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Investigation Into Non-Monosynaptic Corticospinal Excitation of Macaque Upper Limb Single Motor Units

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Olivier, E., S. N. Baker, K. Nakajima, T. Brochier, and R. N. Lemon. Investigation into non-monosynaptic corticospinal excitation of macaque upper limb single motor units. *J Neurophysiol* 86: 1573–1586, 2001. There has been considerable recent debate as to relative importance, in the primate, of propriospinal transmission of corticospinal excitation to upper limb motoneurons. Previous studies in the anesthetized macaque monkey suggested that, compared with the cat, the transmission of such excitation via a system of C_3 – C_4 propriospinal neurons may be relatively weak. However, it is possible that in the anesthetized preparation, propriospinal transmission of cortical inputs to motoneurons may be depressed. To address this issue, the current study investigated the responses of single motor units (SMUs) to corticospinal inputs in either awake ($n = 1$) or lightly sedated ($n = 3$) macaque monkeys. Recordings in the awake state were made during performance of a precision grip task. The responses of spontaneously discharging SMUs to electrical stimulation of the pyramidal tract (PT) via chronically implanted electrodes were examined for evidence of non-monosynaptic, presumed propriospinal, effects. Single PT stimuli (up to 250 μ A; duration, 0.2 ms, 2 Hz) were delivered during steady discharge of the SMU (10–30 imp/s). SMUs were recorded from muscles acting on the thumb (adductor pollicis and abductor pollicis brevis, $n = 18$), wrist (extensor carpi radialis, $n = 29$) and elbow (biceps, $n = 9$). In all SMUs, the poststimulus time histograms to PT stimulation consisted of a single peak at a fixed latency and with a brief duration [0.74 ± 0.25 (SD) ms, $n = 56$], consistent with the responses being mediated by monosynaptic action of cortico-motoneuronal (CM) impulses. Later peaks, indicating non-monosynaptic action, were not present even when the probability of the initial peak response was low and when there was no evidence for suppression of ongoing SMU activity following this peak ($n = 20$ SMUs). Even when repetitive (double-pulse) PT stimuli were used to facilitate transmission through oligosynaptic linkages, no later peaks were observed (16 SMUs). In some thumb muscle SMUs ($n = 8$), responses to PT stimulation were compared with those evoked by transcranial magnetic stimulation, using a figure-eight coil held over the motor cortex. Responses varied according to the orientation of the coil: in the latero-medial position, single peak responses similar to those from the PT were obtained; their latencies confirmed direct excitation of CM cells, and there were no later peaks. In the postero-anterior orientation, responses had longer latencies and consisted of two to three subpeaks. At least under the conditions that we have tested, the results provide no positive evidence for transmission of cortical excitation to upper limb motoneurons by non-monosynaptic pathways in the macaque monkey.

INTRODUCTION

The corticospinal tract represents the major output pathway linking the motor areas of the cerebral cortex to the spinal cord.

The tract makes widespread terminations throughout the spinal gray matter, including direct cortico-motoneuronal (CM) connections to spinal motoneurons (Armand 1982; Armand et al. 1997; Dum and Strick 1996; Kuypers 1981). CM connections appear to be a feature unique to primates and are particularly well developed in the more dextrous species (Bortoff and Strick 1993; Maier et al. 1997; Nakajima et al. 2000; Porter and Lemon 1993). However, the termination of many corticospinal fibers in the spinal gray matter beyond the motor nuclei suggests that the corticospinal tract may control motoneurons through other, non-monosynaptic pathways operating in parallel with the CM pathway (Lundberg 1992).

One such possible pathway is the C_3 – C_4 propriospinal system, which has been extensively studied in the cat and which has been shown to transmit corticospinal excitation to forelimb motoneurons (Illert et al. 1978; see Alstermark and Lundberg 1992). The basic organization of the CM and propriospinal systems is shown in Fig. 1. In the cat, the C_3 – C_4 system provides an important integrative system for motor commands originating from the cortex, superior colliculus, red nucleus, and reticular formation (Alstermark and Lundberg 1992; Baldissera et al. 1981).

Recent investigations by Maier et al. (1998) and Nakajima et al. (2000) found that transmission of corticospinal excitation to upper limb motoneurons via C_3 – C_4 propriospinal neurons in the chloralose-anesthetized macaque monkey was rather uncommon. These studies emphasized major differences between species in the organization of the corticospinal system: we pointed out that in the macaque monkey, with a well-developed CM system, C_3 – C_4 propriospinal excitation of motoneurons was uncommon, whereas in the squirrel monkey, with rather a weak CM projection, such excitation was stronger and more common. We also speculated that in humans, particularly strong and ubiquitous CM connections may have subsumed some of the key functions carried out by the propriospinal system in other species such as the cat. However, the situation remains uncertain because the animal studies were all carried out under general anesthesia, which may selectively enhance transmission through direct pathways while oligosynaptic transmission may be depressed. Indeed one explanation for the paucity of propriospinal-like effects in the chloralose-anesthetized macaque has been that the relevant C_3 – C_4 interneurons

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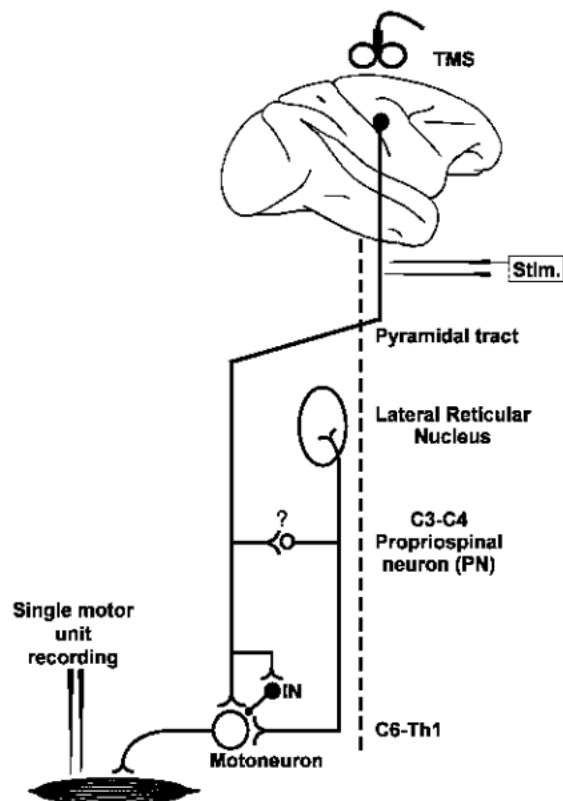


FIG. 1. Testing corticospinal projections in the macaque monkey. Highly schematic diagram of the corticospinal projections influencing cervical motoneurons in the macaque monkey and the methods used to study them. Projections originating in cortical motor areas cross the midline (---) and project to the cervical enlargement (segments C₆–Th₁) where they make direct monosynaptic connections with motoneurons and with inhibitory interneurons (IN). There is also the possibility (?) that corticospinal collaterals in the C₃–C₄ segments excite propriospinal neurons (PN). These PN project monosynaptically onto motoneurons and also have an ascending axon collateral to the lateral reticular nucleus in the brain stem. The corticospinal system was activated either via stimulating electrodes implanted in the medullary pyramidal tract (Stim) or noninvasively using transcranial magnetic stimulation (TMS). Effects on motoneurons were assessed by recording of single motor units from a variety of upper limb muscles.

are under some form of inhibition in this preparation (Alstermark et al. 1999).

In the experiments reported here, we have recorded the responses of single motor units (SMUs) in hand and arm muscles to single stimuli delivered directly to the corticospinal tract via electrodes chronically implanted in the medullary pyramidal tract (PT; see Fig. 1). Because recordings were made from monkeys in either the awake or lightly sedated state, any effects of general anesthesia were avoided. To enhance transmission through oligosynaptic pathways, we also tested double PT stimuli. In addition, we compared responses of the same SMU to PT stimulation and to transcranial magnetic stimulation (TMS) over motor cortex, using either a latero-medial coil orientation, which directly excites corticospinal neurons or a postero-anterior orientation, which excites them indirectly (di Lazzaro et al. 2001; Werhahn et al. 1994). These results can be compared directly with studies carried out in human subjects (de Noordhout et al. 1999; Pierrot-Desilligny 1996).

All SMUs tested showed single, brief response peaks to both PT stimulation and TMS in the lateromedial orientation. Later peaks, possibly resulting from non-monosynaptic actions were

not seen even with double PT stimuli. Thus it would appear unlikely that the lack of non-monosynaptic excitation revealed in our previous studies of the macaque monkey (Maier et al. 1998; Nakajima et al. 2000) was the result of depression by general anesthesia. The findings offer no positive evidence for the transmission of cortical excitation by such pathways in the macaque and indicate that this form of excitation may play only a minor role in the cortical control of upper limb motoneurons, at least under the conditions we have been able to test.

A preliminary account of these results has been published (Lemon et al. 2000).

METHODS

Monkeys

Recordings were taken from three purpose-bred female macaque monkeys. In one *Macaca nemestrina* (monkey 29, 6.0 kg body wt) recordings were made from intrinsic hand muscle SMUs during performance of a precision grip task. Further recordings were made from two *M. mulatta* (monkey 32, 5.3 kg and monkey 35, 5.0 kg) under light sedation with ketamine (5 mg/kg im).

