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Lack of Evidence for Direct Corticospinal Contributions to Control of the Ipsilateral Forelimb in Monkey

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Abstract

Strong experimental evidence implicates the corticospinal tract in voluntary control of the contralateral forelimb. Its potential role in controlling the ipsilateral forelimb is less well understood, although anatomical projections to ipsilateral spinal circuits are identified. We investigated inputs to motoneurons innervating hand and forearm muscles from the ipsilateral corticospinal tract using multiple methods. Intracellular recordings from 62 motoneurons in three anaesthetized monkeys revealed no monosynaptic, and only one weak oligosynaptic excitatory post-synaptic potential following stimulation of the ipsilateral corticospinal tract. Single stimulus intracortical microstimulation of the primary motor cortex (M1) in awake animals failed to produce any responses in ipsilateral muscles. Strong stimulation (>500μA, single stimulus) of the majority of corticospinal axons at the medullary pyramids revealed only weak suppressions in ipsilateral muscles at longer latencies than the robust facilitations seen contralaterally. Spike triggered averaging of ipsilateral muscle activity from M1 neural discharge (184 cells) did not reveal any post-spike effects consistent with monosynaptic corticomotoneuronal connections. We also examined the activity of 191 M1 neurons during ipsilateral or contralateral ‘reach to precision grip’ movements. Many cells (67%) modulated their activity during ipsilateral limb movement trials (compared with 90% with contralateral trials), but timing of this activity was best correlated with weak muscle activity in the contralateral non-moving arm. We conclude that, in normal adults, any inputs to forelimb motoneurons from the ipsilateral corticospinal tract are weak and indirect, and that modulation of M1 cell firing seems to be related primarily to control of the contralateral limb.

Introduction

Many of our everyday actions require the coordinated action of two hands. Much evidence implicates the corticospinal tract, the dominant descending pathway projecting from the brain to the spinal cord in primates, in the control of the contralateral limb. The situation for the ipsilateral limb is less clear: around 40% of corticospinal fibers originate in the primary motor cortex, (M1; (Dum and Strick, 1991)) and terminate mostly in the intermediate and ventral laminae of the cord (Kuypers, 1981). Although the large majority cross at the medullary-spinal junction and descend the contralateral cord, a small fraction (8-10%) do not decussate here but descend ipsilaterally. In addition, contralaterally descending fibers

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have collaterals that re-cross the midline at spinal level, thus also influencing the ipsilateral spinal circuitry (Rosenzweig et al., 2009).

Ipsilaterally descending pathways might play an important role in movement control. Neurons in M1, including pyramidal tract neurons (PTNs), may modulate their activity during both contralateral and ipsilateral movements (e.g. (Matsunami and Hamada, 1980). However, whether the ipsilateral cortex is genuinely involved in controlling ipsilateral muscles, or whether the modulation in activity instead relates to control of contralateral muscles – for example to ensure a stable posture, or to prevent inappropriate contralateral movements, is unclear. Importantly, if they can provide access to limb and hand motoneurons, ipsilateral pathways might provide a substrate for recovery of function following a lesion of the contralateral corticospinal tract (e.g. after motor stroke) (Brus-Ramer et al 2007, Rosenzweig et al 2009), connecting the paralyzed side of the body with an intact cortex capable of effectively relaying voluntary motor commands,.

Anatomical studies do not resolve this issue: many corticospinal projections terminate ipsilaterally in lamina VIII of the spinal cord, a region containing interneurons concerned with control of the axial musculature and many of them commissural (so their axons will cross back to the contralateral side). Reports of terminals in the ipsilateral intermediate zone and ventral horn are more varied: in hindlimb-related segments these terminations have been reported (Lacroix et al., 2004), in cervical cord they are rare (Yoshino-Saito et al., 2010) although some reports describe them (Rosenzweig et al., 2009).

Here we address whether primate ipsilateral corticospinal terminals make corticomotoneuronal connections to forelimb, and especially hand, motoneurons, and whether they influence motor output via more indirect pathways (e.g. involving segmental interneurons). Using electrophysiological recordings in both awake and terminally anaesthetized monkeys, we show that activation of forearm and hand motoneurons by the ipsilateral corticospinal tract is weak or absent. Further, we ask what function modulation of M1 neuron discharge has in ipsilateral hand movements. We show that such modulation is slight compared with that seen during contralateral movements, and most likely related to weak modulations in the activity of contralateral muscles. We conclude that primate ipsilateral corticospinal projections have a quite different function from their more numerous contralateral counterparts.

Methods

All animal procedures were performed under UK Home Office regulations in accordance with the Animals (Scientific Procedures) Act (1986), and were approved by the relevant Local Research Ethics Committee.

Intracellular Motoneuron Recordings

Recordings were made from three female M. mulatta monkeys (monkeys JN, JW and JD. Age: 9 years , weight: 6, 9.2 and 7.6 kg respectively) under terminal anesthesia, using the same methods described in Riddle et al. (2009). Briefly, initial surgical preparation was carried out under deep anesthesia with sevoflurane (3–5% in 100% O_2) and alfentanil (7–23 $\mu g\ kg^{-1}\ h^{-1}$ by IV infusion). A tracheotomy was made, and central venous and arterial lines inserted via neck vessels. Nerve cuff electrodes were implanted around the following peripheral nerves of the right arm: deep radial at the elbow (supplying forearm and digit extensors), median and ulnar nerves in the upper arm (supplying forearm flexors and intrinsic hand muscles), median and ulnar nerves at the wrist (supplying intrinsic hand muscles). Spinal segments C6-T1 were exposed by a laminectomy. The anesthetic regime was then switched to an intravenous infusion of propofol (5–14 $mgkg^{-1}\ h^{-1}$) and alfentanil

(doses as above). To improve recording stability, the vertebral column was clamped at high thoracic and mid-lumbar levels, and the head held in a stereotaxic frame angled to produce ~60° neck flexion. A pneumothorax was carried out to minimize chest movements consequent on ventilation. Neuromuscular blockade was achieved by infusion of atracurium (0.6-1.2 $mgkg^{-1}h^{-1}$). Continuously monitored vital signs included heart rate, arterial and venous blood pressure, blood oxygen saturation, end-tidal CO_2, and core temperature. We verified the depth of anesthesia by ensuring that there were no changes in heart rate or arterial blood pressure in response to peripheral nerve stimulation.

Stainless steel stimulating electrodes insulated with parylene (MS501G, Microprobe Inc), were implanted in both left and right medullary pyramidal tracts (PT) using a double angle stereotaxic technique (Soteropoulos and Baker, 2006), with initial targets A0 ML0.7 DV-6. During electrode placement, antidromic volleys were recorded from epidural electrodes placed over M1 bilaterally (craniotomy centered at A18, ML13), and orthodromic volleys from the cord dorsum. Electrode location was optimized to yield an ipsilateral, but no contralateral M1 response at 300μA stimulating current. The indifferent electrode was a silver wire electrode inserted under the scalp.

Intracellular recordings were made from spinal motoneurons using glass micropipettes broken to a tip of approximately 0.5 μm (tip impedance 3–20 MΩ) filled with 2 M potassium acetate. Motoneurons were identified by antidromic spiking following stimulation through nerve cuff electrodes (intensity 3x motor threshold). Cells were assigned to muscle groups based on the pattern of responses to different cuffs, and known anatomy. Thus, a cell which responded to median nerve at the arm, but not at the wrist, was assumed to project to forearm flexors; response to both median nerve cuffs confirmed projection to intrinsic hand muscles.

Motoneuron responses to ipsilateral and contralateral PT stimulation were recorded to single stimuli and trains of three/four stimuli (300 μA biphasic pulses, 0.2 ms per phase, train frequency 300 Hz, 1 Hz repetition rate). Isolated constant-current stimulators (AM Systems Inc, model 2100) were used to deliver all stimuli. A silver ball electrode on the cord dorsum close to the electrode penetration point recorded surface volleys simultaneously with intracellular potentials. Intracellular waveforms were sampled at 25 kHz (gain 200 or 500, 10 Hz-10 kHz bandpass) via a micro1401 interface (Cambridge Electronic Design, Cambridge, UK) together with M1 and spinal epidural waveforms (12.5 kHz sampling rate, gain 10,000, 30 Hz-5 kHz bandpass) and stimulus markers.

