maintained throughout the experiment, including a mean blood pressure >80 mmHg.

The right medullary PT and left LRN were stimulated with varnish-insulated tungsten electrodes. The optimal location of the PT electrode was based on the threshold and amplitude of the corticospinal volleys evoked from it and recorded from the surface of the dorsolateral funiculus (DLF) at a rostral site (usually C4) and at a caudal site close to the region from which motoneuron recordings were made (C6–Th1) (see Alstermark et al. 1981a; Maier et al. 1998). The LRN electrode was positioned so as to produce a large field potential (Maier et al. 1997) when recording from within upper limb motor nuclei on the ipsilateral side. A number of tracks were made in the region 2.1–2.5 mm lateral from obex and from 0.6 mm rostral to 0.5 mm caudal from obex. The final electrode position was one which gave the field potential with a threshold of <30 μA (see Fig. 7).

Intracellular recordings were made from motoneurons in the C6, C7, C8, and Th1 segments with glass microelectrodes filled with 3 M potassium acetate and having a DC resistance of 4–10 MΩ. A small pressure foot was used in most experiments to reduce movement of the spinal cord. All motoneurons were identified antidromically from the limb nerves. Stimuli to the PT and LRN (1–3 shocks usually at 333 Hz) were delivered at 3 Hz; stimulus duration was 0.1 ms. Intracellular and surface recordings were digitized directly at 10 kHz using a 1401plus interface (CED, Cambridge, UK). Membrane potential was monitored throughout the recording, and only data from stable periods of recording used for analysis (membrane potential <−50 mV). All
short rise time and amplitudes were derived from sets of measurements made from a number of single traces. Population measurements are given as means ± SD. Possible postsynaptic potentials smaller than 200 μV were not measured or counted.

In all four monkeys lesions of the left DLF in the C5 segment were made with fine watchmakers forceps. The lesion was being made corticospinal volleys recorded both above and below the lesion site were monitored. An additional lesion of the left DLF in the C2 segment was made in two of the monkeys. Small electrolytic lesions were placed at the PT and LRN stimulation sites at the end of the experiment, and the animal was killed with an intravenous overdose of barbiturate and perfused through the heart. DLF lesions were reconstructed postmortem from paraffin embedded blocks of spinal cord, and the sites of stimulating electrodes confirmed histologically.

Data from the macaque monkey referred to here consist either of unpublished measurements or results published by Maier et al. (1998) that have been reanalysed. For responses to the PT stimuli, segmental latencies were measured from the positive peak of the corticospinal volley recorded at the same segment as the motoneuron or after the C5 lesion from the equivalent time measured from such a volley recorded before the lesion. For responses to the LRN stimuli, absolute latencies were measured from the stimulus artifact.

**RESULTS**

We searched for evidence of propriospinal transmission of corticospinal excitation, generated by stimulation of the contralateral pyramidal tract (PT; Fig. 1). The characteristic signs of such transmission have been established in the cat. They are as follows: 1) the EPSPs evoked from the corticospinal tract have a disynaptic latency (Illert et al. 1976, 1977); 2) the indirect nature of the PN linkage means that repetitive stimulation of the PT is needed to evoke these EPSPs; they are not obtained in response to single stimuli (Baldissera et al. 1981; Illert et al. 1976, 1977); 3) the location of the descending axons of C3–C4 PNs in the ventral part of the lateral funiculus (Fig. 1, inset) means that these effects survive a lesion of the DLF at the C5 level, cutting the fibers of the lateral corticospinal tract projecting to the lower cervical segments (C0–Th1) containing the forelimb motor nuclei (Alstermark and Lundberg 1992; Illert et al. 1977); 4) the EPSPs are, however, abolished by a DLF lesion at the C2 level, since this interrupts the corticospinal input to the C3–C4 PNs (Fig. 1) (Illert et al. 1977).