Behavioral task

The precision grip task (Lemon et al. 1986) required the monkey to position its thumb and index finger on two fixed levers about 25 mm apart and to exert a steady isometric force of around 1.0 N on each of them. The forces exerted were registered by sensitive strain gauges attached to the levers. A tone indicated to the monkey when both finger and thumb forces were in target; successful trials were rewarded with small pieces of fruit. In some sessions, thenar muscle surface electromyographic (EMG) activity was rectified and smoothed and used as a biofeedback signal to control the "in target" tone signal; the levels were then set so as to encourage the monkey to adopt a contraction level appropriate for steady discharge of the sampled SMU.

Implant of PT-stimulating electrodes

Each monkey underwent an operation for implantation of a stainless steel headpiece (for head fixation) and pyramidal tract stimulating electrodes. Surgery was carried out after induction with 10 mg/kg ketamine intramuscularly and maintained with 2–2.5% isoflurane in a 50:50 mixture of O₂:N₂O. Two fine epoxy-insulated tungsten wire electrodes (tip impedance, 10–20 kΩ at 1 kHz) were positioned in the medullary pyramids, at stereotaxic coordinates A3 and P2 and 1.0 mm from the midline. Their location was confirmed during the implant by stimulating and recording the antidromic field potential from the surface of the ipsilateral motor cortex. The final position of each electrode was adjusted to obtain the lowest possible threshold for the antidromic volley (range, 20–30 μA). After surgery, antibiotics (20 mg/kg im terramycin LA, Pfizer, Sandwich, Kent, UK) and an analgesic (buprenorphine hydrochloride 5–10 μg/kg im Vetergesic, Reckitt and Colman, York, UK) were administered. Monkey 29 underwent a terminal experiment under deep general anesthesia (see Maier et al. 1998 for details) during which it was confirmed that stimulation through the PT electrodes evoked a large orthodromic volley in the corticospinal tract, recorded from the surface of the dorsolateral funiculus. At the end of the experiment, monkeys were killed by an overdose of general anesthetic (pentobarbital sodium, Nembutal, 80 mg/kg ip) and perfused through the heart. The location of both electrode tips in the PT was confirmed histologically in all three monkeys. All procedures were carried out under appropriate licences from the UK Home Office.

Experimental protocol

AWAKE MONKEY. EMG and SMU recording. A pair of surface EMG electrodes was placed over the thenar muscles. For SMU recordings from adductor pollicis (AdP), the method described by Lemon et al. (1990) was adopted. In brief, pairs of fine (25 μm) nylon-insulated microwires (Karma wire, Californian Fine Wire) were cut across at the tip to produce a selective recording electrode (tip impedance typically 1 M Ω at 1 kHz) and introduced into the AdP muscle using a single sterile 27 gauge needle, which was then immediately removed. Signals were amplified and high pass filtered at 1 kHz using Neurolog modules (Digitimer, Welwyn Garden City, UK) and sampled at 10 kHz using a CED 1401plus interface (CED, Cambridge, UK). In most experimental sessions, it was possible to record stable SMU action potentials during the hold period of the task (Lemon et al. 1990). The precise orientation of the manipulandum was adjusted until steady discharge (20–30 imp/s) of one clearly discriminable SMU was obtained.

PT stimulation. At the beginning of each recording session, the monkey's head was restrained by metal bars fixed to the implanted headpiece. A constant current stimulator was connected to the electrodes through a fast relay device, and PT stimuli of 0.2-ms duration and up to 250- μA intensity were delivered during the steady hold period of the task. The terminal experiment in this monkey demonstrated that with the electrode configuration used, an intensity of around 250 μA was necessary to activate all the fastest corticospinal fibers. This was shown by saturation of the fast-conducting volley with stimuli above this intensity (see Maier et al. 1997).

Transcranial magnetic stimulation. A small figure-eight coil (outside diameter of each coil, 70 mm) was rigidly fixed with its central region over the motor cortex contralateral to the performing hand. Stimuli were delivered using a Magstim 200 stimulator (2.2 T maximum magnetic field; Magstim, Dyfed, UK). Initially, the central region of the coil was positioned such that initial current flow in the coil was from lateral to medial (LM orientation); current induced in this way is most effective for direct activation of corticospinal neurons in motor cortex hand area (see Werhahn et al. 1994). The final coil position was adjusted to optimize the amplitude of the short-latency thenar EMG response. Recordings were also made with the coil center positioned over the same point but with the central region orientated such that the initial coil current flowed from posterior to anterior (PA orientation), which activates corticospinal neurons indirectly (Werhahn et al. 1994). Just before TMS delivery, the relay device connected to the PT electrodes was activated so as to isolate them and prevent induction of currents through them.

Timing of stimuli relative to SMU discharge. The timing of stimuli was manually controlled on a trial-by-trial basis: whenever the SMU of interest had been discharging at a steady rate for a few hundred milliseconds, TMS was delivered at a rate of 0.3 Hz until the SMU stopped firing. Interleaved with TMS, single stimuli were delivered to the PT at a rate of 2 Hz, beginning 500 ms after the first TMS pulse and ending 500 ms before subsequent TMS pulses. In this way, the effect of TMS and PT stimulation could be tested on the same segment of SMU activity. Between 50 and 200 stimuli at a given intensity were tested (PT or TMS). For some SMUs, it was possible to test a range of TMS intensities.

SEDATED MONKEYS. SMU recordings. These were made from muscles acting on the thumb (adductor pollicis, AdP and abductor pollicis brevis, AbPB), wrist (extensor carpi ulnaris radialis, ECR) and elbow (biceps), using a 26-gauge needle bipolar electrode containing two 50- μm insulated stainless steel wires, each having an impedance of around 0.5–1 M Ω at 1 kHz (Bawa and Lemon 1993). With the low dose of ketamine given, there was considerable muscle tone present, and the steady firing rate of the selected SMU could be maintained by either cutaneous or proprioceptive manipulation (brushing or squeezing the skin, bending joints, etc). Throughout each experimental run, the precise form and amplitude of the action potential was carefully

monitored using a digital storage oscilloscope and audio amplifier to ensure that records were made from the same SMU.

PT stimulation. Stimuli (duration, 0.2 ms) were delivered at 1 Hz while the SMU was discharging at a regular rate. Intensities of between 40 and 150 μA were tested. In *monkey 35*, SMUs were tested with both single and paired PT stimuli (inter-stimulus interval 3 ms).

Analysis

The difficulty of obtaining stable recordings from SMUs is well recognized, especially in awake behaving animals. We took particular care to ensure that our analysis was based on reliable recordings from only one SMU. We used custom-written spike software (Getspike, SN Baker) to discriminate the potentials generated by a single unit. This process first displayed five sets of superimposed potentials, discriminated by crossing a preset voltage/time threshold and selected at random from five different sections of the total spike train (Fig. 2A). The similarity of the discriminated waveforms in this display was then confirmed using principle component analysis and cluster cutting techniques (Eggermont 1990) to select the population of waveforms most likely to belong to a given SMU (Fig. 2B). We then examined the interval histogram of the discriminated events that this analysis generated (Fig. 2C) to confirm that there were no physiologically implausible short intervals: the presence of such intervals is a clear indication that events from more than one SMU have been discriminated. Peri-stimulus time histograms (PSTHs) of SMU discharge were constructed for TMS and PT stimuli, using a 0.2-ms binwidth (Fig. 2E). Each sweep was also checked by hand to ensure that the SMU of interest could be clearly identified (Fig. 2D). We included in the analysis only those sweeps in which there was steady discharge of the unit for the last 200 ms before stimulus delivery (see Fig. 3A); this ensured that the excitability of the motoneuron was comparable across all stimulation protocols. The discharge times of all identified SMU discharges in the period 50 ms before and after the stimulus were measured either by hand or using Spike2 software (Cambridge Electronic Design). We measured the entire duration of the response peak in the PSTH (not its half-width); the onset and offset of the peak were determined from the cumulative sum (CUSUM) (Ellaway 1978) (see Figs. 3–5). Mean latencies and response peak durations are given together with $\pm$ SD.

RESULTS

Responses to PT stimulation were recorded in 56 SMUs that met all the strict criteria for well-discriminated SMUs. Nine AdP SMUs were investigated in the awake monkey (*monkey 29*); eight of these were also tested with TMS (7 responded to LM and 6 to PA orientation). In the sedated monkeys, a further 9 SMUs were recorded from either AdP (6) or AbPB (3) in *monkey 32*, as well as 13 SMUs from ECR and 9 from biceps. In *monkey 35*, 16 SMUs were recorded from ECR.