Postsynaptic responses (PSPs) in motoneurons were identified from superimposed single sweep and averaged records. Intracellular potentials were compared with field recordings made just extracellular to the motoneuron to ensure deflections represented genuine intracellular effects. Segmental latencies (SLs) of excitatory PSPs (EPSPs) were measured from the first inflection of the corresponding epidural volley to the onset of the postsynaptic response. Latencies <1 ms were considered to be monosynaptic (Jankowska et al., 2003, Riddle et al., 2009). Response amplitudes were measured from the onset to peak of the EPSP.

At the end of the experiments, stimulating electrode positions were marked with electrolytic lesions (50 μA for 20 s), anesthesia was increased to a lethal level and the animals were perfused through the heart with phosphate buffered saline (PBS), followed by 4% paraformaldehyde in PBS. Brains were removed and, after cryoprotection in 30% sucrose PBS solution, sectioned at 75 μm on a microtome. Sections were mounted and stained with cresyl violet before reconstruction of the location of stimulating electrode tips.

Behavioral Paradigm

Two female rhesus macaques (monkeys T and E, 4 years old, ~6kg) were trained on the precision grip task described in Soteropoulos and Baker (2006). The animal was presented with two precision grip manipulanda for left and right hands. Access to the manipulanda were obstructed by plastic flags. The monkey commenced a trial by placing both hands on homepad switches in front of the flags. After ~500 ms, a 1 s-long audiovisual cue indicated the required movement (left hand only, right hand only or bimanual), chosen at random. After an instructed delay period (0.7-1.3 s), during which the animal kept the hands on the homepad switches, both flags then moved down ('Go Cue') permitting access to the manipulanda. The animal reached out with the correct hand and grasped the levers between finger and thumb in a precision grip. The levers were held above a criterion displacement for 1 s, before being released to obtain a food reward. Motors opposed lever movement simulating the action of springs (force for initial lever movement, 0.15 N; spring constant, 0.03 N/mm). Incorrect movements or premature homepad switch release resulted in a failure tone and termination of that trial. In this report, we analyze only data from the unimanual trials, which are referred to as "contralateral" or "ipsilateral," referring to the side of the moving hand relative to the M1 recording site.

Surgical Preparation for Awake Recordings

All surgical operations were performed under deep general anaesthesia (2–2.5% isoflurane in 50 : 50 O_2 : N_2O) and were followed by a full course of antibiotics (coamoxyiclav 140/35, 1.75 mg kg^{-1} clavulanic acid, 7 mg kg^{-1} amoxycillin, Synulox, Pfizer Ltd) and analgesic (buprenorphine; Vetergesic, 10 μg kg^{-1}, Reckitt & Coleman, Hull, UK) treatment. In an initial surgery, epimysial patch electrodes (Miller et al., 1993) were implanted over the following muscles bilaterally, with wires routed subcutaneously to a connector on the back: first dorsal interosseus (1DI), abductor pollicis brevis (AbPB), abductor pollicis longus, (AbPL) flexor digitorum superifcialis (FDS), extensor digitorum communis (EDC), biceps (Bic), and triceps (Tri). In a subsequent surgery, the monkeys were implanted with a headpiece to allow atraumatic head fixation (Lemon, 1984) and recording chambers (craniotomy center A18 ML13) allowing access to M1 bilaterally. Two insulated tungsten stimulating electrodes (LF501G, Microprobe, Potomac, MD) were chronically implanted in each pyramidal tract (PT) for antidromic identification of pyramidal tract neurons (PTNs; see (Lemon, 1984; Baker et al., 1999).

Awake Recordings

A sixteen channel Eckhorn microdrive (Thomas Recording, Giessen, Germany) was used to make up to 14 simultaneous microelectrode penetrations into M1 during daily recording sessions (average number of electrodes used per session: 9, range 3 to 14). Electrodes were platinum insulated with quartz glass and had a shaft diameter of 80 μm and impedance of 1–2 MΩ (Thomas Recording). Cells were identified as PTNs if they responded at constant latency to stimulation through the chronically implanted PT electrodes (maximum stimulus intensity: 400 μA, 0.2 ms pulse, 1 Hz) and if the evoked spikes could be collided by orthodromic spikes occurring shortly before the stimulus. Cells that could not be so activated were classified as unidentified neurons (UIDs). Single-unit activity (bandpass, 300 Hz to 10 kHz, sampled at 25 kHz) was recorded while the animal performed the task, together with lever displacement and EMG activity (bandpass, 30 Hz to 2 kHz, sampled at 5 kHz). Off-line, action potential waveforms were discriminated to generate the occurrence times of single spikes using custom-written cluster-cutting software (Getspike, SN Baker, Spikelab, (Dyball and Bhumbra, 2003)). Only single units with a consistent spike waveform and no inter-spike intervals <1 ms were used in subsequent analysis.

The hand representation of M1 was identified by multiple pulse stimulation (13 biphasic stimuli, 0.2 ms per phase, 300 Hz train frequency, 1 Hz repetition rate) through the recording electrodes and visual observation of muscle twitches at low (<20 μA) current intensities. In some sessions we recorded the EMG responses to single stimulus intracortical microstimulation (sICMS, 3.3 or 6 Hz repetition rate).

Corticospinal Stimulation in an Awake Behaving Monkey

A third monkey (monkey O, age 5 years, weight 6.5 kg) trained on a similar bimanual task as described above was implanted with two stimulating electrodes in the left medullary PT, rostral to the decussation. Bilateral M1 epidural recordings (25kHz sampling rate, gain 10-20k , 30 to 10KHz bandpass) during the implantation procedure were used to optimize the location of the electrodes so that there was an ipsilateral, but no contralateral antidromic field potential in M1 with a stimulus intensity of 400μA. In later experiments biphasic stimuli (0.1ms duration pulse, 2Hz) were delivered through the PT electrodes, using a DS4 Stimulus Isolator (Digitimer, UK) at two intensities (500μA and 1mA) while the monkey performed the task so that both forelimbs were active during stimulation The same EMGs were recorded as in monkeys E&T, with the exception of FDS and AbPB on the right, and the addition of the lateral deltoid bilaterally.

Data analysis

Stimulus Triggered Averages (SmTA)—Stimulus triggered averages of all ipsilateral and contralateral rectified EMGs were compiled following sICMS (monkeys E&T), covering a period 70 ms before to 70 ms after the stimulus. Because the earliest onset latency was expected to be approximately 4 ms (for Triceps/Biceps muscles), we examined a standardized response region from 4 to 20 ms after the stimulus; as a control region we used 4-20 ms before the stimulus. The time of the maximum value within the response region was found, and the average of the 12 ms-long data section centered on this time found for each stimulus presentation. The control region was similarly processed. To detect significant responses, single sweep mean values from control and response regions were compared with paired t-tests (significance level $P<0.05$).

Any significant responses were further checked by re-compiling the averages, excluding sweeps which included large amplitude artifacts or EMG modulation. This typically rejected <5% of stimuli. Statistical comparisons were repeated; only responses which remained significant in this further analysis are considered here.

Spike Triggered Averages (STA)—For each cell where more than 5000 spikes were recorded, we calculated a spike triggered average (STA, ± 2s) of each rectified EMG to assess the cell's connectivity with motoneuron pools innervating the recorded muscles. Co-modulation of cell firing rate and muscle activity can lead to a non-stationary baseline in STAs. We estimated this baseline by convolving the STA with a Gaussian kernel of unit area and width parameter σ=30 ms; this was then subtracted from the STA (Williams et al., 2009). The STA was then truncated by 60ms on either end to remove the convolution edge effects. The standard deviation (SD) of this baseline-corrected STA was calculated, excluding the middle region within 50 ms of the triggering spike. To detect significant effects, the maximal and minimal values were found within a standard window 3 to 20 ms post-spike. Averages were classified as facilitations or suppressions, depending on whether the maximum or minimum showed the largest deviation from baseline. The number of bins within the 17ms-long response region (total of 85 bins) which were larger (for facilitations) or smaller (for suppressions) than the 2SD level was counted. The rest of the baseline-corrected STA (excluding the middle ±50ms region) was subdivided into a total of 222 sections 17ms long, and the same procedure repeated. If the number of bins in the response

region exceeding 2SD of the mean was larger than or equal to the maximum number found in the control region, this was considered a significant effect ($P<0.0045$).

