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Changes in descending motor pathway connectivity after corticospinal tract lesion in macaque monkey

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Damage to the corticospinal tract is a leading cause of motor disability, for example in stroke or spinal cord injury. Some function usually recovers, but whether plasticity of undamaged ipsilaterally descending corticospinal axons and/or brainstem pathways such as the reticulospinal tract contributes to recovery is unknown. Here, we examined the connectivity in these pathways to motor neurons after recovery from corticospinal lesions. Extensive unilateral lesions of the medullary corticospinal fibres in the pyramidal tract were made in three adult macaque monkeys. After an initial contralateral flaccid paralysis, motor function rapidly recovered, after which all animals were capable of climbing and supporting their weight by gripping the cage bars with the contralesional hand. In one animal where experimental testing was carried out, there was (as expected) no recovery of fine independent finger movements. Around 6 months post-lesion, intracellular recordings were made from 167 motor neurons innervating hand and forearm muscles. Synaptic responses evoked by stimulating the unlesioned ipsilateral pyramidal tract and the medial longitudinal fasciculus were recorded and compared with control responses in 207 motor neurons from six unlesioned animals. Input from the ipsilateral pyramidal tract was rare and weak in both lesioned and control animals, suggesting a limited role for this pathway in functional recovery. In contrast, mono- and disynaptic excitatory post-synaptic potentials elicited from the medial longitudinal fasciculus significantly increased in average size after recovery, but only in motor neurons innervating forearm flexor and intrinsic hand muscles, not in forearm extensor motor neurons. We conclude that reticulospinal systems sub-serve some of the functional recovery after corticospinal lesions. The imbalanced strengthening of connections to flexor, but not extensor, motor neurons mirrors the extensor weakness and flexor spasm which in neurological experience is a common limitation to recovery in stroke survivors.

Introduction

The corticospinal tract is the major descending pathway linking brain and spinal cord in mammals. In primates, it is especially important for control of the hand, where considerable evidence points to its pivotal role in subserving dexterity (Lemon, 1993). Damage to the corticospinal tract can be caused by stroke and by spinal cord injury, and commonly results in a flaccid paralysis of

the affected side. However, in the weeks following the lesion, patients show some recovery of function reflecting a reorganization of neural pathways (Twitchell, 1951).

Most current concepts of recovery focus on surviving corticospinal fibres as the substrate for functional restoration. For example, many studies have identified changes in the unlesioned hemisphere following corticospinal damage in humans (Cramer *et al.*, 1997; Netz *et al.*, 1997; Marshall *et al.*, 2000; Calautti *et al.*, 2001; Ward *et al.*, 2003*a, b*) and in macaques (Nishimura *et al.*, 2007; Nishimura and Isa, 2009), suggesting that corticospinal fibres arising there may contribute to recovery. The large majority of projections from motor cortex to the spinal cord decussate in the medulla, but a proportion (up to 15% in man) fail to cross and descend into the spinal cord ipsilaterally, in either the lateral or the ventral funiculus (Kuypers, 1981). Additionally, many pyramidal tract fibres that do decussate before descending generate intraspinal collaterals that cross back within the spinal cord, providing a further route by which the intact hemisphere may influence ipsilateral circuits (Rosenzweig *et al.*, 2009; Yoshino-Saito *et al.*, 2010). The recent demonstration that spinally decussating corticospinal fibres may sprout within the cord following spinal lesions (Rosenzweig *et al.*, 2010) provides a possible substrate for recovery of function via the undamaged corticospinal pathways, either by forming new direct connections or through detour pathways (Bareyre *et al.*, 2004).

Alternatively, phylogenetically older brainstem pathways may provide a route for motor commands to reach the spinal cord. After corticospinal lesions in monkey, outputs from the red nucleus have been shown to strengthen (Belhaj-Saif and Cheney, 2000). However, projections from the red nucleus to the spinal cord in man may be weak or absent (Nathan and Smith, 1955; Onodera and Hicks, 2010), calling into question the relevance of these findings from monkey—where the rubrospinal tract is clearly an important pathway—to patients recovering from brain lesions.