Responses to PT stimulation

COMPARISON BETWEEN THE AWAKE AND SEDATED CONDITIONS. Figure 3A illustrates a single-sweep recording from the awake monkey. It shows delivery of a single PT stimulus during steady discharge of an AdP SMU at around 20 imp/s. The PSTH (binwidth of 0.2 ms) and CUSUM of this unit's response to 104 PT stimuli are given in Fig. 3B. The inset shows five superimposed sweeps of the responding SMU to illustrate the stability of the recording. The stimulus intensity (100 μA , 0.2 ms) was adjusted so as to produce a low response probability ($P = 0.16$). The response consisted of a single, brief peak with

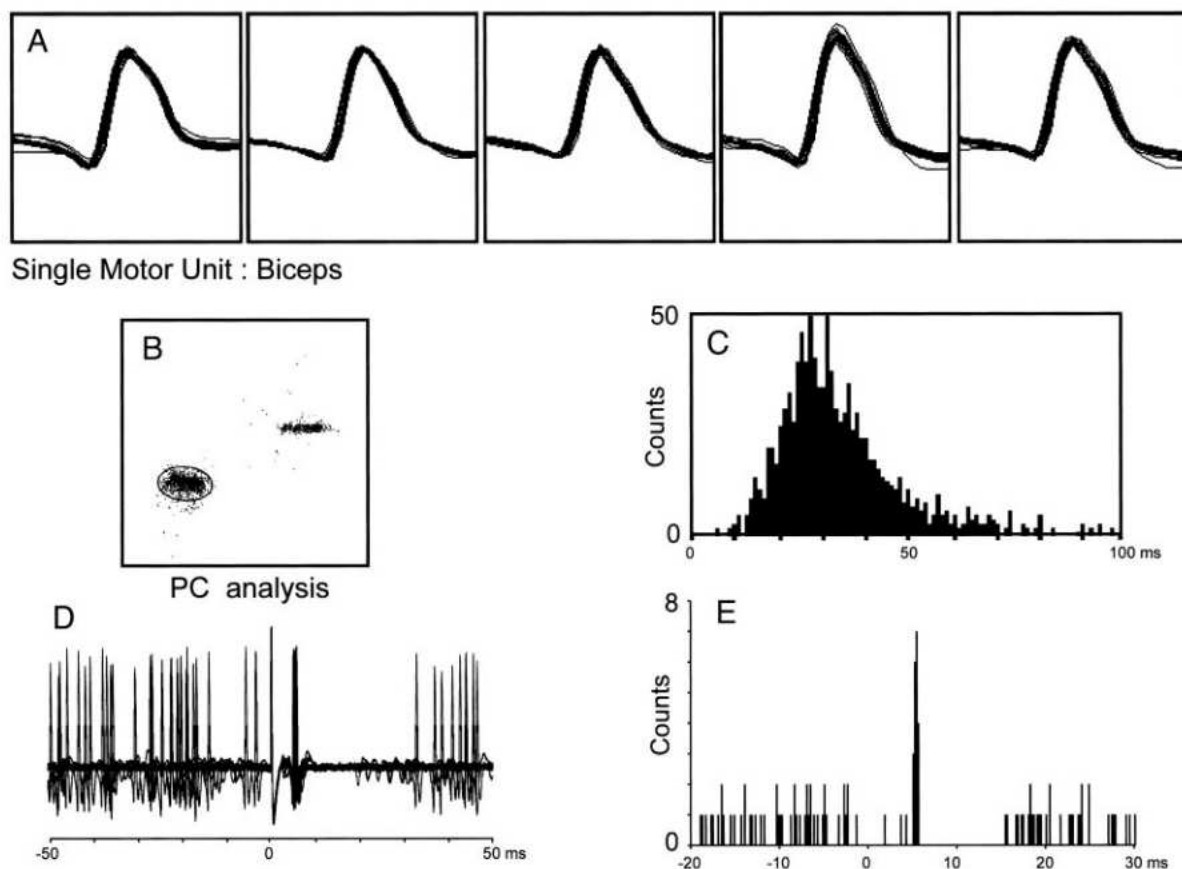


FIG. 2. Method used for discrimination of single motor units (SMUs). *A*: 5 sets of superimposed motor unit action potentials (MUAPs) recorded from a biceps SMU in a sedated monkey. MUAPs were discriminated using a time-voltage threshold. These MUAPs are taken from different sections of a 312-s-long recording of the SMU and were selected at random. Note the reproducibility of the MUAPs throughout. *B*: principle component (PC) analysis of the waveforms to identify clustering of MUAPs from the same SMU. In this case, the 1st principle component has been plotted against the peak-to-peak voltage of the potential; this plot allowed complete discrimination from the SMU (circled) from other recorded signals (mainly stimulus artifacts, at upper right). The circled cluster was admitted for analysis. *C*: interval histogram of the SMU's discharge. Note absence of spuriously short intervals (less than 10 ms). *D*: superimposition of 20 sweeps showing response to pyramidal tract (PT) stimulation (at time 0, 85 μ A). Note that the SMU was firing spontaneously before PT stimuli were delivered and these evoked a highly synchronized short-latency response at 5 ms. *E*: peri-stimulus time histogram of the SMU to 120 PT stimuli (85 μ A). Binwidth, 0.2 ms; response probability, 0.17.

an onset latency of 12.0 ms. The duration of the peak was only 0.8 ms. There was no evidence of later excitatory peaks. The CUSUM shows that there was no suppression after the initial peak that might have masked any later peaks.

Figure 3*C* shows the response of an SMU in another thenar muscle (AbPB), but this time recorded in a monkey under light sedation (monkey 32). The recording stability was better than in the awake monkey, and this allowed a larger number of stimuli ($n = 444$) to be tested. This SMU fired at around 30 imp/s during PT stimulation. Once again, the PSTH shows a single peak with a sharp onset latency and brief duration (1.2 ms), with a low response probability ($P = 0.12$), and there was no sign in the CUSUM of any postpeak suppression. Comparison of Fig. 3, *B* and *C*, suggests very similar responses in both the awake and sedated state for thenar SMUs. There was no significant difference in the mean duration for the nine thenar SMUs tested in the awake monkey and for the eight tested in the sedated monkeys (0.93 ± 0.40 and 0.86 ± 0.21 ms, respectively, t -test, $P = 0.31$). The distribution of peak response durations in all SMUs is shown in Fig. 7, *A* (awake) and *B* (sedated). The onset latencies for the responses were on average slightly shorter in the sedated monkeys (11.1 ± 2.0

ms) than in the awake one (13.0 ± 1.7 ms), a difference that was just significant (t -test, $P = 0.046$).

SMU RESPONSES IN DIFFERENT MUSCLES. Examples of SMU recordings taken from biceps, ECR and AbPB are shown in Fig. 4, *A–C*, respectively. All were recorded from the same monkey (32) under sedation. The PT stimulation intensity (50–55 μ A) was again adjusted to give a low response probability (~ 0.1). The responses all have a very similar form: a single, brief peak at a fixed latency. In both the biceps and ECR units, the responses peak was followed immediately by a period of suppression. The mean duration of the response peak for all thenar SMUs (0.91 ± 0.32 ms, $n = 17$) was significantly longer than that for ECR + biceps (0.66 ± 0.17 ms, $n = 35$, t -test, $P < 0.001$). All SMUs had a similar low response probability (mean: 0.13 ± 0.01 , $n = 56$). Mean onset latencies were 12.0 ± 2.0 ms for thenar muscles ($n = 18$), 7.8 ± 1.9 ms for ECR ($n = 29$), and 6.2 ± 0.9 ms for biceps ($n = 9$). These mainly reflect differences in peripheral conduction distance from spinal cord to the sampled muscles.

Of the 56 SMUs recorded, 20 (11 thenar, 8 ECR, 1 biceps) showed no evidence of any suppression of discharge after the