All significant responses were further examined by re-compiling the averages excluding sweeps with artifacts or other large changes in the EMG; only responses which were still visible in these averages are considered in the results.

Analysis of Cell Rate Modulation—For each cell, activity was aligned to the end of the 1 s-long lever squeeze ('End Hold' event) for ipsilateral and contralateral trials, and a peri-event time histogram (PETH, 20 ms bin width) was generated. Baseline rate was estimated from a period 3-4 s prior to the End Hold event; in this period, the monkey rested both hands on the home pads. The maximal and minimal rate relative to the baseline, and the time at which these occurred relative to End Hold, was measured over the 2.5 s period prior to End Hold.

Latency Regression Analysis—We assessed the temporal relationship between cell firing and muscle activity by measuring the extent to which the latency of the peak of cell and EMG activity was correlated from trial to trial (Schepens and Drew, 2004). The instantaneous firing rate of the cell was estimated by Gaussian convolution ((Baker and Lemon, 2000; Nawrot et al., 2000); kernel with 30 ms width parameter), and the rectified EMG was smoothed using the same kernel. The time of maximum cell firing, and maximum EMG, was measured for each trial over the period 1 s before to 1.2 s after the Go Cue. The linear correlation between these two latencies was measured across trials. A similar analysis was performed for the minimum cell and EMG activity.

In cases where the greatest difference from baseline was a suppression, and the rate dropped fully to zero, we took the first bin in the epoch of interest whose rate dropped below 1Hz as the latency of the response.

Results

Synaptic Responses of Motoneurons to PT Stimulation

Intracellular recording affords a direct window on the functional connectivity of the corticospinal tract with motoneurons. A total of 62 motoneurons were recorded in three monkeys, of which 34 were tested only with a single stimulus; the remaining 28 were also tested with three or more stimuli to the ipsilateral PT (iPT). Example recordings from two different motoneurons are illustrated in Fig. 1AB. Fig. 1A shows the mean intracellular responses in a forearm flexor motoneuron following a single stimulus (300 μA) delivered to the iPT (black traces) and contralateral PT (cPT, grey traces). The cord dorsum (volley) recordings are also shown underneath. The descending volleys produced by iPT and cPT stimulation were of similar amplitudes, confirming that each electrode activated a similar fraction of PT fibers on either side. The intracellular recordings showed a clear EPSP following cPT stimulation, but no response to iPT stimulation. Figure 1B shows the response of a different motoneuron (also projecting to a forearm flexor muscle) following a train of stimuli to each PT. By delivering a train of stimuli, we would expect to produce temporal summation in interposed interneurons, and to potentiate any indirect (di- or oligo-synaptic) responses. Once again, there was a clear response to the cPT; this followed each stimulus, as expected for a monosynaptic response. By contrast, trains of three or four stimuli to the iPT (train of four illustrated) did not elicit detectable synaptic potentials. In this experiment the volley from iPT stimulation was larger than that from cPT stimulation (both with 300μA stimulus intensity).

Figure 1C shows the distribution of sampled motoneurons, divided by the category of the projection muscle (d: distal, intrinsic hand motoneuron, f: forearm flexor, e: forearm extensor), and the maximum number of stimuli tested in a train to the iPT or cPT. Responses were assumed to be monosynaptic if their segmental latency was shorter than 1 ms. No monosynaptic responses were seen from iPT stimulation; by contrast, the great majority of motoneurons (30/38 cells) in all three muscle categories received robust monosynaptic EPSPs following cPT stimulation (Fig. 1C, bars to the right of the dotted line).

In just 2/62 cells weak oligo-synaptic effects could be detected (grey shaded bars in Fig. 1CD) following iPT stimulation. These are illustrated in Fig. 1E&F; one was excitatory, the other inhibitory (segmental latencies 6.1 and 4.1 ms respectively). Figure 1D shows the distribution of the amplitudes of the monosynaptic EPSPs from cPT; for comparison, the amplitudes of the single oligosynaptic EPSP (Fig. 1E) and IPSP (Fig. 1F) found from iPT are marked as grey bars. The ease with which cPT responses could be seen serves to emphasize the indirect, weak and rare nature of any effects from iPT.

As in our previous intracellular recordings (Riddle et al., 2009), we often observed IPSPs following cPT stimulation (20/38 cells); these were always superimposed on the falling phase of monosynaptic EPSPs, making further analysis difficult. Additionally, stimulation of the medial longitudinal fasciculus in the brainstem as part of a different experiment in these animals generated disynaptic EPSPs (as in Riddle et al., 2009). This suggests that our anesthetic regime left spinal circuits sufficiently excitable for disynaptic responses to be observed. The lack of effects from iPT stimulation therefore probably indicates that iPT axons do not generate either mono- or disynaptic responses in motoneurons.

The drawings of histological sections in Fig. 1G show the locations of the stimulating electrode tips in the corticospinal tract of the three monkeys used to gather the above data.

Responses in Awake Behaving Monkeys to M1 Stimulation

We recorded bilateral EMG responses during sICMS delivered to 27 forearm M1 sites in two awake behaving monkeys. During stimulation, animals performed the bilateral behavioral task described in Methods. Figure 2A illustrates the results from a single representative example, in which the stimulus intensity was 30 μA. The EMGs were rectified and the responses normalized relative to the mean EMG level during the pre-stimulus epoch. At this site, the threshold stimulus to elicit visible movements following a train of pulses was estimated as 6 μA. Significant responses were seen in all contralateral muscles except biceps; by contrast, no responses were seen in the corresponding ipsilateral muscles.

Significant contralateral responses were elicited from 23/27 sites tested. The stimulus intensity tested varied from 10-30 μA (mean 18.8 μA); this was 1-5 times greater than the threshold (mean threshold: 8.4μA) to elicit twitches from a train of stimuli (mean 2.9 times). The mean number of stimuli given was 2474 (range:1087 to 6444).

Overall 65 significant muscle responses ($P<0.05$) were seen, six of which were in ipsilateral muscles. However, all six apparent ipsilateral effects (and five contralateral ones) were produced by artifacts in a small number of sweeps, as excluding these sweeps from the average abolished the effect. Although no genuine ipsilateral responses were seen from individual sites, it might be that the effects were too weak to reach significance with the number of stimuli available to average. Accordingly, Fig. 2B presents grand-averaged traces over all 23 sites which showed a significant contralateral response; although these will blur distinctions between sites, they will improve the available signal-to-noise ratio and allow the detection of weak effects, if they are present in many recordings. Although clear effects can

be seen in contralateral forearm and hand muscles (grey traces), no modulation is visible in the ipsilateral EMGs (black traces). Fig. 2C shows the overall incidence of significant effects for each contralateral muscle.

Responses in Awake Behaving Monkeys to PT Stimulation

Because focal M1 stimulation is likely to activate only a small number of PTNs (both directly and indirectly; (Baker et al., 1998)), it is possible that this was insufficient to generate significant responses in ipsilateral muscles, especially if connections are weak or polysynaptic. To test for ipsilateral effects when a large fraction of PT axons are active, we stimulated the PT directly at the medullary pyramids with high stimulus currents in a single monkey. The stimulating electrodes were placed in the left PT rostral to the decussation of the pyramidal fibers, so that they should activate both contralaterally and ipsilaterally descending fibers from the left PT. Recordings were made during the performance of a similar bilateral behavioral task as described in Methods, ensuring that both arms were active during stimulation.

The results are shown in Fig. 3. The two columns of Fig. 3A show stimulus-triggered averages from left (ipsilateral) and right (contralateral) muscles. The grey and black traces correspond to the different stimulus intensities tested (black: 500μA, grey: 1mA). Clear effects can be seen in contralateral muscles following stimulation at 500μA (the lowest threshold for seeing effects in contralateral muscles was 100μA for EDC & 1DI), while in ipsilateral EMGs only a weak suppression is visible, which occurs at a longer latency than the contralateral effects. Figure 3B shows M1 local field potentials evoked by the stimuli, which were recorded simultaneously with the EMG. For a stimulus intensity of 500μA, there was an antidromic response only in the left M1, confirming that there was no current spread to the PT contralateral to the stimulating electrode. This was therefore a purely unilateral activation of the PT. By contrast, when the intensity was increased to 1mA, a small antidromic response was seen in M1 on both sides, suggesting current spread from the electrode tip to both pyramids. Even at such a high intensity however there was no visible facilitation of ipsilateral EMGs. In comparison contralateral EMGs, especially those from more distal muscles, were clearly facilitated. Even when a large number of PT axons are activated, there is thus no evidence for any monosynaptic responses in ipsilateral muscles.