Against this background, we recently showed that the descending pathways originating in the medial brainstem including the reticulospinal tract can activate primate hand muscles, albeit weakly (Riddle *et al.*, 2009). These tracts have a highly divergent and non-selective output, making it likely that they control gross hand movements rather than the fine and relatively independent finger movements, which have been the focus of much previous work. We hypothesized that reorganization and strengthening of these tracts might underlie some of the functional recovery seen after corticospinal lesions, but that the divergent nature of the outputs could also act as a constraint on that recovery (Baker, 2011). Here, we report experiments that tested this idea directly by making focal unilateral pyramidal tract lesions and allowing functional recovery. Using intracellular recordings from motor neurons, we searched for functional pathways from the unlesioned pyramidal tract to ipsilateral motor neurons, and for changes in pathways from the medial brainstem to motor neurons. We found no evidence for the establishment of novel connections from the intact ipsilateral pyramidal tract, but there was a non-uniform strengthening of reticulospinal connections to particular muscle groups, in a pattern that may underlie the selective recovery of function often observed in stroke survivors.

Materials and methods

Results are reported from nine female adult *Macaca mulatta* monkeys; of these, three underwent unilateral lesions of the left pyramidal tract (ages at terminal experiment 4.5, 4.6 and 4.9 years, weights 6.0, 5.1 and 6.3 kg for Monkeys C, D and M, respectively) and six provided control data. The control animals provided the datasets reported in Riddle *et al.* (2009) and Soteropoulos *et al.* (2011). All experimental procedures were carried out under UK Home Office regulations and were approved by the Newcastle University Research Ethics Committee.

Corticospinal tract lesions

Corticospinal tract lesions were made during surgery under deep anaesthesia (sevoflurane 3–4% in 100% O_2; intravenous alfentanil 7–23 mg kg^{-1} h^{-1}). Craniotomies made over the left and right motor cortex allowed cortical field potential recording using epidural electrodes. Stimulating electrodes were implanted into the pyramidal tract bilaterally (initial stereotaxic target coordinates 3 mm posterior and 10 mm ventral to the inter-aural line, 1.3–2 mm from the midline), and fixed at the location of lowest threshold for evoked cortical antidromic volleys (thresholds at final location all ≤40 μA, except left pyramidal tract in Monkey C, threshold 180 μA). A thermocoagulation probe (TC-0.7-2-200, Cosman Medical Inc.) was inserted into the left pyramidal tract rostral to the stimulating electrode [target coordinates 1mm (Monkeys C and D) or 2.8 mm (Monkey M) anterior and 6mm ventral to the inter-aural line, 1 mm lateral to the midline; final location was also determined with reference to the threshold for evoked cortical antidromic volleys]. Repeated lesions (between one and four) were made by raising the probe tip temperature to 65–70°C for 20 s, until the antidromic volley evoked from the pyramidal tract electrode (300 μA, 0.1 ms biphasic pulses) on the left side was abolished (Fig. 1B). At the end of the surgery, all electrodes and the probe were removed, wounds repaired and the animal allowed to recover from anaesthesia. Animals received a full programme of postoperative care, including steroids to reduce brain oedema (methylprednisolone 20mg/ kg intramuscular), analgesia (10 μg/kg buprenorphine intramuscular; 5 mg/kg carprofen intramuscular; 0.1 mg/kg meloxicam oral daily) and prophylactic antibiotics (8 mg/kg cefovecin sodium subcutaneous, daily). Supportive therapy was provided as necessary in the immediate postoperative period, including intravenous fluids and tube feeding.

Intracellular recordings

Between 4.8 and 8.6 months after the lesion, a terminal experiment was carried out to assess the strength of inputs via the intact (ipsilateral) pyramidal tract and the medial brainstem pathways to upper limb motor neurons.