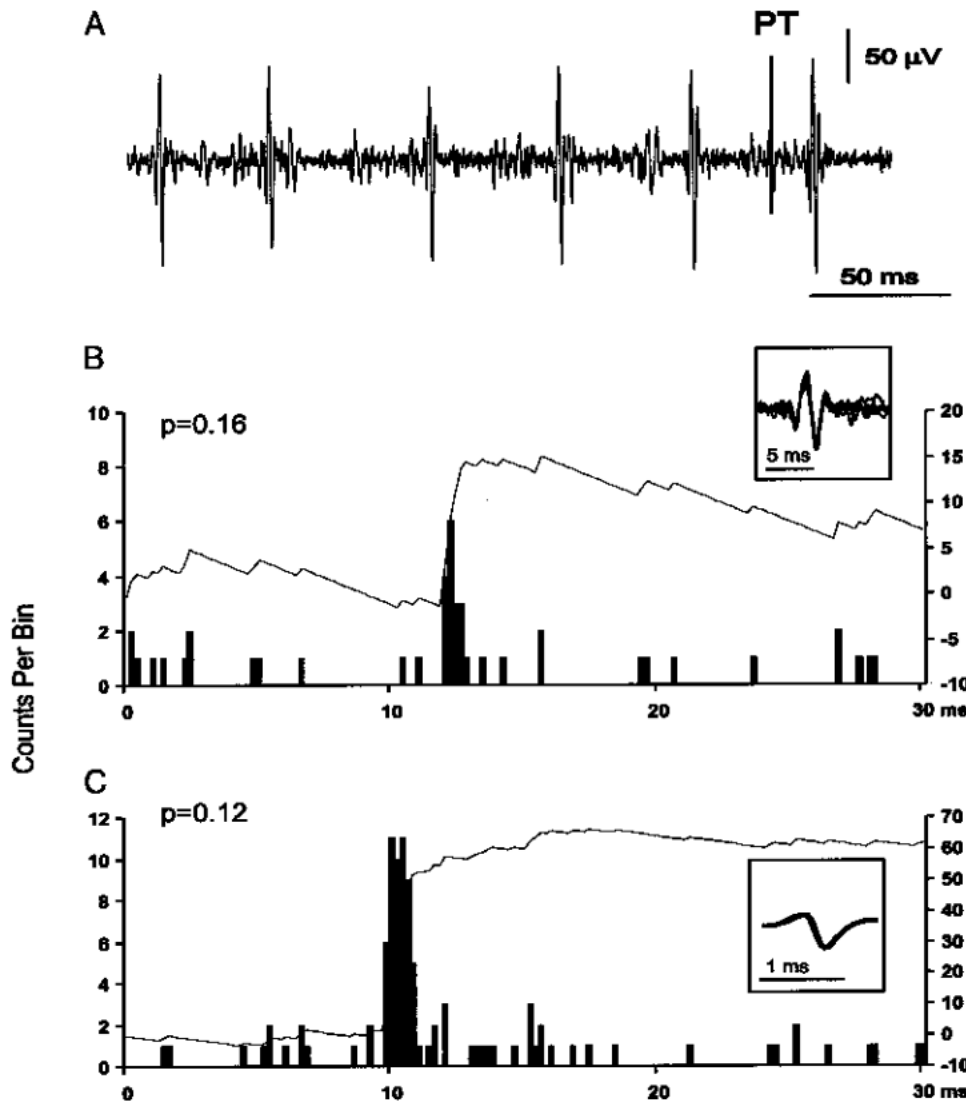


FIG. 3. Comparison of SMU responses to PT stimulation in awake and sedated monkeys. *A*: thenar muscle (AdP) SMU recorded in a monkey while performing the precision grip task. This SMU was firing at about 20 Hz when the PT stimulus ($100 \mu\text{A}$; duration, 0.2 ms) was given (vertical dotted line). *B*: peri-stimulus time histograms (PSTH, filled bins) computed from the same SMU in response to PT stimulation ($n = 104$). Binwidth: 0.2 ms; peak onset latency: 12 ms; duration: 0.8 ms, response probability $P = 0.16$. PSTH scale (counts per bin) is shown on the left. *Inset*: 5 superimposed MUAPs from the SMU. The cumulative sum (CUSUM) is superimposed on the PSTH; scale on the right. *C*: PSTH for a thener (AbPB) unit recorded in another monkey under light ketamine sedation ($n = 444$). Binwidth: 0.2 ms; PT intensity: $50 \mu\text{A}$; peak onset latency: 9.8 ms; duration: 1.2 ms, $P = 0.12$.

main peak. The responses in these SMUs also consisted of single peaks of brief duration (mean: 0.8 ± 0.24 ms) with no evidence of later peaks. In the remaining 36 SMUs, the initial response peak was followed by suppression of discharge lasting between 3 and 35 ms, which could have masked subsequent excitatory peaks.

EFFECT OF PT STIMULATION INTENSITY. Figure 5 shows responses of an AbPB SMU recorded in a sedated monkey in which the effects of different intensities of PT stimulation were tested. The background discharge rate of the SMU was around 12 imp/s; the threshold for excitation of this SMU lay somewhere between $40 \mu\text{A}$ (Fig. 5*A*, no clear response) and $50 \mu\text{A}$ (Fig. 5*B*, response present). The response was stronger at the higher intensities used (55 and $75 \mu\text{A}$; Fig. 5, *C* and *D*, respectively), but the duration of the peak was unchanged. Weak suppression of SMU discharge was already present at $40 \mu\text{A}$ (Fig. 5*A*) and became steadily more prolonged with higher intensities. Similar results were obtained in a further 14 SMUs tested with two or more intensities.

RESPONSES TO PAIRED PT STIMULI. In the anesthetized preparation, it is necessary to use repetitive PT stimuli to ensure transmission of corticospinal excitation to motoneurons

through the oligosynaptic propriospinal pathway (Illert et al. 1978; Maier et al. 1998; Nakajima et al. 2000). We reasoned that even without the depressive effect of general anesthesia, single PT stimuli might be ineffective in bringing significant numbers of PNs to discharge, in which case no PN-mediated late excitation of SMUs would be seen. We therefore tested 16 ECR SMUs recorded in monkey 35 with pairs of PT stimuli, using an inter-stimulus interval of 3 ms. PSTHs from two SMUs are shown in Fig. 6, *A1* and *A2*, and in Fig. 6, *B1* and *B2*, respectively. A number of interesting features can be discerned: the SMUs showed a clear response to both the first and second stimulus with identical latency. There was no evidence for either broader or additional peaks after the second stimulus: the duration of the peaks was identical. Finally, the response to the second stimulus had a higher probability than the first. This occurred despite the postpeak suppression of discharge that occurred after a single stimulus; this is particularly noticeable for the SMU shown in Fig. 6*B*. For the 16 ECR SMUs tested with double PT stimuli, there was no significant difference between the duration of the first and second response peaks (means 0.61 ± 0.2 and 0.65 ± 0.19 , respectively, paired *t*-test, $P = 0.57$). The distribution of second peak durations is shown

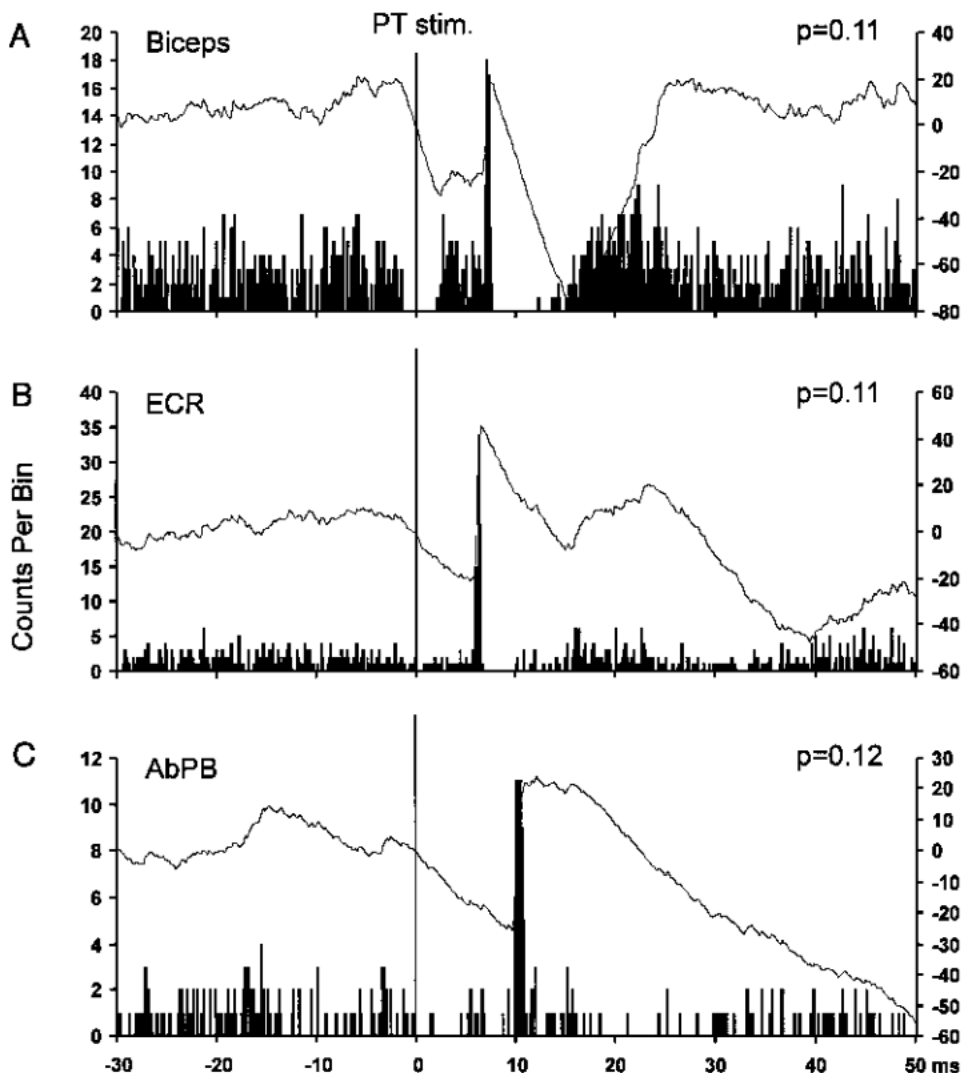


FIG. 4. Responses to PT stimulation in SMUs from different muscles. PSTHs (left-hand scale) and CUSUMs for 3 SMUs recorded from biceps (A), extensor carpi radialis (B; ECR) and abductor pollicis brevis (C; AbPB) from the same monkey under light sedation. PT intensity: 55 μ A, $n = 243$ (A), 50 μ A, $n = 621$ (B) and 50 μ A, $n = 444$ (C). Note pronounced suppression of SMU discharge after the initial peak in A and B. Bin-width: 0.2 ms. Stimulus artifacts prevented SMU discrimination close to the PT shock; this particularly affected the PSTH in A.

in Fig. 7C. The mean probability of the first and second peaks was 0.12 ± 0.06 and 0.13 ± 0.05 , respectively.

Responses to TMS

Figure 8 shows the responses of an AdP unit recorded in the awake monkey during performance of the precision grip task. This SMU was tested with several different intensities of TMS with the coil in the LM orientation. The SMU fired at a steady rate of about 30 imp/s for several hundred milliseconds before delivery of TMS. The PSTHs all show a short single response peak at just under 12 ms, followed by a suppression of spontaneous SMU discharge. At the lowest intensity tested (14% maximum stimulator output, MSO), response probability was low ($P = 0.2$), but a clear peak with a duration of 1 ms can be seen (Fig. 8A). Raising the intensity steadily increased the response probability (Fig. 8D), which rose to 0.55 at 16% (Fig. 8B) and to 0.78 at 20% MSO (Fig. 8C). There were no changes in either latency or duration of the response over the range 14–25% MSO (Fig. 8, E and F). This result was repeated for all five SMUs tested with different intensities of TMS.