Spike Triggered Averaging

Activity from a total of 211 neurons was recorded from M1 during performance of the behavioral task (monkey T 142 cells (65 PTNs), monkey E 69 cells (43 PTNs)); the activity of 184 cells with 5000 or more spikes was used to perform STA of bilateral EMGs (a total of 2576 STAs). A total of 145 significant post-spike effects (facilitation in 104; suppression in 41) were seen, with twelve occurring in ipsilateral muscles. The nine clearest (most significant) potential ipsilateral effects are shown in Fig. 4A.

Although STA is a powerful method to detect a monosynaptic connection between a cortical cell and motoneurons innervating a given muscle, care must be taken in the interpretation of effects. Significant features can be produced if the triggering cell is synchronized with other cells which make monosynaptic connections to motoneurons, even if the triggering cell makes no connections of its own (Fetz and Cheney, 1980; Lemon et al., 1986; Baker and Lemon, 1998). Such synchrony effects often have an earlier onset latency than possible for a causal influence from cell to muscle, given known central and peripheral conduction delays. Additionally, jitter in the synchronization leads to wider post-spike effects when generated by synchrony than by direct corticomotoneuronal connections; a peak-width at half-maximum (PWHM) larger than 7 ms has been suggested as a criterion to exclude pure synchrony effects (Baker and Lemon, 1998). All of the significant effects seen in ipsilateral

muscles were either too broad or had too early an onset latency to be accepted as evidence for a monosynaptic corticomotoneuronal connection. Some had clear oscillations (e.g. Fig. 4A2&4); it is well known that there is oscillatory synchronization between M1 bilaterally (Murthy and Fetz, 1996a, b; Kilner et al., 2003). In other cases, significant ipsilateral effects were caused by a small number of sweeps containing high-amplitude artifacts in the EMG (five effects, two of which are shown in Fig. 4A8&9). These effects disappeared when the sweeps with artifacts were excluded.

By contrast, effects seen in contralateral EMGs (Fig. 4B) included examples of likely monosynaptic effects (Fig. 4B1,2,4,5,6), based on the PWHM measure (listed to the right of each plot) falling below the criterion of 7 ms. Of the 133 contralateral effects, 19 were excluded as caused by artifacts, and 33/114 had PWHM below 7 ms (mean 4.6ms); 29/33 effects were from PTNs. Figure 4C&D show the grand average of all significant ipsilateral and contralateral effects. Whilst such grand averages loose many of the features of the individual effects, they do reveal clearly that the ipsilateral effects were much broader than those in contralateral muscles. The effect in Fig. 4C had an estimated PWHM of 13.8 ms, and an onset of 0 ms relative to the spike trigger time. These figures are well outside what would normally be considered as a causal post-spike effect.

Modulation of Cells in M1 with Ipsilateral and Contralateral Movements

The activity of 191 cells (104 PTNs) in M1 was recorded for at least 5 trials of the behavioral task performed with each hand. Figure 5A shows the mean PETH, averaged across all PTNs, for ipsilateral (thin line) and contralateral (thick line) trials. Figure 5B presents a similar display for the unidentified cells (UID). Both populations showed a clear modulation in average activity for contralateral trials. Although there were fluctuations in the mean PETH for ipsilateral trials, these were small.

These averages PETHs are useful as summary measures of the population activity; however, they obscure modulations in firing which differ between cells. When we calculated the size of the modulation for single cells, they were larger than the population modulation shown in Fig. 5AB. For contralateral trials, the modulations were 62±39 Hz (mean± STD) for PTNs and 68±52 Hz for UIDs. For ipsilateral trials, the corresponding modulations were 18±10 Hz for PTNs and 24±17 Hz for UIDs. There was no significant difference in modulation between PTNs and UIDs for either trial laterality (unpaired t-test, $P>0.2$).

To assess the fraction of each cell category which modulated with a given trial type, we counted the number of cells where rate deviated from a pre-task baseline period (between 3 and 4 s before End Hold) by more than two standard deviations, in successive 20 ms bins. This is illustrated in Fig. 5C-F, for PTNs and UIDs, and contralateral and ipsilateral trials. Grey plots illustrate bins with a rate significantly lower than baseline, black significantly higher. Fewer cells modulated with ipsilateral than contralateral trials.

For each cell, we counted the number of bins significantly different (by ±2 SD, $P<0.05$) from baseline over the 3 s period prior to End Hold. Since there are 150 bins in this period, we required 13 or more significant bins for the cell to be categorized as significantly modulated ($P<0.05$, binomial correction for multiple comparisons). Figure 5G is a cluster plot of the number of significant bins for each trial type for each cell. Figure 5H presents the fraction of the PTNs and UIDs which were significantly modulated by either ipsilateral, contralateral, or both trial lateralities. The great majority of cells with an ipsilateral modulation were also modulated by contralateral trials (93% for both PTNs and UIDs).

Figure 5I shows the deviation from baseline firing rate for all cells during ipsilateral and contralateral trials. During this task, the majority of cells (PTN: 92%, UID: 90%) showed an

overall increase in rate for contralateral trials – most of the points are to the right hand side of the abscissa zero line. For ipsilateral trials however, there was a larger fraction of PTN cells which had a suppression in their activity as their largest response (relative to baseline, PTN:31% compared to 8% in contralateral trials); the fraction of UID cells which showed a suppression relative to baseline for ipsilateral trials was 25%.

M1 Cell Firing Latency Correlation with EMG Activity

Although cell responses during ipsilateral trials were weak, they were still significant and present in more than half of the M1 cells recorded. It may be that this activity is used to control the movements of the ipsilateral limb. However, another possibility is that it relates to contralateral movements. Although the non-cued hand was required to remain on the home pad during execution of the precision grip task, some weak modulation in EMG was still seen. Representative data (rectified EMG) from a single muscle (left AbPL) is illustrated in Fig. 6A. Although this muscle modulates clearly with left arm trials, there is also a small modulation in activity during trial performance with the right hand (grey shading). Figure 6B presents an average of the rectified EMG from this muscle, aligned on the End Hold task marker. There is a robust modulation during left handed trials (black line), but also a small consistent modulation during right handed trials (grey line; note different scales used for each trace).

To determine whether cell firing was likely to relate to contralateral or ipsilateral muscle activity during ipsilateral trials, we took advantage of trial-by-trial fluctuations in the timing of both cell discharge and EMG (Schepens & Drew, 2004). Figure 7 presents an example of the analysis for a single cell. Figure 7A shows the PETH (black) and mean EMG for the contralateral AbPL muscle (grey). Both traces showed a clear peak around the time of the Go Cue, as expected for the fast ballistic movements of reaching out to the manipulandum and squeezing the levers into target. Figure 7B presents five single trial estimates of these measures. It is apparent that the time of the peak in both cell instantaneous firing rate and rectified EMG amplitude varied from trial to trial. The times of these peaks were measured (arrows in Fig. 7B); Fig. 7C shows the correlation between the EMG and cell peak latencies. There was a strong and significant correlation (r^2=0.69,P<0.001), which persisted even when the four outlier trials were excluded (outside the dotted square). The correlation provides evidence that this cell probably contributes to the control of this muscle.

This analysis was performed for all cells that showed significant rate modulation during ipsilateral trials, for each muscle and laterality of trial. For each cell, we noted the muscle with the largest significant correlation coefficient, as the muscle that the cell was best related to. The results across the recorded cell population are illustrated in Fig. 8A for PTNs, and Fig. 8B for UIDs; black bars correspond to contralateral trials, grey to ipsilateral. The upward projecting bars bars show the mean correlation coefficients for each muscle; the downward going bars show the fraction of cells which had that muscle as the best correlated. For PTNs, 35/68 cells showed a significant correlation with muscle during contralateral trials; by contrast only 4/68 showed a significant correlation with muscle during ipsilateral trials. In all cases, however, the best correlated muscle was always contralateral to the recording site. For UIDs, 23/56 cells had significant correlation with muscles during contralateral trials, in all cases the best muscle was contralateral. Only 9/56 unidentified cells had significant correlation during ipsilateral trials; of these, in two cases the best muscles were ipsilateral (EDC for one cell, and triceps for the other). In both cases the r^2 value was less than 0.2. Overall therefore, in cells where we could detect a timing correlation of spiking with muscle during ipsilateral trials, the best related muscle was contralateral to the recording site in 11/13 cases.