**Responses of motoneurons before a lesion to the corticospinal tract at C5**

From monkeys with intact spinal cords, we analyzed the recordings from 29 spinal motoneurons, identified from the median (18), ulnar (9), or DR (2) nerves. In most of these motoneurons (79%), single PT shocks evoked early EPSPs (see median motoneuron in Fig. 2B) or an early EPSP followed by an IPSP (ulnar motoneuron in Fig. 2F). These EPSPs were identified as monosynaptic by the short segmental delay between the arrival of the descending corticospinal volley at the recorded spinal segment and the onset of the EPSP (0.7 ms in Fig. 2, B and F). The mean segmental delay of monosynaptic EPSPs was 0.9 ± 0.1 (SD) ms (n = 23); the distribution is plotted in Fig. 5A (hatched columns). These CM EPSPs were generally small (0.6 ± 0.3 mV, n = 23; Fig. 9A) and relatively slow rising (cf. Maier et al. 1997).
Responses of motoneurons after a lesion to the corticospinal tract at C5

After these initial recordings, a lesion was made in the DLF at the C5 spinal level; reconstructed lesions from two of the experiments are shown in Fig. 3. These lesions abolished most of the negativity in the PT-evoked volley recorded caudal to the lesion. Similar lesions and volleys were recorded in the other experiments.

Subsequently, recordings were made in all four animals from a further 37 motoneurons (median, 26; ulnar, 8; DR, 3). A typical set of records is shown in Fig. 4, A–D. In this median motoneuron, there was no response, either EPSP or IPSP, to a single PT shock after the lesion (Fig. 4B). Only 3 of 37 (8%) motoneurons sampled after the lesion showed an early CM EPSP. The response to repetitive PT stimulation was quite different: two or three PT shocks evoked a large, late EPSP (e.g., Fig. 4, C and D) with a segmental delay of 1.8 ms (Fig. 4D). Responses of this type were seen in the great majority of tested motoneurons (32 of 37, 86%). A further example, this time from a DR motoneuron, is given in Fig. 4, E–H, which shows a typical late EPSP to PTx3 (Fig. 4H; segmental delay 1.5 ms), but which was unresponsive to PTx1 (Fig. 4F). With single PT shocks, only one motoneuron showed an IPSP; even with repetitive stimulation only 6 of 37 (16%) showed an IPSP.

The segmental latencies of all responses recorded before and after the C5 lesion are plotted in Fig. 5. The occurrence of monosynaptic CM EPSPs with short segmental delays (0.7–1.1 ms) was dramatically reduced by the C5 lesion (cf. Fig. 5, A and C), and this was also true of the IPSPs (Fig. 5, B and D), all of which fell within the disynaptic range (1.3–1.9 ms). Late EPSPs evoked by PTx3 were common both before and after the lesion, although in the intact cord they had a wider spread of latencies (1.4–3.0 ms), with relatively few in the disynaptic range. IPSPs were evoked in the majority of the motoneurons before the C5 lesion and this made the precise onset of late EPSPs difficult to identify. This may have lead to an overestimation of the segmental latency, and this is also suggested by the finding that after the lesion, the late EPSPs were much less broadly distributed and of 15 of 37 tested motoneurons (41%), almost one-half of those showing late EPSPs responded within the disynaptic range. The mean segmental delay for all late EPSPs after the lesion was 2.0 ± 0.3 ms.