Monkeys were initially anaesthetized as above. Surgical preparation included tracheotomy; insertion of central arterial and venous lines; implantation of nerve cuff electrodes around nerves in the right arm (median and ulnar at the wrist; median and ulnar in the upper arm; deep radial nerve at the elbow); laminectomy and pneumothorax. Stimulating electrodes were inserted into the right medial longitudinal fasciculus (ipsilateral medial longitudinal fasciculus) and the unlesioned right pyramidal tract, guided by antidromic volleys observed from epidural recordings from motor cortex, and orthodromic volleys recorded from the cervical spinal cord (Riddle *et al.*, 2009). Both medial longitudinal fasciculus and pyramidal tract electrodes were implanted at the anterio-posterior location of the inter-aural line, close to the level of
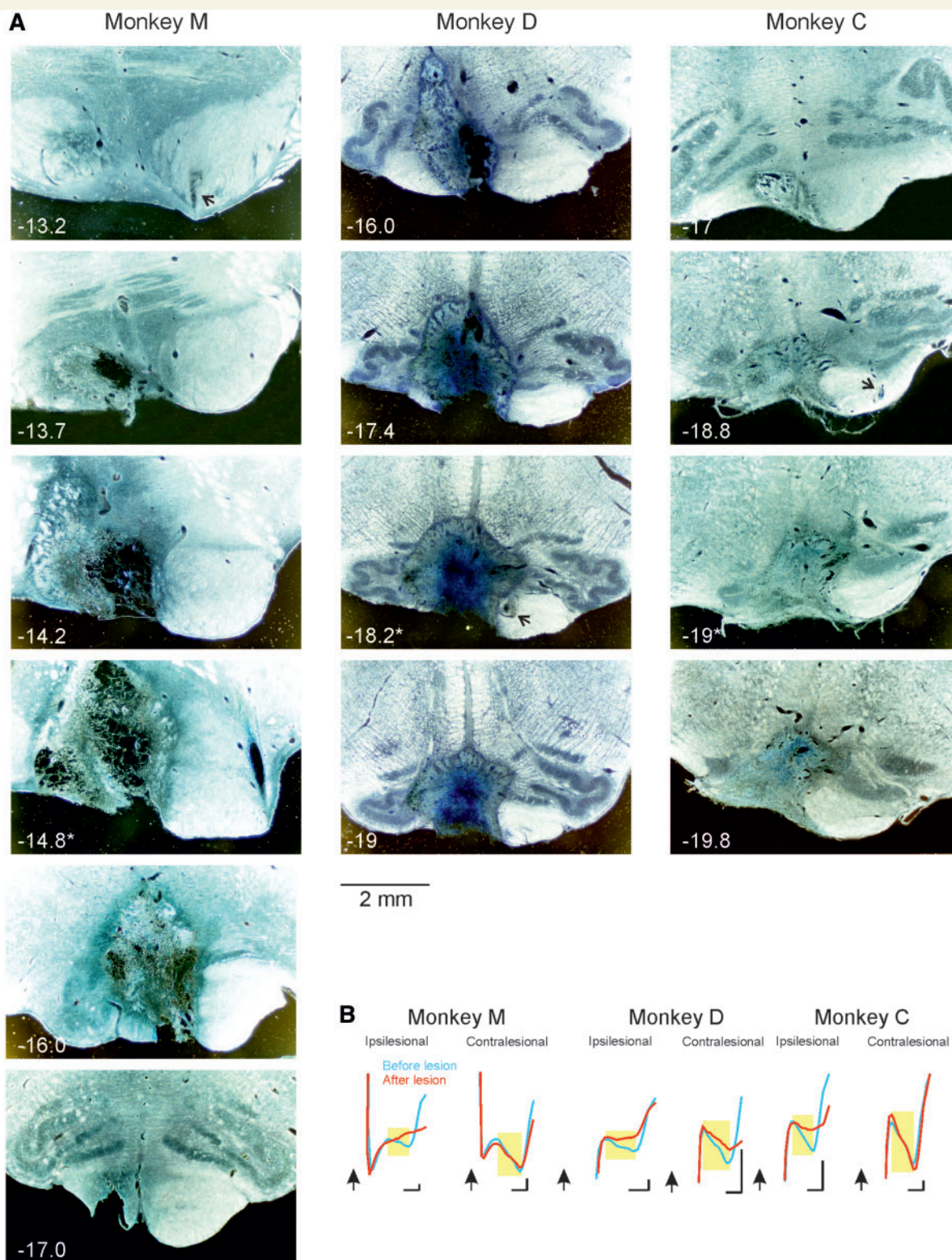
Figure 1 Histological and electrophysiological characterization of pyramidal tract lesions. (**A**) Histological sections of the brainstem from the three experimental animals. Each column presents material from a different animal. Numbers in the bottom left of each photomicrograph are estimated rostro-caudal location of each section, using the anterior-commissure referenced coordinates of Martin and Bowden (1996). Asterisks after these coordinates mark the section at the largest extent of the lesion. Arrows indicate the location of the tip of the pyramidal tract stimulating electrode used in the terminal experiment. Sections were photographed under dark field illumination. (**B**) Epidural recordings from motor cortex before (blue) and after (red) the lesion, showing potentials evoked by pyramidal tract stimulation caudal to the lesion site. Arrows mark time of the stimulus; traces are blank in the immediate post-stimulus period due to large stimulus artefacts. Yellow shading indicates the earliest (antidromic) part of the response. Calibration bars are 0.25 ms, 20 µV.