Discussion

How supraspinal motor centers communicate with motoneurons constrains how they operate. Here, we assessed the monosynaptic ipsilateral actions of the corticospinal tract, and measured M1 neuron activity during behavioral tasks to determine ipsilateral contributions to coordinated hand use, and to assess normal connectivity that may be a substrate for recovery of function following lesions. Our data show that M1 activity carried down the corticospinal tract exerts almost exclusively contralateral actions on motoneurons.

Ipsilateral Corticospinal Connectivity

Several potential routes might allow motor cortex to control the ipsilateral limb. Around 10% of primate corticospinal fibers descend ipsilaterally to the spinal cord, some of which terminate ipsilaterally (Rosenzweig et al., 2009) although many decussate before terminating (Yoshino-Saito et al, 2010). Spinal commissural collaterals can arise from contralaterally descending corticospinal tract fibers (Rosenzweig et al. 2009). Anatomical studies describe ipsilateral corticospinal terminations principally in lamina VIII (Satomi et al., 1988; Rosenzweig et al., 2009); Yoshino-Saito et al, 2010), which contains commissural interneurons. This suggests a role in the control of axial muscles, and organizing posture. Ipsilateral terminations outside lamina VIII exist (LaCroix et al., 2004; (Rosenzweig et al., 2009) but are sparse (Yoshino-Saito et al, 2010). The single published illustration of ipsilateral corticospinal terminations among motoneurons (Rosenzweig, 2009, figure 9) shows terminations in the medial (axial) motoneurons caudal in the T1 segment. Thus potential anatomical substrates for ipsilateral corticospinal tract actions exist, but their functionality has not been assessed.

We used several complementary methods to assess direct ipsilateral corticospinal influences on hand and arm motoneurons. Intracellular recordings revealed strong monosynaptic input from the contralateral corticospinal tract, but no effects from the ipsilateral tract, even after stimulus trains. The single excitation we detected during lengthy experiments in three animals was a small poly-synaptic EPSP (4% of mean monosynaptic EPSP size). Anesthesia will have depressed spinal interneuron activity, so we may have underestimated of the frequency of indirect connections. However, as we often observed oligosynaptic IPSPs after cPT stimulation, the level of anesthesia clearly did not completely suppress oligosynaptic effects.

Anesthesia is not a confounding factor in awake monkeys. Results from three different approaches consistently point to the same conclusion. Unilateral stimulation of most corticospinal axons at brainstem level facilitated contralateral muscles, but gave no response, or weak, late suppressions in ipsilateral muscles. Such gross activation of the tract is unphysiological, but weak sICMS should activate circuitry with similar functional outputs, yet it too never elicited ipsilateral responses. Finally, spike-triggered averaging provides information on corticospinal connectivity at single cell level; again, no monosynaptic ipsilateral effects were detected. Compared with the robust monosynaptic input to motoneurons from the contralateral tract, direct ipsilateral actions appear insignificant.

Our failure to detect facilitation of ipsilateral muscles contrasts markedly with the literature in humans using transcranial magnetic brain stimulation (TMS), which reports ipsilateral effects (Wassermann et al., 1994; Ziemann et al., 1999; Eyre et al., 2001; Chen et al., 2003; MacKinnon et al., 2004). Although it is sometimes assumed that these responses are mediated via the ipsilateral corticospinal tract (Eyre et al., 2001), good evidence implicates other descending pathways e.g. reticulospinal tracts (Ziemann et al., 1999), which connect to hand and forearm muscles in primates (Riddle et al., 2009) and are bilaterally organized

(Davidson and Buford, 2006; Davidson et al., 2007). Strong TMS stimuli elicit multiple corticospinal volleys (Edgley et al., 1990) that may be more likely to excite cortico-reticular fibers than weak sICMS. TMS may thus reveal ipsilateral responses via a cortico-reticulospinal route, as proposed for ipsilateral responses generated in cat hindlimb (Jankowska et al., 2005).

The only significant effects of PT stimulation found in ipsilateral muscles were weak suppressions (Fig. 3). These had longer latencies than the facilitations of homologous contralateral muscles, suggesting an indirect pathway. Several possibilities exist. Antidromic activation of corticospinal axon collaterals could lead to suppression in the contralateral hemisphere via a transcallosal route. Whether these collaterals project directly via the corpus callosum is unclear (Catsman-Berrevoets et al., 1980; Matsunami and Hamada, 1984), but even if not they may still activate transcallosal neurons. Alternatively, reticulospinal axons can both suppress and/or facilitate upper limb muscles (Davidson and Buford, 2006; Schepens and Drew, 2006; Davidson et al., 2007). Ipsilateral corticospinal fibers acting through segmental inhibitory interneurons, or contralateral corticospinal fibers acting via spinal commissural interneurones provide further alternatives (Jankowska and Stecina, 2007; Stecina and Jankowska, 2007).

Our findings slightly contrast with the report by Aizawa et al. (1990) that trains of microstimuli delivered to a region of M1 lying between the face and hand representations could elicit ipsilateral hand movements. Our study was focused more medially, in the conventional hand representation. Since ipsilateral responses were not seen following PT stimulation, it is likely that – like ipsilateral responses following TMS in humans – the stimulus trains used by (Aizawa et al., 1990) generated responses indirectly via callosal or reticulospinal pathways. Similarly, Boudrias et al (2010) recently reported activation of ipsilateral muscles following sICMS in supplementary motor area (SMA). Since PT stimulation which activates corticospinal axons from all cortical areas does not generate ipsilateral effects, a probable substrate for the responses to SMA stimulation, given their longer latencies than M1 evoked responses, is reticulospinal pathways. These terminate bilaterally in the spinal cord (Peterson et al., 1975; Davidson and Buford, 2006), can access distal muscles (Riddle et al., 2009; Riddle and Baker, 2010) and receive SMA projections (Keizer and Kuypers, 1989).

Modulation of M1 Discharge with Ipsilateral Movement

As in previous reports (Matsunami and Hamada, 1978, 1980, 1981; Tanji et al., 1988; Donchin et al., 1998; Kermadi et al., 1998; Kazennikov et al., 1999; Cisek et al., 2003), we found discharge modulation in M1 neurons during ipsilateral limb movements. This could serve several functions.

One possibility is that ipsilaterally-modulated discharge is related to control of the contralateral limb. During unimanual movements weak muscle activity can occur in the non-moving hand (mirroring; (Armatas et al., 1994; Mayston et al., 1999); we also demonstrated activity of this type in our experiments (see also Soteropoulos and Baker, 2008). Most cells in which timing of discharge and ipsilateral EMG correlated were nevertheless better correlated with activity in a contralateral muscle, suggesting that mirroring at least partially explains the ipsilateral movement-related modulation. The two neurons (UIDs) that correlated best with an ipsilateral muscle may have correlated better with a contralateral muscle that was not sampled.

Alternatively, the M1 discharge modulation during ipsilateral movements may suppress unwanted movements contralaterally. Corticospinal activity can generate powerful disynaptic inhibition in motoneurons (Jankowska et al., 1976; Kasser and Cheney, 1985).

Patients with proprioceptive loss often develop involuntary movements (pseudoathetosis, (Spitz et al., 2006)), and mirroring of movements is common (Armatas et al., 1994), especially in children (Mayston et al., 1999), suggesting that preventing limb movement requires active, carefully controlled inhibition. More than 30% of M1 PTNs recorded showed a suppression in activity during ipsilateral movements (Fig 5I)

When a hand moves, widespread anticipatory postural adjustments are required to maintain posture. These have been extensively studied in cat, and shown to involve both the reticular formation and M1 (Schepens and Drew, 2006; Yakovenko and Drew, 2009). These postural adjustments are tailored to the situation; during our recordings, seated with the head fixed, they might be expected to be small. Nevertheless, ipsilateral limb movement-related activity may contribute to postural adjustments.

A component of M1 cell discharge during ipsilateral movements may reflect ipsilateral limb control. Although unlikely to exert effects on motoneurons via the ipsilateral corticospinal tract, subtle modulation of spinal interneuron circuits or brainstem pathways is a possibility. It is important to emphasize the likely relative importance of ipsilateral and contralateral M1. Modulation across the population of M1 cells was very small during ipsilateral compared to contralateral movements (Fig. 4AB). For the reach-to-grip movements studied here, ipsilateral M1 is likely to contribute minimally, although we cannot rule out a significant role in more proximal movements (Brinkman and Kuypers, 1973).