Abolition of late EPSPs by a C2 lesion

The latency of the late EPSPs, their dependence on repetitive stimulation of the PT, and their persistence after the C5 DLF lesion are all consistent with their production via PNs in the upper cervical segments. Further confirmation of this conclusion was obtained by demonstrating that, in four motoneurons (2 monkeys), an additional DLF lesion at the C2 level abolished the late EPSPs, as explained in Fig. 1. The lesion and corticospinal volleys from one experiment are shown in Fig. 6, A and B; the median motoneuron in Fig. 6, C–F, was unresponsive to either single or repetitive PT stimuli (Fig. 6, D and E), although it still responded to activation of other inputs, such as from peripheral nerves and from the LRN (Fig. 6F; see next section). This result indicates that the late EPSPs evoked from the PT were not mediated by a supraspinal, brainstem pathway receiving corticospinal collaterals (cf. Illert et al. 1977).
Responses to stimulation of the lateral reticular nucleus

In further tests to identify the source of the late EPSPs we stimulated the brainstem lateral reticular nucleus (LRN) ipsilateral to the recorded motoneurons. In the cat, this nucleus receives ascending collaterals of the C3–C4 PN neurons (Fig. 1) and therefore LRN stimulation evokes monosynaptic EPSPs in forelimb motoneurons (Alstermark et al. 1981a; Illert and Lundberg 1978). At the beginning of each experiment the region of the LRN was explored with a stimulating microelectrode while recording field potentials in motor nuclei in C6 or C7. The electrode tip was located at the optimal position (Fig. 7A) for evoking the characteristic field potential shown in Fig. 7B, with a slow negativity beginning at ~1.5 ms after the stimulus, reaching a maximum at 3.0 ms and lasting for ~10–15 ms. The timing and form of this field potential response is identical to that evoked by LRN stimulation in the cat (Alstermark et al. 1981a) and macaque monkey (Maier et al. 1998). The potential in Fig. 7B had a threshold of 20 μA; its amplitude increased steadily with intensities up to 200 μA, but it showed little further increase with stronger shocks (Fig. 7B).

Stimulation in the LRN evoked short latency EPSPs in all 18 motoneurons tested before the C5 lesion and in most (34 of 36) after it. Figure 7, C and D, shows typical EPSPs evoked from the LRN in a DR and in a median motoneuron, respectively. The mean amplitude of the EPSP evoked by a 200 μA stimulus was 1.7 ± 0.9 mV (n = 18) before and 1.7 ± 0.8 mV (n = 34) after the C5 lesion. Figure 7E shows the amplitudes of the EPSPs in Fig. 7C plotted against stimulus strength, together with a similar curve from another motoneuron. These curves are typical and very similar in form to the equivalent measurements in the cat by Alstermark and Sasaki (1986a).

The latencies and rise times of the EPSPs evoked from the LRN are plotted in Fig. 8. It was not possible to estimate the segmental latency of the postsynaptic responses evoked by LRN stimulation, because no clear volley responsible for the effects could be discerned in the surface recording; therefore only the absolute latency, measured from the time of the stimulus, is plotted for the LRN responses. The monosynaptic nature of many of these EPSPs was indicated by their short and consistent absolute latencies, and the lack of facilitation or latency shortening with repetitive LRN stimulation (Fig. 7D).

The distributions of EPSP latencies are plotted before (Fig. 8C) and after (Fig. 8D) the C5 lesion; the mean postlesion latency was 1.8 ± 0.3 ms (n = 34).

As in the cat (Alstermark and Sasaki 1986a), there was a clear positive correlation between the amplitude and rise time of the EPSPs evoked from the LRN, measured as indicated by the dotted lines in Fig. 8, A and B. This correlation was significant both before the C5 lesion (Fig. 8E; P = 0.002) and after it (Fig. 8F; P = 0.0046). Figure 8, G and H, shows a similar relation between amplitude and rise time for motoneu-
rons of the macaque monkey \((G, P = 0.0062 \text{ after lesion}; H, P = 0.0035 \text{ after lesion})\).