Comparison of responses to TMS and PT stimulation

Figure 9 compares responses of the same AdP unit to TMS and to direct stimulation of the PT (100 μ A). The latter produced a single, brief response peak with a sharp onset latency of 10.9 ms and short duration of 0.6 ms (Fig. 9A). The response probability of this peak was low (0.15), and there was no evidence for later excitatory peaks nor of any obvious postpeak suppression. TMS in the LM orientation also produced a single, brief response with a duration of 1.0 ms (Fig. 9B). This response had an onset latency of 11.2 ms, 0.3 ms longer than that from the PT (dashed vertical line). Suppression was evident after this initial peak. With the coil in the PA orientation (Fig. 9C), a quite different pattern of response was observed, with three separate peaks, each having a duration of around 1–1.4 ms. The first peak had an onset latency of 14 ms i.e., 2.8 ms later than that in the LM orientation. The third peak was followed by suppression of discharge.

Figure 9D summarizes the findings for those AdP units tested with both TMS and PT. To remove variation in latency across SMUs due to differences in the location of the intramuscular electrode (see Lemon et al. 1990), the timing of the responses was normalized to that obtained with PT stimulation,

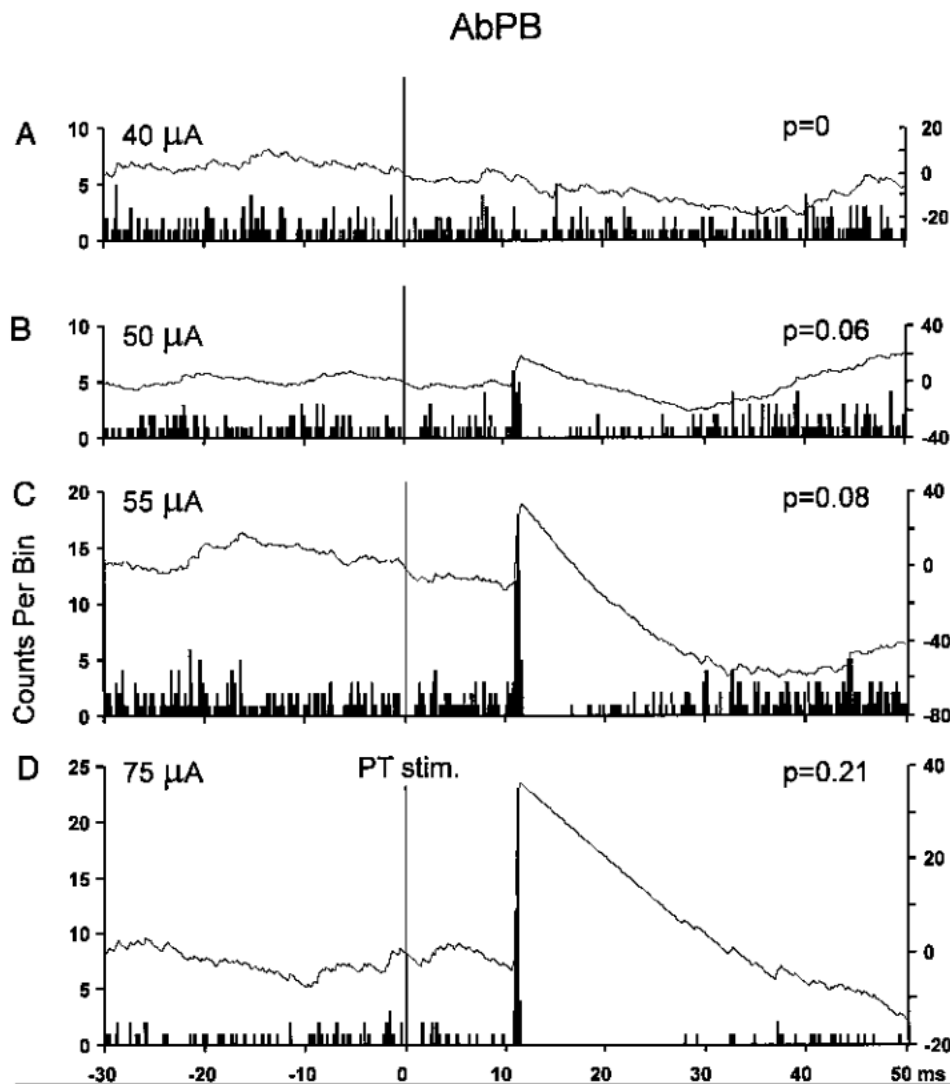


FIG. 5. Effect of PT intensity on SMU response. PSTHs (left-hand scale) and CUSUMs for an AbPB unit recorded in a sedated monkey during application of PT stimuli at four different intensities. There was no excitatory response at 40 μ A (A), and higher intensities at 50 μ A (B), 55 μ A (C), and 75 μ A (D) produced steadily higher response probabilities (P). Number of stimuli A: 385; B: 386; C: 611, and D: 197. Binwidth: 0.2 ms.

by subtracting the onset latency to PT stimulation from the TMS response latency. Thus the PT response would appear at 0 ms in Fig. 9D. All SMUs responding to TMS ($n = 7$) showed a single brief peak with the LM orientation, and there was little difference in the duration of the excitatory response compared with that from the PT (PT: 0.93 ± 0.4 ms; TMS: 1.05 ± 0.34 ms). The TMS responses occurred, on average, 0.5 ± 0.2 ms later than that from the PT. In contrast, the PA orientation ($n = 6$ responding SMUs) usually produced a much more variable response pattern, with 1, 2, or 3 response peaks, which had longer latencies and total duration than the LM response. For the PA orientation, the mean latency difference from the PT response was 3.9 ± 1.1 ms. The interval between the different subpeaks, when present, was around 1.5 ms. The threshold for evoking responses for the PA orientation was always higher than that for the LM response; for example, Fig. 9 shows that an intensity of 16% in the LM orientation produced a similar level of response probability to 40% in the PA orientation.

DISCUSSION

The important conclusion resulting from this study is that macaque upper limb SMUs show brief response peaks to both PT stimulation and TMS (LM orientation) consistent with

monosynaptic, cortico-motoneuronal activation of the parent motoneuron. These SMUs showed no sign of any later peaks that might be expected if there were significant transmission of corticospinal excitation of motoneurons through non-monosynaptic pathways. Our sample of SMUs offers the unique opportunity of comparing responses to TMS with those evoked from direct stimulation of the PT. Interpretation of the motor effects produced by TMS is often complicated by its repetitive activation of the corticospinal system. It generates a succession of direct and indirect waves (Day et al. 1987; di Lazzaro et al. 1998; Edgley et al. 1997; Rothwell et al. 1991), and it is therefore difficult to be certain about the origin of motor responses or reflex facilitation that occur after the initial, presumably monosynaptic component. Such later responses could result from the later arrival of I waves from the corticospinal tract, as has been widely assumed (Day et al. 1989; Olivier et al. 1995), but could also be due to non-monosynaptic excitatory action mediated by propriospinal neurons (see Pauvert et al. 1998).

In this study, we found SMU responses were highly consistent from one SMU to another, and the response characteristics of units in thumb muscles, a wrist extensor (ECR), and elbow flexor (biceps) to be remarkably similar. Responses to TMS

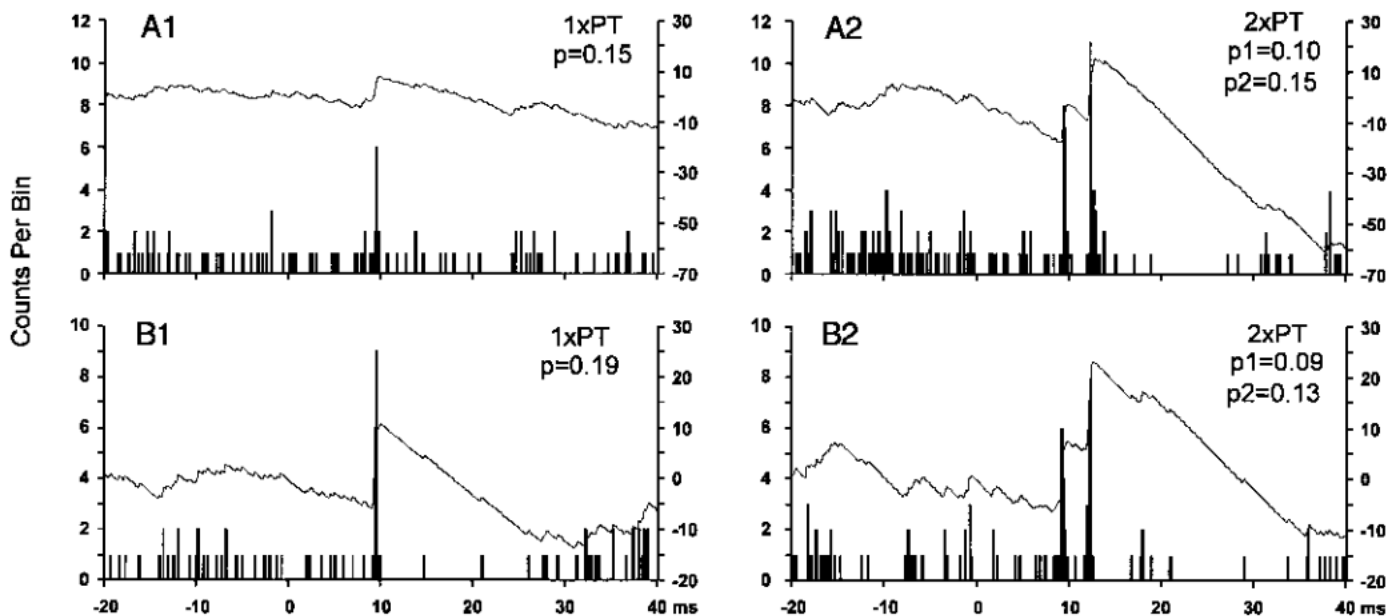


FIG. 6. Effect of repetitive, double-pulse, PT stimulation on SMUs in ECR. Responses of 2 ECR units (*A* and *B*) recorded in a sedated monkey to either single ($1 \times$ PT) or double ($2 \times$ PT) stimuli, with an inter-stimulus separation of 3 ms, to promote transmission through oligosynaptic pathways. Number of sweeps *A1*: 58, *A2*: 150, *B1*: 83, *B2*: 138. Binwidth: 0.2 ms.

varied between simple, single peaks similar to those evoked from the PT, to more complex responses consisting of several subpeaks. We argue that all of these responses are probably due to CM action.