Implications for Recovery from Lesion

Our data suggest that ipsilateral corticospinal axons are unlikely to play an important role in mediating the motor command to hand and wrist motoneurons in healthy adult primates. However, during recovery from corticospinal lesion, ipsilateral M1 activity can play an important role (Marshall et al., 2000; Feydy et al., 2002; Nishimura et al., 2007), although this is not always the case (Liu and Rouiller, 1999; Feydy et al., 2002). Recent evidence implicates substantial sprouting of surviving decussating corticospinal axons, both from ipsilateral and contralateral cortex, in functional recovery following spinal hemisections in primates (Rosenzweig et al, 2010). Our data suggest that if direct connections to motoneurons from ipsilateral corticospinal axons contribute to recovery of hand and arm movement after damage, this must occur through the formation of new connections, since we find no pre-existing connections in normal animals. Our own preliminary work suggests that changes in brainstem pathways are important in recovery (Zaaimi et al., 2009), as proposed by Jankowska and Edgley (2006). Better understanding the limitations on the role of ipsilateral pathways in health may enable more rational therapeutic approaches to enhancing their actions during functional recovery.

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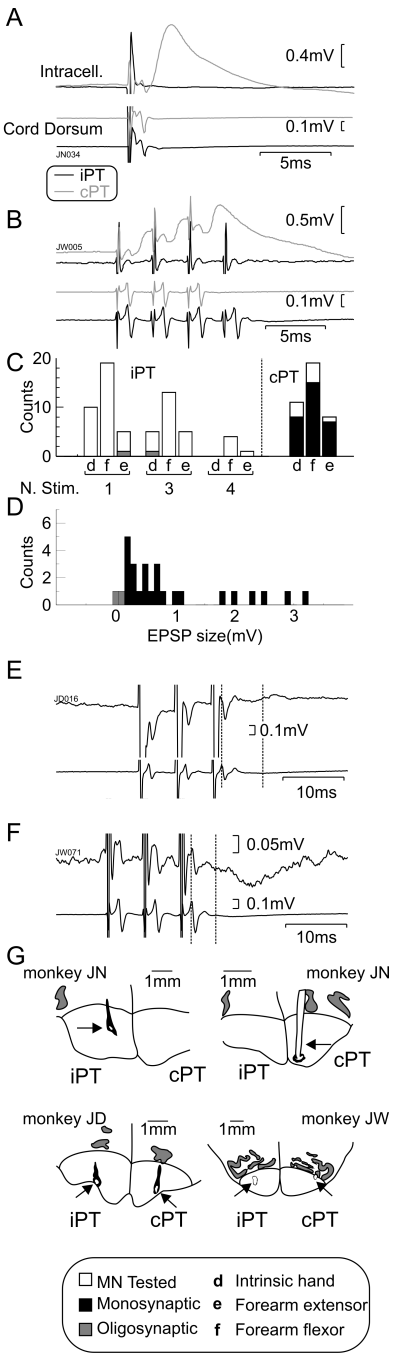

Figure 1. Intracellular Motoneuron responses to Pyramidal Tract (PT) stimulation

A, Example averaged intracellular recordings from a forearm flexor motoneuron in which an EPSP is evoked by a single stimulus to cPT (n=36) but not to iPT (n=37). B, Averaged intracellular recordings from a different forearm flexor motoneuron showing EPSPs evoked by multiple stimuli to cPT (3 stimuli, n=30) but not to iPT (4 stimuli, n=55). C, Histogram showing the types of motoneurons tested with iPT/cPT, and maximum number of stimuli used. Bars to the right of the dotted line correspond to cPT (single stimulus). Grey bars indicate oligosynaptic responses, black bars monosynaptic responses, white bars no responses. D, Distribution of postsynaptic response amplitudes from PT stimulation; black corresponds to cPT effects, gray bars to the iPT effects seen. E, Example of weak

polysynaptic facilitatory response following a train of three stimuli to iPT. F, Example of weak polysynaptic inhibitory response to a train of three stimuli to iPT. G, Drawings showing the locations of tips of PT stimulating electrodes (arrows) reconstructed from histology. In (A,B,E,F), intracellular recordings are shown above cord dorsum records. In E&F dotted vertical lines indicate the arrival of the PT volley to the cord and the measured onset of the response.

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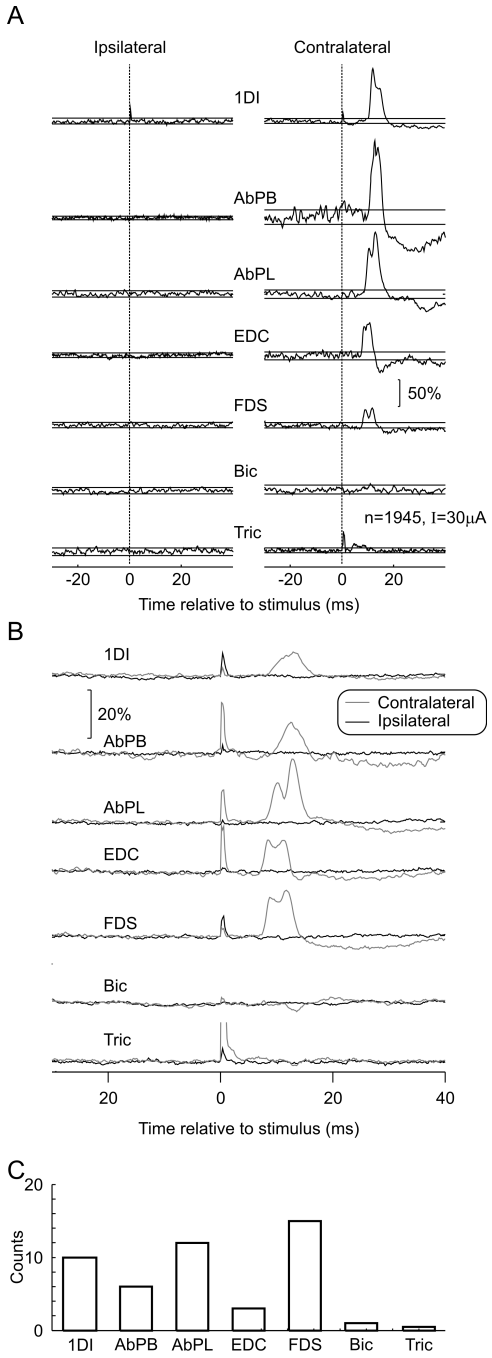

Figure 2. Responses to single-pulse intracortical microstimulation in M1

A, Average EMG responses evoked by sICMS delivered to a single site in M1 (intensity 30 μA) while the monkey was performing the behavioral task. . Traces are normalized as a percentage of the mean baseline level. B. Average across all 23 stimulation sites (black: ipsilateral muscles, grey: contralateral muscles). C, Frequency of effects in different contralateral muscles