**Comparison of responses to stimulation of PT and LRN before and after the C5 lesion in squirrel and macaque monkeys**

Many of the results reported here are strikingly different from those in our previous study of the macaque monkey (Maier et al. 1998). With respect to the amplitude of the responses to PT and LRN, Fig. 9, A and B, shows that for the ulnar, median, and DR motoneurons sampled in the two studies, the CM EPSPs (recorded before the C5 lesion) were significantly smaller in the squirrel monkey \((0.6 \pm 0.3 \text{ mV}, n = 23; \text{Fig. 9A})\) than in the macaque \((1.9 \pm 1.0 \text{ mV}, n = 62; \text{Fig. 9B}; \text{Mann-Whitney U test, } P < 0.0001)\) (cf. Maier et al. 1997). In contrast, the late EPSPs, on the third PT shock and recorded after the C5 lesion (Fig. 9, C and D), were generally, but not significantly, larger in the squirrel monkey \((1.2 \pm 0.6 \text{ mV}, n = 32)\) than in the macaque \((0.9 \pm 0.5 \text{ mV}, n = 16, P = 0.063)\). EPSPs evoked from the LRN (Fig. 9, E and F) were significantly larger in the squirrel monkey \((P < 0.0003)\).

The occurrence of late EPSPs was also markedly different. This is shown in Fig. 10 which summarizes all the responses to PT and LRN in the two studies. Whereas in the squirrel monkey late EPSPs were common both before and after the C5 DLF lesion (79 and 86%, respectively), far fewer macaque motoneurons responded in this way (18 and 19%, respectively). Although the C5 lesion had little effect on the frequency of occurrence of late EPSPs in either species, it did affect the distribution of the segmental latencies (see Fig. 5, A and D). In the squirrel monkey the proportion of responses within a synapsic range increased from 14 to 41% after the lesion and in the macaque from 3 to 14%. This shift probably resulted from the general absence of contaminating IPSPs after the lesion (16% with PTx3; see above). IPSPs evoked by single PT shocks were common before the lesion in both species, but were found in only 3% of motoneurons after the lesion in the squirrel monkey and in only 24% in the macaque. The C5 lesion produced a striking reduction in both CM EPSPs and IPSPs in both studies, although the change was more marked in the squirrel monkey. Whereas in the macaque, with relatively large compound CM EPSPs, the corticospinal axons surviving the lesion could be expected to evoke effects in some motoneurons (36% of which still gave EPSPs, but nearly all with amplitudes <1.0 mV), the small amplitude of CM EPSPs in the intact squirrel monkey (Fig. 9A) may well mean that postlesional effects were too small to detect. The C5 DLF lesion had no effect on the occurrence of EPSPs from the LRN in either species.

**Correlation of responses in single motoneurons to LRN and PT stimulation**

In the cat, Alstermark and Sasaki (1986a) have demonstrated that across a population of forelimb motoneurons there is a positive correlation between the amplitudes of the LRN-evoked EPSP and the disynaptic EPSP evoked by PTx3 in the same motoneuron. Their data, recorded from motoneurons supplying muscles acting at the shoulder and elbow, has been replotted in Fig. 11A (triangle, C5 lesion, \(r = 0.54, P < 0.002\)). Figure 11A also shows that in both the squirrel monkey (filled circle) and macaque (open circle), a similar positive correlation exists (squirrel monkey, thick regression line, \(r = 0.38, P = 0.035\); macaque, thin regression line, \(r = 0.62, P = 0.015\)). All three regression lines fall below the line of identity (dotted line). The distribution of data points with small amplitudes is replotted for clarity in Fig. 11B.

The arrowheads on the abscissa in Fig. 11A show important
The major finding reported here is that in a primate species with a rather weak CM system we have found significant transmission of corticospinal excitation to arm and hand motoneurons by a C₃–C₄ propriospinal pathway. This is in distinct contrast to the findings of our previous study, under identical experimental conditions, of the macaque monkey, in which CM effects are stronger but where there is little evidence for PN transmission (Maier et al. 1998).