Responses to PT stimulation

Direct stimulation of the PT produces a relatively simple corticospinal volley, discharging each corticospinal axon once only, compared with the repetitive discharge evoked by surface stimulation of the cortex, intracortical microstimulation or non-invasively with transcranial magnetic (TMS) or electrical (TES) stimulation (Burke et al. 1990; Day et al. 1987; Edgley et al. 1990, 1997; Jankowska et al. 1975; Kemell and Wu 1967). Thus PT stimulation allows us to examine the response of upper limb motoneurons to a much less complex input than is generated by TMS or TES; this can in turn illuminate the likely origin of responses to noninvasive stimulation.

The most plausible explanation for the single peaks of short duration evoked from the PT is that they result from monosynaptic excitatory postsynaptic potentials (EPSPs) evoked in the motoneurons by CM connections. A high proportion of macaque upper limb motoneurons receive a monosynaptic EPSP from single stimuli delivered to the PT, and these are particularly common and large for intrinsic hand muscle motoneurons (Fritz et al. 1985; Maier et al. 1998; see Porter and Lemon 1993). Maier et al. (1998) found that in the chloralose anesthetized macaque monkey, 73% of upper limb motoneurons showed such monosynaptic EPSPs; their sample included motoneurons supplying elbow, wrist, and hand muscles. The lack of variation in the SMU response latency and its brief duration (less than 1.1 ms in 52/56 SMUs, mean: 0.74 ms) are both consistent with a monosynaptic origin (Fetz and Gustafsson 1983; Kirkwood 1995). The duration of these peaks probably reflects the brief rise time of CM EPSPs in hand and forearm motoneurons (0.93 ± 0.18 ms) (Maier et al. 1998). If

is also similar to or less than the duration of peaks observed in cross-correlations between CM cells and SMUs (Lemon and Mantel 1989; Mantel and Lemon 1987; see Porter and Lemon 1993).

The PSTHs obtained provide no evidence for later non-monosynaptic excitation from the PT, at least under the present experimental conditions. It would be expected that non-monosynaptic inputs, mediated either through a C_3 – C_4 propriospinal system or through segmental excitatory interneurons, would either broaden the initial response peak or even result in later separate subpeaks of activity. Maier et al. (1998) did find a small proportion of motoneurons that showed non-monosynaptic EPSPs after repetitive PT stimulation. These EPSPs had latencies 0.7–2.5 ms longer than the earliest CM monosynaptic EPSPs (see also Alstermark et al. 1999). Therefore it could be predicted that any SMU discharges due to such non-monosynaptic effects should be clearly discriminable from the early, brief monosynaptic peak.

There are a number of reasons why non-monosynaptic actions may have been missed in the present experiments. First, it could be argued that the sample of motor units tested was dominated by those from more distal muscles and that we may have missed propriospinal-mediated excitation in more proximal muscles. There is evidence in the cat that such excitation may be particularly directed to more proximal muscles concerned in the act of reaching rather than to more distal muscle groups involved in movements such as food-taking (see Alstermark and Lundberg 1992). However, it is also essential to note that although behavioral and other evidence has implicated the C_3 – C_4 system in reaching *all* cat forelimb motoneurons exhibit corticospinal excitation via C_3 – C_4 PNs, including those supplying distal muscles. In support of this, stimulation of the lateral reticular nucleus (which has been shown to activate ascending axons of C_3 – C_4 PNs; see Fig. 1) evokes large EPSPs in most cat forelimb motoneurons, and some of the largest effects were in ECR motoneurons (Alstermark and

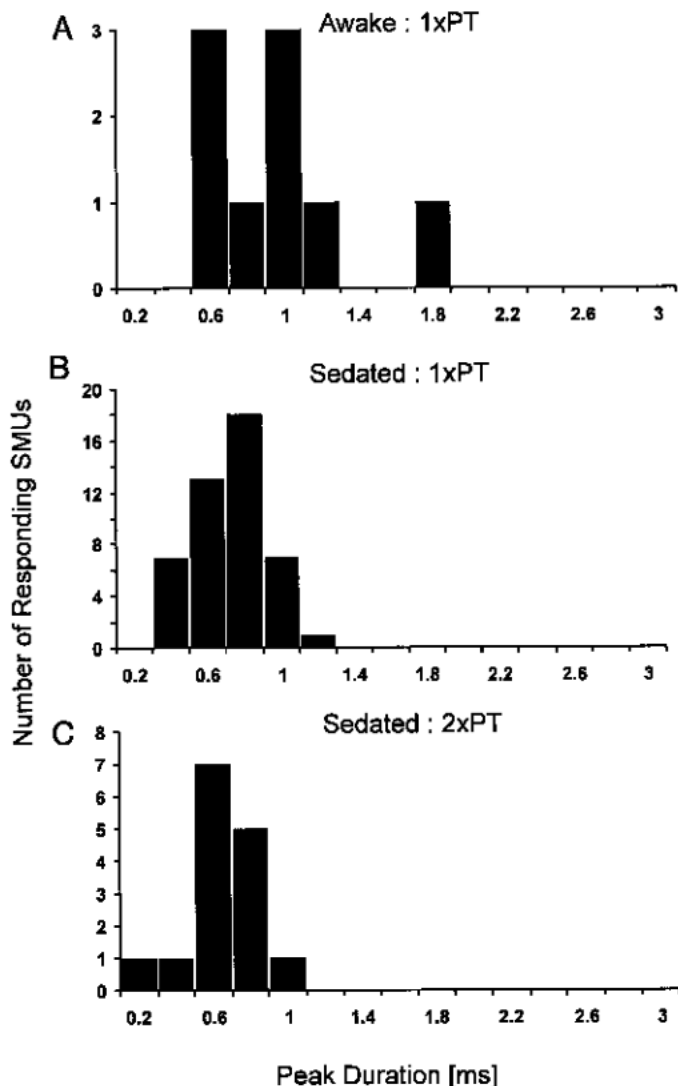


FIG. 7. Duration of response peaks in macaque SMUs to PT stimulation. Distribution of response peak duration (in ms) for 9 SMUs recorded in the awake monkey with single stimuli (A) and for SMUs recorded in 2 sedated monkeys and tested with either single (B; 46 SMUs) or double PT stimuli (C; 15 SMUs).

Sasaki 1986). Thus if a C_3 – C_4 propriospinal system is similarly organized in the macaque monkey, we should expect PN-mediated effects in most motoneurons.

Second, it is possible that neither single- nor double-pulse PT stimulation is capable of bringing C_3 – C_4 PNs to discharge, preventing transmission through to the tested motoneurons. In the deeply anesthetized preparation, repetitive stimulation with up to three or four shocks is sometimes necessary to obtain C_3 – C_4 transmission. However, in an awake or lightly sedated animal, one would expect the PNs to be more excitable and therefore able to transmit effects evoked by even single PT shocks. We should add that in both the chloralose-anesthetized cat and squirrel monkey, transmission through PNs was clearly evident after double shocks (see Illert et al. 1976, Fig. 2; Nakajima et al. 2000, respectively).

Third, there is the issue of whether the fast monosynaptic CM-EPSPs dominated the response of the motoneuron and that discharges resulting from these EPSPs prevented any response to later EPSPs mediated by non-monosynaptic pathways. To

avoid this, we specifically selected a rather weak PT stimulation intensity so that the response probability was low (Figs. 3–6). Under these circumstances, there should be a disproportionately large number of sweeps (80–90%) in which the early CM-EPSP fails to fire the motoneuron but would summate with any later oligosynaptic EPSPs to discharge it at a longer latency (cf. Malmgren and Pierrot-Deseilligny 1988). We should add that primate PN-mediated EPSPs, when they do occur, have rise times similar to those in the cat (Maier et al. 1998; Nakajima et al. 2000).

Finally, it is possible that PT-evoked inhibition could mask non-monosynaptic excitation (see Maier et al. 1998). Such inhibition could arise either from local, segmental inhibitory interneurons (Jankowska et al. 1976; Perlmuter et al. 1998) or indeed from propriospinal inhibitory neurons (Alstermark et al. 1999) with corticospinal input. In this respect, the results from the 20 SMUs in which there was no clear postpeak suppression is of critical importance: examples of such responses are shown in Figs. 3, B and C, 5B, and 6A. In these cases, any significant non-monosynaptic effects should have been detectable, but none was observed.