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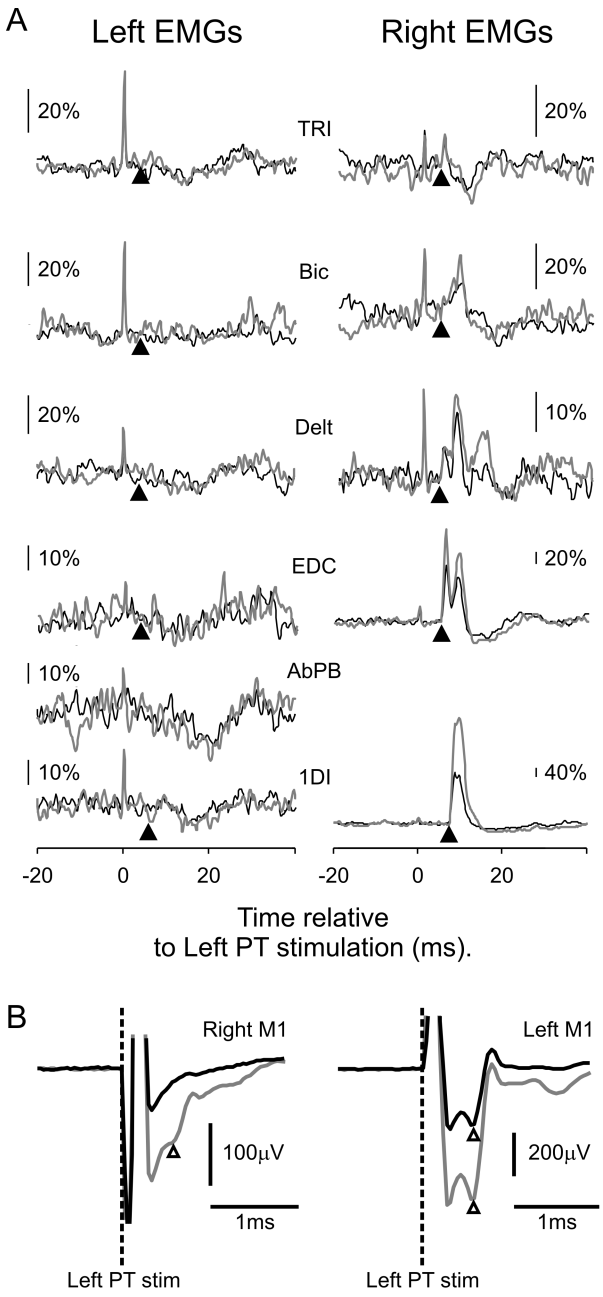

Figure 3. Responses to single-pulse stimulation of the pyramidal tract

A, Stimulus triggered averages of bilateral rectified EMGs, using left PT stimulation at intensities of 500 μA (black) and 1000 μA (gray); n=1511 and 919 respectively. The arrows under each trace indicate the onset latency of the response in that muscle after stimulation on the contralateral side. B, Antidromic field potentials (onsets marked by white arrows) recorded from M1 bilaterally following left PT stimulation (indicated by dotted lines). Note that 500 μA stimuli evoked a response in left M1 only (black traces) whereas stimulation at 1000μA (grey traces) also elicited a small response on the left side indicating stimulus spread to the contralateral PT.

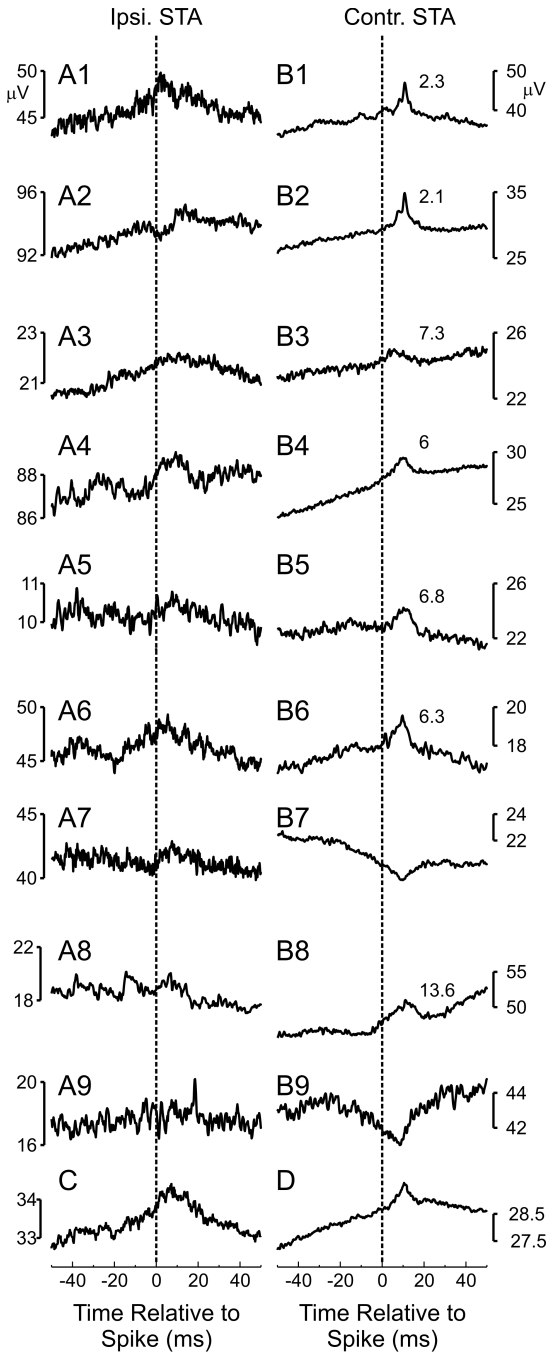

Figure 4. Spike-triggered averages of EMG from M1 cell activity

A1-9, Nine example ipsilateral averages, showing the clearest significant effects found. B1-9, same as A1-9 but for contralateral muscles. C, average across all significant ipsilateral effects. D, average across all significant contralateral effects. Note that the averages are differently scaled. The numbers to the right of the contralateral effects correspond to the peak width at half maximum.

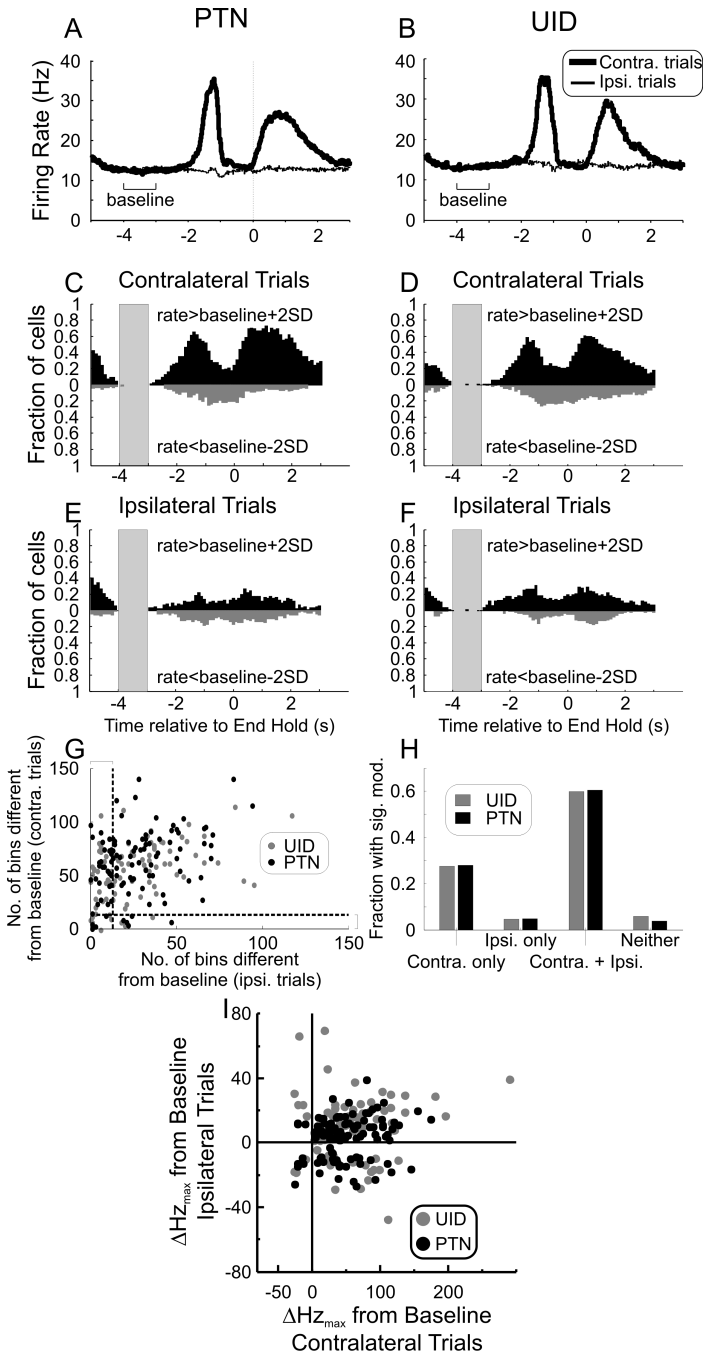

Figure 5. Task related activity of cells in M1 during ipsilateral and contralateral limb movements

A. Mean PSTH of 104 pyramidal tract neurons (PTNs) during ipsilateral (thin line) and contralateral (thick line) trials aligned to End Hold task marker. B, same as A but for 87 unidentified cells (UIDs). C. Number of bins across the population of PTNs with rates higher than baseline+2SD (upward, black bars) and with rates lower than baseline – 2SD (downward, grey bars), for contralateral trials. D, same as C but for UIDs. E, same as B but for ipsilateral trials. F, same as D but for ipsilateral trials. Shaded area on (C-F) indicates region used as baseline. Cell activity aligned to end of hold event (time 0). Time axis is the same for panels A to F. G, cluster plot of the number of bins crossing the 2SD limit for

ipsilateral and contralateral trials. Each dot corresponds to a single neuron (grey for UIDs, black for PTNs). The vertical and horizontal dotted lines indicate the minimum number of bins needed before a cell can be judged to have significant modulation with the particular trial. H, histogram of number of cells showing modulation with the different trial lateralities. Simply by chance, we would expect a certain number of false positives in each category, and only the ‘Contra only’ and ‘Contra. + Ipsi.’ categories have counts above the number expected by chance. I, cluster plot of maximal rate modulation during ipsilateral (ordinate axis) and contralateral (abscissa) trials for PTNs and UIDs. The rate modulation is defined as the maximal absolute deviation relative to a baseline epoch. For both PTNs and UIDs during contralateral trials the majority showed a rate increase; for ipsilateral trials a higher proportion of cells showed a rate suppression.