There are four pieces of evidence that the late EPSPs evoked from the PT in squirrel monkey motoneurons were mediated by a C₃–C₄ propriospinal pathway: 1) most EPSPs had a segmental latency in the di- or trisynaptic range; 2) all EPSPs were seen with repetitive but not single PT stimuli, indicating an indirect pathway; 3) the EPSPs persisted after a C₅ lesion was made that abolished most of the corticospinal input to the lower cervical segments where the target motoneurons are located. PNs with axons running in the ventral parts of the lateral funiculus, as in the cat (Illert et al. 1977, 1978) (Fig. 1, inset), are likely candidates for mediating these effects (Maier et al. 1998); and 4) the effects were abolished by a second lesion to the DLF at C₅, which interrupted corticospinal input to the C₃–C₄ segments (Illert et al. 1977).

Comparison of motoneuron responses in cat, squirrel monkey, macaque monkey, and human

It is particularly interesting to compare the results obtained in studies of corticospinal excitation in three different species: cat, squirrel monkey, and macaque (Fig. 12), and to explore the significance of this comparison for the human motor system. A similar anesthetic regime and experimental approach has been employed in all the animal studies. In motoneurons recorded after the C₅ lesion, late EPSPs from the PT were common in the squirrel monkey (86% of sampled motoneurons; Fig. 12), which is slightly less than in the cat, where all motoneurons show such effects (Alstermark and Lundberg 1992; B. Alstermark, personal communication). The proportion in the squirrel monkey is in striking contrast to the macaque, in which only 18% of 88 tested upper limb motoneurons showed evidence of di- or oligosynaptic EPSPs evoked by repetitive PT stimulation after a C₅ lesion (Maier et al. 1998). Both the latency and the amplitude of the late EPSPs in monkeys tend to suggest that C₃–C₄ transmission of corticospinal excitation is weaker than in the cat. Thus in the squirrel monkey, as in the macaque, some of the effects we have observed were beyond the presumed disynaptic range (Fig. 5). However, the distribution is not bimodal, as might be expected if the longer latency effects were trisynaptic. If there is a relatively weak convergence of PN neurons onto individual motoneurons in monkeys, then EPSPs derived only from slowly conducting PN fibers, become more likely and disynaptic EPSPs would be expected at latencies longer than those in this limited range; there is evidence in the cat for such slowly conducting fibers (Alstermark and Sasaki 1986a; Alstermark et al. 1981a). In any case, even if
only those late EPSPs that were strictly within the presumed disynaptic range are compared, the same species differences are observed (after C5 lesion: cat, 100%; squirrel monkey, 41%; and macaque, 14%; see Fig. 10). These results suggest that, under the experimental conditions used, PN neurons transmit a significantly higher proportion of corticospinal excitation in the cat than in the squirrel monkey, which in turn is higher than in the macaque.

The decline in the contribution of PN-transmitted corticospinal excitation from cat, to squirrel and macaque monkey is accompanied by a clear increase in the influence of the CM system. CM connections are absent in the cat (Fig. 12B, *), present but relatively weak in the squirrel monkey, and well developed in the macaque. In the squirrel monkey, the mean amplitude of the CM-EPSP in motoneurons supplying hand and forearm muscles was 0.6 ± 0.3 mV (n = 23) compared with 1.9 ± 1.0 mV (n = 62) in the macaque.
It is significant that the human CM system is the most highly developed among primates (Heffner and Masterton 1975, 1983; Kuypers 1981). The estimated value given for CM-EPSPs in Fig. 12B is 4.2 mV, substantially larger than in either monkey species. This estimate is based on responses of single motor units in hand and forearm muscles to transcranial electrical stimulation of the motor cortex (de Noordhout et al. 1999); similar estimates have been found in other human studies (Day et al. 1989; Palmer and Ashby 1992).