In other SMUs we did observe suppression of ongoing SMU activity shortly after the initial excitatory peak (Figs. 2E, 4, A and B, 5, C and D, and 6C). However, in some of these SMUs, CUSUM analysis indicated that suppression did not begin until 1–4 ms after the peak (e.g., Fig. 4C), i.e., it was not present during the 0.7- to 2.5-ms period after the initial peak in which non-monosynaptic effects would be expected and therefore could not have masked any later peaks. Further, robust responses to double PT stimuli proved that the inhibitory postsynaptic potential (IPSP) induced by the first volley was not sufficiently large to obscure responses to the second (Fig. 6); however, this might not apply to rather a small and weak oligosynaptic input.

The present results are in keeping with recent investigations by Maier et al. (1998), who, in chloralose-anesthetized monkeys, made intracellular recordings from a large number of upper limb motoneurons in response to PT stimulation. Late EPSPs reflecting oligosynaptic transmission were rarely observed. Repetitive PT stimulation was used to facilitate transmission through non-monosynaptic pathways but only 19% of motoneurons showed late EPSPs and only 3% had EPSPs with a disynaptic latency, appropriate for a C_3 – C_4 propriospinal linkage (see Alstermark and Lundberg 1992). A C_5 lesion was made to reduce the corticospinal input to the lower cervical segments, with the aim of reducing segmental excitatory and inhibitory actions and thereby revealing any propriospinal actions. However, even after such a lesion, the proportion of disynaptic EPSPs was still small (14%). Maier et al. (1998) provided a number of arguments to show that their results were not due to selective depression of non-monosynaptic pathways by general anesthesia. The absence of late peaks in both the awake and lightly sedated monkey reinforces that conclusion. The use of repetitive (double-pulse) PT stimulation, which should have enhanced propriospinal transmission, also failed to produce any evidence of later peaks (Fig. 6). If the C_3 – C_4 system is indeed under some form of inhibition, as suggested by recent experiments by Alstermark et al. (1999), then our data indicate that transmission through this system remains suppressed even in the awake state.

Our results raise fundamental questions as to the functional

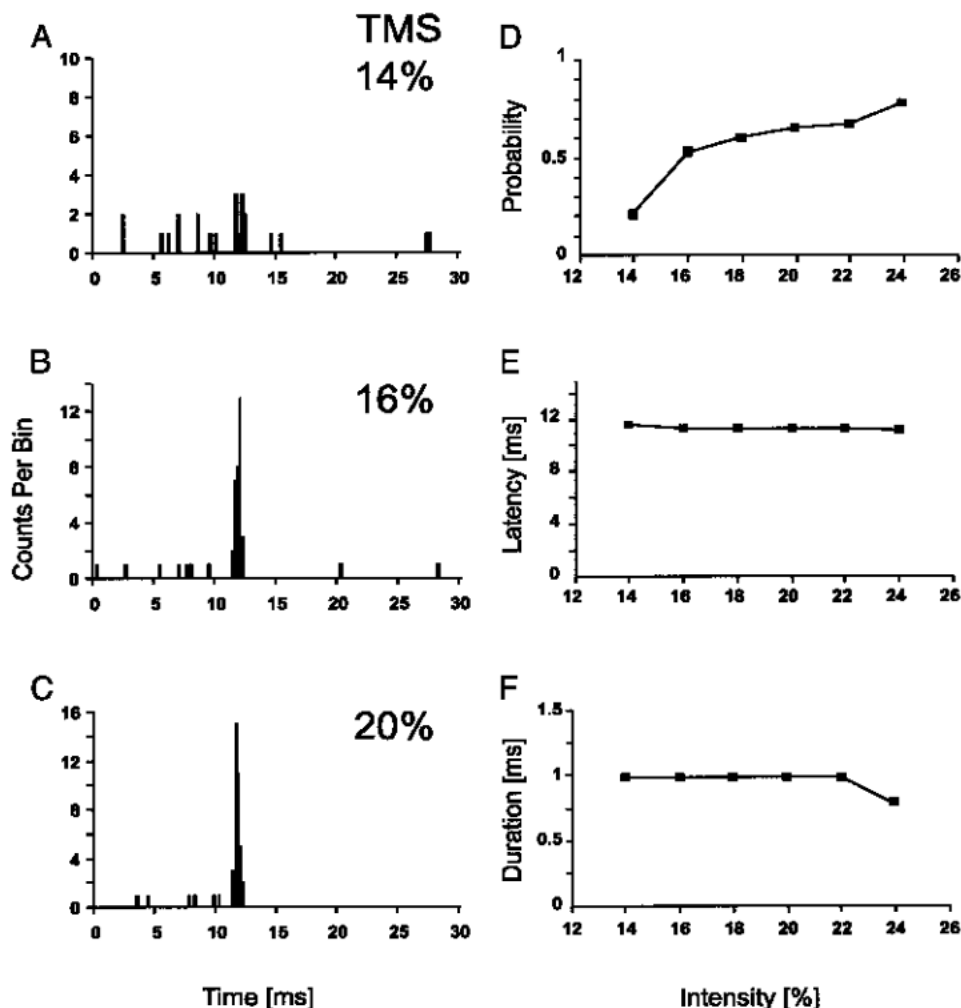


FIG. 8. SMU responses to TMS in the awake monkey. *A–C*: PSTHs of responses of an AdP unit recorded during the hold period of the precision grip task to TMS applied with the coil in the lateromedial (LM) orientation using intensities of 14 (*A*), 16 (*B*), and 20% (*C*) maximum stimulator output (MSO). Binwidth: 0.2 ms. *D–F*: relationship between TMS intensity (% MSO) and peak probability (*D*), onset latency (*E*), and duration (*F*) in the PSTH computed for 6 different intensities tested for this SMU.

importance of C_3 – C_4 transmission under natural conditions. Of course we cannot exclude the possibility that such transmission might occur under different experimental conditions than we have tested here. There are plentiful examples in the literature of pathways that are patent only under particular behavioral conditions. For instance, disynaptic excitation of external intercostals motoneurons by muscle spindle afferents during inspiration (Kirkwood and Sears 1982), disynaptic excitation of flexor and extensor motoneurons during fictive scratching (Degtyarenko et al. 1998), or during particular phases of locomotion (McCrea 1998; McCrea et al. 1995; Quevedo et al. 2000). Feed-forward corticospinal inhibition of the PN system is likely to be enhanced by strong stimulation (Nicolas et al. 2001); note however that all of our experiments were done with rather weak PT stimuli, which evoked low response probabilities, and that PN-type effects were absent even at the weakest intensities (Fig. 5).

The absence of non-monosynaptic excitation is perhaps surprising given the widespread termination of the corticospinal tract in regions beyond the motor nuclei, i.e., in laminae VI–VIII (Armand et al. 1997). Many of these terminations are probably concerned with pre- and postsynaptic control of reflex inputs; many excite spinal interneurons with inhibitory actions on upper limb motoneurons (Baldissera et al. 1981; Cheney et al. 1985; Jankowska et al. 1976; Rothwell et al. 1984) (see Fig. 1). Inhibitory effects from the PT are widespread: Maier et al. (1998) found that 65% of sampled motoneurons had IPSPs

within the disynaptic range. These IPSPs probably explain the early suppression by PT stimulation of ongoing activity in some SMUs. The presence of this suppression demonstrates that non-monosynaptic pathways other than the propriospinal one were operating effectively under the conditions of our experiments. Recent work in the awake behaving monkey has demonstrated the presence of many segmental interneurons which facilitate upper limb motoneurons (Perlmutter et al. 1998; Prut and Fetz 1999); whether or not these interneurons receive a significant corticospinal input is as yet unresolved. Clearly this approach would be able to clarify the issue of C_3 – C_4 propriospinal transmission still further.

Responses to TMS

The responses of AdP units to TMS in the LM orientation consisted of single brief peaks, identical to those evoked from the PT, but with a slightly longer onset latency (mean: 0.5 ± 0.2 ms). This is similar to the conduction delay from cortex to medullary pyramid in the fastest corticospinal fibers (Baker et al. 1994, 1995) and is therefore consistent with direct activation of corticospinal neurons at or close to their initial segment (Edgley et al. 1997). The SMU responses are most likely the result of the monosynaptic action of impulses in the direct or D wave that can be recorded from the corticospinal pathway (Baker et al. 1994, 1995; Edgley et al. 1990, 1997).