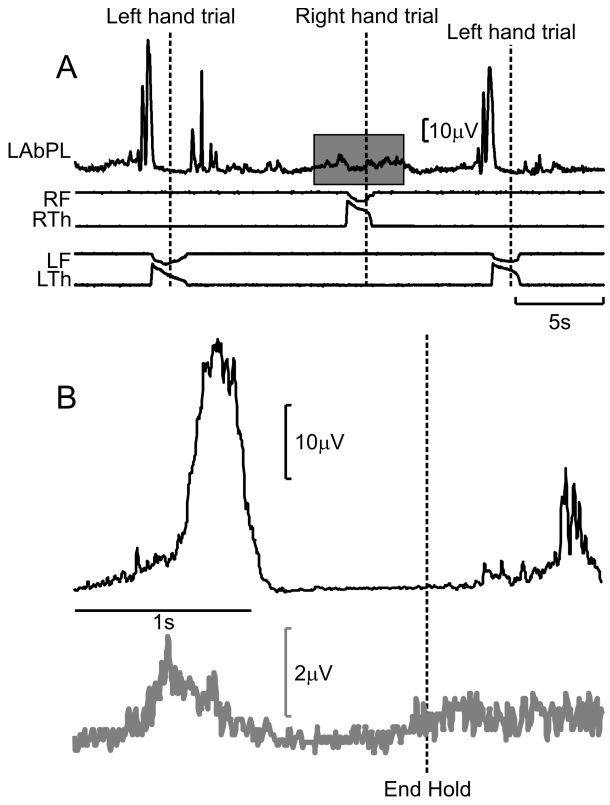

Figure 6. Muscle Mirroring

A, Excerpt from a single recording session showing the rectified activity of the left abductor pollicis longus (AbPL) muscle during ipsilateral and contralateral trials. RF,RTh,LF,LTh correspond to Right Finger, Right Thumb, Left Finger and Left Thumb lever displacement traces respectively. Vertical dotted lines indicate the ends of the hold period. Shaded box marks modulation of left muscle activity during a right handed trial. B, activity of the left AbPL muscle averaged relative to the End Hold task marker, during ipsilateral (grey) and contralateral (black) trials. There is a weak modulation in activity during ipsilateral trials (note the difference in scale bars between the two trial types).

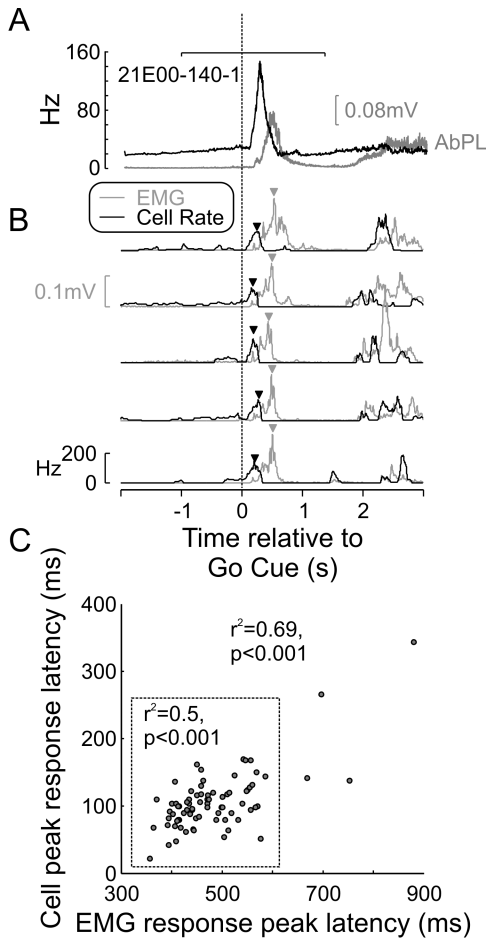

Figure 7. Latency Correlation between M1 Cell Activity and EMG

A, mean EMG activity of contralateral AbPL (grey line) and PSTH of M1 PTN (black line) aligned to the 'Go' Cue. Horizontal bar at top indicates the region used to search for the peak in cell and EMG response on a trial by trial basis. B, five example trials showing the cell's instantaneous firing rate (black line) and EMG activity (grey line), with triangles indicating peak response times of both. C, cluster plot showing good correlation between EMG peak response latency and neuronal peak response latency. When including all trials correlation coefficient was 0.69, and when extreme values (data points outside the dotted square) were excluded this correlation was still highly significant at 0.5.

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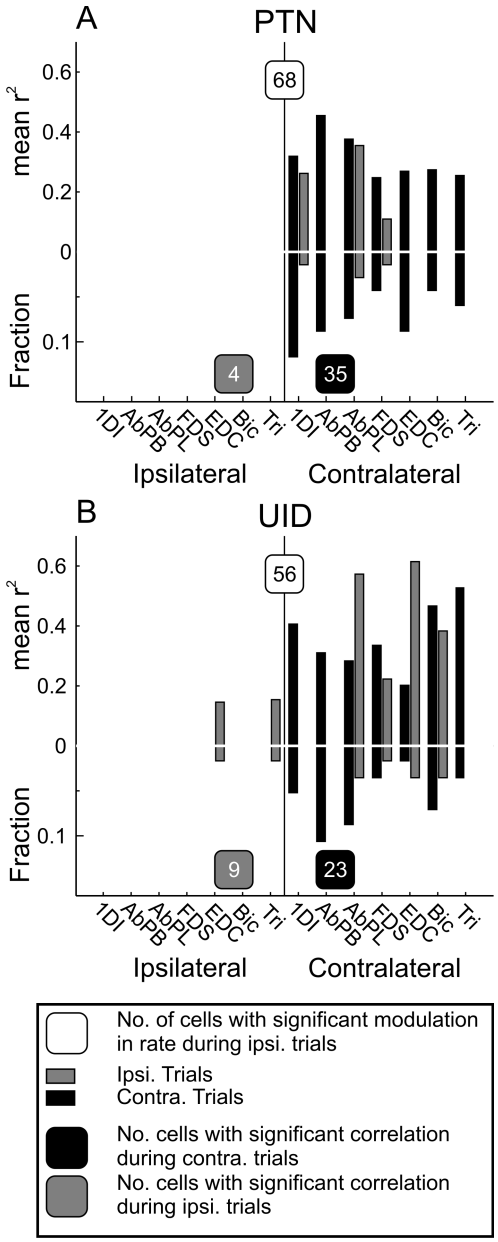

Figure 8. Population results for latency correlation analysis

For this analysis only cells that showed a significant modulation with ipsilateral trials were considered. In cases where the cell had a significant correlation with multiple muscles, the one with the strongest correlation was used for this plot. A. Mean peak correlation coefficients per muscle for PTNs showing a significant correlation with EMG. Black bars are for contralateral trials and grey bars are for ipsilateral trials. Upward going bars show correlation coefficients, downward going bas the proportion of cells with best correlation with that muscle. For both ipsilateral and contralateral trials, maximal correlations were with contralateral muscles. The numbers in the shaded boxes indicate how many cells showed a significant correlation with EMG during trials of the particular laterality. B, same as A but for unidentified cells (UIDs).