Factors that might explain species differences in transmission of corticospinal excitation

There are a number of possible explanations for the species differences in PN transmission. The first possibility is that the corticospinal excitatory projection to the C3–C4 PNs is much weaker in the macaque than squirrel monkey or cat. This can only be established by direct recording from these PNs. A second possibility, already referred to above, is that the projection of the macaque PNs to upper limb motoneurons is relatively weak. Some insights into this second explanation can be gained from the properties of the responses to LRN stimulation. The field potentials (Fig. 7B) and EPSPs (Fig. 7, C and D and Fig. 8, A and B) evoked from the LRN were very similar to those reported in great detail in the cat (Alstermark et al. 1981a) and the macaque monkey (Maier et al. 1998). Further, the relationship between EPSP rise time and amplitude is remarkably similar in both monkey species (Fig. 8, E–H) as compared with that seen in the cat (Alstermark and Sasaki 1986a). These features all suggest that the EPSPs are likely to be due, at least in part, to antidromic activation of ascending axons of PNs to the LRN (see Discussion in Maier et al. 1998). The amplitude of EPSPs evoked in upper limb motoneurons from the LRN appears to be largest in the cat, smaller in the squirrel monkey and smallest in the macaque (see arrowheads in Fig. 11). Note that because proximal upper limb muscles were represented in the macaque and not in the squirrel monkey study (Maier et al. 1998), any bias in motoneuron selection between the two primate studies should have resulted in the macaque EPSPs being larger than those in the squirrel monkey and more like those in the cat. Figure 11 shows that this was not the case. Also, the squirrel monkey EPSP amplitudes are clearly generally closer to the origin in Fig. 11 than the EPSPs from even the fast motoneurons in the cat population (Alstermark and Sasaki 1986a).

We recognize the dangers of making these comparisons both between laboratories and between species, especially because motoneurons were not type-identified in either of the primate studies and nor were the muscles as precisely identified as in the cat studies. Nevertheless, these results tend to support the idea that some of the species differences in PN-mediated excitation from the PT are due to variations in the strength of the C3–C4 PN projection to upper limb motoneurons. In Fig. 12 this is indicated by the thickness of the line linking the C5–C4 PNs to MNs.

Inhibition of C3–C4 transmission

A complicating factor in estimating the strength of PN transmission of corticospinal excitation is that stimulation of the corticospinal tract gives rise to feedforward inhibition of PN neurons (Alstermark et al. 1984a,b). Alstermark and Sasaki (1986a) considered that this feedforward inhibition was one of the reasons why, for a given motoneuron, the size of the EPSP from the LRN was always larger than that evoked from the PT. The regression line relating the amplitudes of these two effects always lay below the line of identity (Fig 11, fine dotted line), and the slope of the regression line was taken to represent the degree of feedforward inhibition (Alstermark and Sasaki 1986a). It is interesting that in all three species investigated, there is a significant relationship between the LRN EPSP and late EPSP from the PT and that the regression lines have similar slopes. Although there is considerable scatter in Fig. 11, this data provides no support for a different degree of feedforward inhibition across the three species.

A recent report from Alstermark et al. (1999) challenges the
now seems that the need for re-evaluation [of human studies interpreted via PN studies in the cat] is not so urgent."

Although the results from Alstermark et al. (1999) are important and lead to a number of new questions, we believe that these results are open to more than one interpretation and that they therefore do not invalidate our conclusions. First, the claim that the inhibitory control is stronger in the macaque monkey than in the cat must remain a hypothesis until further detailed experiments with strychnine are performed in the cat. Second, it is important that the issue of the definition of the "C₃–C₄ propriospinal system" is carefully considered. The results of Alstermark et al. (1999) suggest that a significant number of C₃–C₄ PNs which project via the ventral portion of the lateral funiculus to upper limb motoneurons are under inhibitory control. The authors' interpretation implies that when released from this inhibition the PN system in the macaque is comparable in strength to that in the cat. However, an important element in investigating this system in many of the publications from Alstermark and his colleagues has been the observation that "the vast majority" of PNs (86% of those terminating above T₀ (Alstermark et al. 1981a) have an ascending collateral to the LRN, and many properties of the system have been investigated on this basis, as described above. Thus if the macaque has a PN system comparable to that in the cat, the EPSPs from the LRN should also have similar amplitudes to those in the cat. This prediction is in conflict with our data, which shows that these EPSPs are in fact substantially smaller than in the cat (Fig. 11); note that these EPSPs, if they reflect antidromic activation of C₃–C₄ PNs, should not be affected by inhibition of these neurons. We contend that it is a much more prudent assumption that if other interneurons are released from inhibition by strychnine, then they should be taken as a previously unknown category, which may not have the same properties and connections as the well-known C₃–C₄ interneurons investigated in the cat in the absence of strychnine.