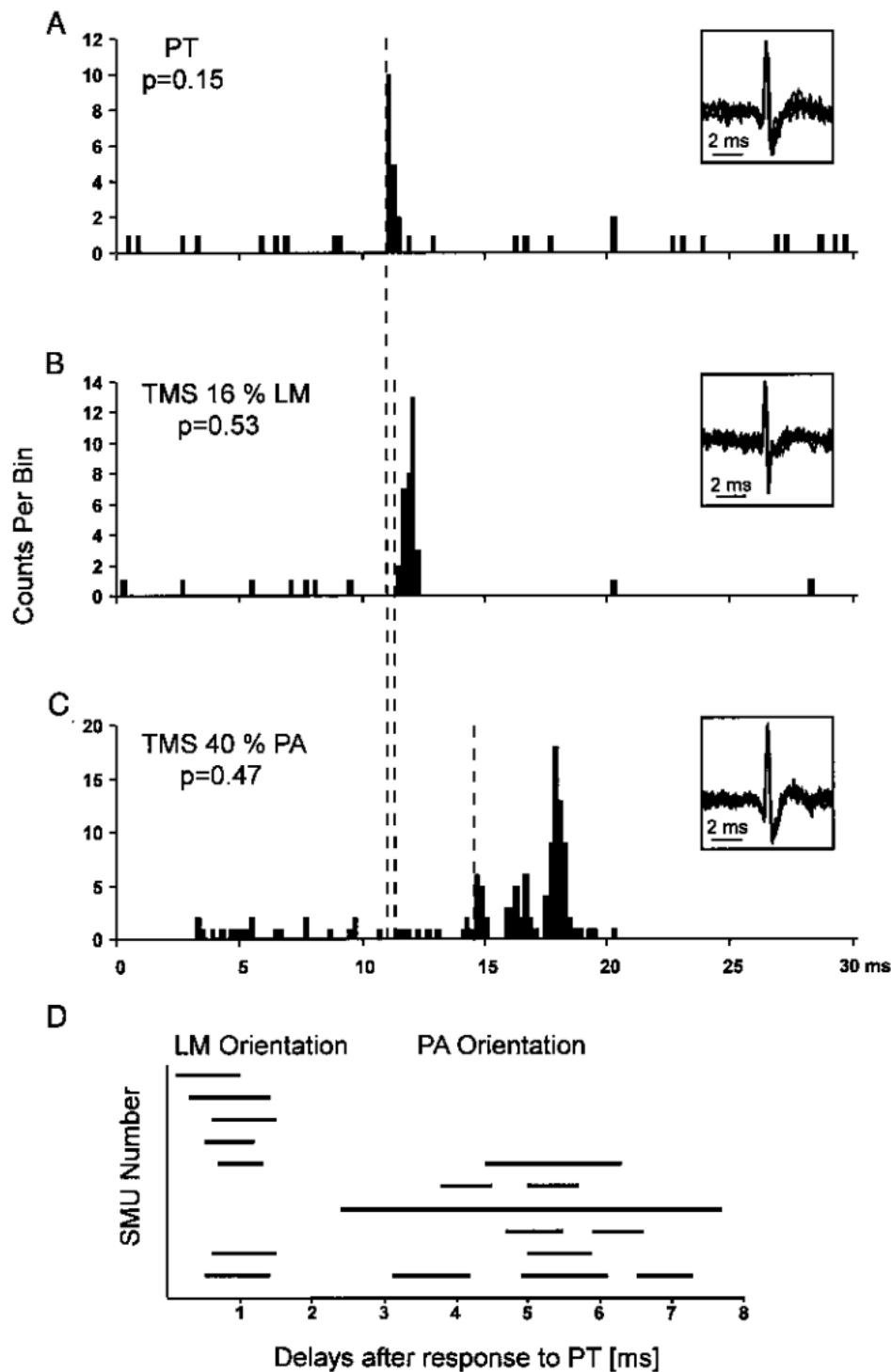


FIG. 9. Comparison of SMU response to PT stimulation and to TMS. Responses in the same rhythmically firing AdP unit responses to PT stimulation and to TMS in the LM and PA coil orientations. *A*: PSTH for PT stimulation (intensity: 100 μ A, duration: 0.2 ms, $n = 117$). Peak onset latency: 11 ms, duration: 0.6 ms, response probability, 0.15, binwidth: 0.2 ms. Note the absence of any later subpeaks in the PSTH following PT stimulation. *B*: PSTH for responses to TMS applied over the motor cortex (LM orientation; intensity: 16% MSO, $n = 55$). Peak onset latency: 11.4 ms, duration: 1.0 ms, response probability: 0.53. *C*: PSTH for TMS (PA orientation; intensity: 40% MSO, $n = 205$). Total response probability was 0.47. Onset latencies of the first, second and third peaks were 14, 15.8 and 17.4 ms, respectively. *D*: horizontal bars indicate latencies and durations of response peaks for SMUs that responded to TMS in either the LM ($n = 7$ responsive SMUs) or PA orientation ($n = 6$). Latencies are plotted relative to the onset of the peak to PT stimulation; this value has been subtracted from the latency to TMS. Note the short relative latency and brief duration of the LM evoked effects, compared with the longer latency, more complex effects of PA responses, which in 3 SMUs showed multiple peaks.

Several earlier studies have shown that the direction of the induced cortical current flow can markedly alter the type of corticospinal activation (Amassian et al. 1990) and the latency and form of the EMG and SMU responses (Amassian et al. 1989; Davey et al. 1994). Werhahn et al. (1994) and di Lazzaro et al. (2001) have shown that when current is induced in the LM direction, there is direct activation of corticospinal neurons and significantly shorter EMG response latencies than for currents flowing in the PA direction, which induces mainly indirect, *trans-synaptic* activation. The precise reason for this

difference probably depends on the orientation the corticospinal cells' soma, dendrites and axon initial segment (Henn et al. 1962) and on the trajectory of their axons within the subcortical white matter with respect to the induced current flow (Amassian et al. 1992).

The similarity in the duration and form of the response peaks after PT stimulation and after TMS (LM orientation) suggests that both activate a rather similar population of the CM cells projecting to the sampled SMU. The absence of later peaks in the response to TMS in the LM orientation means that its

principal effect on these cells, at least in the monkey, is D-wave activation; our earlier work suggested relatively little I-wave activity using this coil orientation (Baker et al. 1995). In contrast, the responses to TMS in the PA orientation had longer latencies and were composed of multiple peaks with interpeak intervals of 1.2–1.5 ms, similar to those reported in many earlier human studies (Day et al. 1989; Olivier et al. 1995; Werhahn et al. 1994) and primate studies (Edgley et al. 1990, 1997; Maier et al. 1997). This interval corresponds exactly with that between successive I waves in epidural recordings from the spinal cord in man (Burke et al. 1993; di Lazzaro et al. 1998) and from recordings of single corticospinal axons in the monkey (Edgley et al. 1997). Thus it is probable that each of the peaks in the PSTH reflects monosynaptic activation of the motoneuron by successive volleys of corticospinal I-wave activity rather than by non-monosynaptic inputs. The presence of later peaks after TMS in the PA orientation demonstrates that the motoneurons remain excitable in this period and again argues against masking of non-monosynaptic excitation by inhibition. For example the probability of discharge in the first subpeak in Fig. 9C ($P = 0.08$) is not very different to that evoked from the PT in Fig. 9A ($P = 0.15$): with TMS, there were subsequent subpeaks, but with the single volley set up by PT stimulation, no later peaks were seen. The experiments with double PT stimuli also confirmed the excitability of SMUs in the postpeak period (see preceding text).

Propriospinal transmission of corticospinal excitation in humans?

Compared with TMS, low-intensity anodal electrical stimulation (TES) of the motor cortex in human subjects produces a much simpler pattern of descending activity that is dominated by direct activation of corticospinal axons (Burke et al. 1990; Day et al. 1987). Recent studies by de Noordhout et al. (1999) using this type of stimulation demonstrated that SMUs recorded from a wide range of human upper limb muscles responded with brief single peaks almost identical to those reported here (mean duration: 0.97 ms), again with no sign of later excitation. This suggests that in man, as in the macaque monkey, direct stimulation of the corticospinal tract does not lead to activation of motoneurons via oligosynaptic pathways. Responses to TES are followed by profound suppression of SMU discharge, and one criticism of the results obtained by de Noordhout et al. (1999) is that propriospinal excitation may have been masked by this suppression. The results from our study showing absence of any clear PN-mediated effects in those SMUs without such suppression suggests that such objections may be unfounded.

The conclusion that propriospinal transmission of cortical excitation is not present in humans is in conflict with the long series of experiments on human subjects carried out by Pierrot-Deseilligny and his colleagues indicating that a significant proportion of cortical excitation may be transmitted through a propriospinal-like system, organized along similar lines to the C₃–C₄ system in the cat (Burke et al. 1994; Gracies et al. 1994; Marchand-Pauvert et al. 2000; Pauvert et al. 1998; Pierrot-Deseilligny 1996). Using cortical TMS alone, these authors have also not reported any longer-latency effects in SMUs that could be attributed to non-monosynaptic pathways. Rather

evidence for non-monosynaptic transmission has been obtained by interacting the descending effects set up by TMS with activation of non-monosynaptic reflex inputs to upper limb motoneurons. These inputs have been generated by stimulating particular peripheral nerves (those without any monosynaptic connections to motoneurons of the tested muscle) or using particular stimulus intensities at which non-monosynaptic reflex effects are present (Pauvert et al. 1998; Pierrot-Deseilligny 1996).

Thus it seems possible that in humans, as in macaque monkeys, propriospinal transmission from the fast-conducting corticospinal system that is activated by TMS, TES, or PT is weak and is not revealed in either EMG or SMU recordings unless the propriospinal neurons are facilitated by additional excitatory peripheral inputs. We should point out that such inputs were probably present in our study, both in the awake monkey performing the precision grip task, and even in the sedated animals, since natural proprioceptive and cutaneous stimuli were applied to sustain steady SMU discharge. Thus facilitation from these sources should have been present. We did not attempt spatial facilitation with electrical stimulation of peripheral nerves; a pathway that transmits only when highly synchronous corticospinal and peripheral inputs generated by electrical stimulation are presented simultaneously probably reflects a rather weak transmission system, which may have little functional relevance for transmission of asynchronous inputs present under natural conditions.

Conclusion

Our experiments do not provide any evidence in favor of non-monosynaptic excitation of macaque upper limb motoneurons from the corticospinal tract but rather serve to emphasize the strength of the CM input. Our results in the macaque, like those of de Noordhout et al. (1999) in humans, do not discount the existence of a C₃–C₄ propriospinal pathway but rather suggest that this pathway may be too weak to be responsible for transmission of a significant proportion of corticospinal input to motoneurons.

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