The function of feedforward or other sorts of inhibition of C₃–C₄ PNs is still unknown; it may be concerned with focusing motor acts (Alstermark et al. 1999; Pierrot-Desilligny 1996), but this has yet to be demonstrated.

The primary importance of the analogy between responses in man (Pierrot-Deseilligny 1996) and the well-known system in the cat is the ability to use some of the properties of the cat system to help evaluate the human measurements. If it is likely, as we suggest above, that the system now revealed in the experiments of Alstermark et al. (1999) is substantially different from that well-known in the cat, then the analogy is not a safe one for these purposes, and it remains the case that "deductions . . . based on data from the cat should be regarded with great caution" (Maier et al. 1998). We suggest that, at the very least, new experiments are needed to investigate whether or not the properties of the connections revealed by strychnine (in either monkey or cat) correspond to those of the well-known C₃–C₄ system, before extrapolation from cat to man is considered to be safe.

**The relative contribution of the CM and PN systems to upper limb control in different species: a hypothesis**

There are, of course, many differences between the motor behaviors of the four species considered here. One is the striking variation in dexterity (Figure 12D) (Heffner and Masterton 1975).
terton 1975, 1983). In the cat, with an index of dexterity of 2, there is a very restricted capacity for independent digit movements (Boczek-Funcke et al. 1998). Although the squirrel monkey (index = 5) has much more advanced hand function than the cat, independent finger movements are limited and there is no precision grip or capacity to manipulate objects within the hand (Costello and Frasgasy 1988; Frasgasy 1983). The macaque monkey (index = 6) has both the capacity for true tip-to-tip opposition of thumb and index in the precision grip and employs tactile exploration and manipulation of objects (Heffner and Masterton 1975, 1983; Lawrence and Hopkins 1976), and these functions are further refined in the human hand (index = 7) (Napier 1980; Wiesendanger 1999). Thus it has been concluded that the relative development of the CM system in the four species is important for the degree of dexterity which they exhibit. One could therefore speculate that the apparently converse relationship with the influence of the PN system is similarly related to the degree of dexterity.

However, in attempting to assess the relative contribution of CM and PN transmission of cortical commands, it is important to point out that in the cat the C3–C4 PN system has been implicated in the control of reaching movements but not grasping (taking food with the paw) (Alstermark and Lundberg 1992; Alstermark et al. 1981b). In keeping with this function, it has been observed that motoneurons supplying proximal upper limb muscles receive a strong PN input in this species (Alstermark and Sasaki 1986b). In contrast, the observations of Maier et al. (1998) suggest that this input to motoneurons supplying this same group of muscles is generally absent in the macaque monkey. This prompts us to suggest that the PN system is of less importance in the control of reaching in primates than in the cat.

Thus we hypothesize that in addition to the already well-established function of the CM system in the control of the most distal upper limb muscles, there is also a CM contribution to the control of reaching that effectively takes the role of that mediated by the C3–C4 system in the cat. There is evidence that in primates, upper limb motoneurons supplying muscles involved in reaching receive CM connections. In humans, short-latency motor evoked potentials are evoked in muscles acting at the shoulder and elbow by non-invasive cortical stimulation (de Noordhout et al. 1999; Palmer and Ashby 1992). In macaque monkey, CM EPSPs have been recorded in motoneurons supplying proximal arm muscles (Maier et al. 1998; Porter and Lemon 1993). A recent study reported CM cells showing postspike facilitation of muscles such as deltoid, latissimus dorsi, biceps, and brachialis during reaching movements (McKiernan et al. 1998). Indeed, one can argue that the highly coordinated acts of reach and skilled grasp in primates (Jeanerod 1988) requires the elaboration of the CM system to involve not only selective control of the most distal extremity but also that of more proximal upper limb muscles. This latter control is essential for stabilizing and orienting the hand (Humphrey and Reed 1983; McKiernan et al. 1998; Porter and Lemon 1993).

Thus there is evidence for the existence of some appropriate projections, as well as teleological justification, for our proposal that the CM system in primates (in particular in the human) could fulfill the functional role that has been assigned to the PN system in the cat.

Other factors

SEGMENTAL INHIBITION. In both the macaque and squirrel monkey most motoneurons showed disynaptic inhibition from the PT. This inhibition was facilitated by repetitive stimulation and was greatly reduced by the C5 DLF lesion. This suggests that an important part of the corticospinal projection to the lower cervical segments may be to segmental inhibitory interneurons in accordance with anatomical findings (Armand et al. 1997; Dum and Strick 1996; Kuypers 1981) and consistent with the effects presumed to be mediated in C3–C4 segments under strychnine (Alstermark et al. 1999). Recordings of spinal interneurons in the awake monkey (Perlmutter et al. 1998) showed that many of these exerted postspike suppression of upper limb EMG activity. Inhibition of unwanted muscle activity is an important component of skilled arm and hand control (Schieber 1995), and can be used to sculpt the final motor output by changes during both preparation and execution of movement (Prut and Fetz 1999). In the squirrel monkey with an intact cord, these inhibitory effects could have masked at least some of the late EPSPs from the PT, especially those strictly within the disynaptic range (cf. Fig. 5, A and C). However, in the macaque monkey with a C5 lesion, such masking was definitely not a significant factor since Maier et al. (1998) reported that only 34% of motoneurons tested after the lesion with repetitive PT shocks showed IPSPs and these were small. Moreover, 26% of motoneurons were found with neither a monosynaptic EPSP nor a disynaptic IPSP that could have masked any later effects: none of these motoneurons showed late EPSPs.

ANESTHESIA. The investigations of the C3–C4 PN system in different animal species have all been carried out under chloralose anesthesia. Although there are good arguments for refuteing a species-specific anesthetic depression of C3–C4 transmission (see Maier et al. 1998), the results of these studies can only strictly apply to the particular experimental conditions that have been tested, which were the ones used in the original definition of the C3–C4 PN system (Illert et al. 1977, 1978). As we discussed above with regard to the recent experiments of Alstermark et al. (1999), this can be regarded in many ways as a virtue, rather than as a limitation. However, it is clear that more information is needed about the transmission of corticospinal excitation under other pharmacological regimes and in the ananaesthetized state.

Conclusion

These findings suggest that the positive correlation across species between more advanced hand function and the strength of the CM system is accompanied by a negative correlation between this function and the strength of the PN system. The results indicate that in primates with more advanced hand function the CM system may have replaced PN-mediated control and also predicts that in man, where the CM system is more highly developed than in any other species, the C3–C4 PN system, as described in the cat, is unlikely to be responsible for significant transmission of cortical commands to upper limb motoneurons.

We acknowledge the expert advice and help of T. Morris and K. Thompson in carrying out these experiments. We thank N. Philbin, C. Seers, K. Sunner, H.-W. Yang, and R. Spinks for technical assistance, and L. Williams and staff
at the University of South Alabama. We received useful comments on an earlier version of this manuscript from M. Illert and J. Nielsen.

This study was funded by the Wellcome Trust and International Spinal Research Trust.

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