the facial and abducens motor nuclei. In six monkeys (including all of those with lesions), an electrode was also inserted in the left medial longitudinal fasciculus (contralateral medial longitudinal fasciculus). Due to the proximity of the medial longitudinal fasciculus to the midline, stimulation through the left or right medial longitudinal fasciculus electrode probably excites preferentially, but not exclusively, the fibres on the side of implantation.

Anaesthesia was then switched to propofol (5–14 mg kg^{-1} h^{-1}) and alfentanil (7–23 mg kg^{-1} h^{-1}), and neuromuscular blockade commenced (atracurium, 0.6–1.2 mg kg^{-1} h^{-1}). Heart rate and arterial blood pressure were monitored to ensure deep anaesthesia throughout. Slow increases in these parameters, or sharp rises following noxious stimulation, were taken as evidence of possibly lightening anaesthesia. Immediate supplementary doses were then given, and infusion rates increased.

Intracellular recordings from motor neurons were made with glass pipettes filled with 2 M potassium citrate or potassium acetate. Motor neurons were identified by antidromic activation following peripheral nerve stimulation (3× motor threshold, determined just prior to the onset of neuromuscular blockade). This not only confirmed that the cell was a motor neuron, but also yielded information on the muscle group innervated. Cells that responded to stimulation of the median or ulnar nerves in the arm, but not to the corresponding nerves at the wrist, were classified as forearm flexor motor neurons. Those that also responded to stimulation at the wrist were intrinsic hand muscle motor neurons. Cells that responded to the deep radial nerve at the elbow were classified as forearm extensor motor neurons.

Single and trains of three or four stimuli were delivered to the medial longitudinal fasciculus and pyramidal tract electrodes, at an intensity of 300 µA (0.2 ms biphasic pulses). This intensity was chosen because it activated a substantial number of fibres in the vicinity of the electrode (as judged by the robust spinal volleys that they elicited); however, spread to adjacent structures was unlikely. We verified that medial longitudinal fasciculus and pyramidal tract electrodes activated non-overlapping populations of fibres using occlusion tests (Riddle *et al.*, 2009). As a consequence of using a relatively low stimulus intensity, it is unlikely that all fibres within a given targeted pathway were activated. Nevertheless, the total number of axons activated should be consistent, allowing valid comparisons of responses to stimulation between lesioned and unlesioned animals.

The segmental onset latency of excitatory post-synaptic potentials (EPSPs) was measured from the first inflexion of the cord dorsum volley (recorded from the spinal surface close to the penetration point) to the EPSP onset (marked by vertical dotted and solid lines, respectively, in Figs 2 and 4). Potentials with segmental latencies below 1 ms, and consistent responses to each stimulus in a train, were classified as likely to be monosynaptic in origin. Those with longer segmental latencies were classified as disynaptic; these usually augmented in amplitude following successive stimuli, reflecting temporal summation in the interposed inter-neurons (Riddle *et al.*, 2009).

Results are given as the mean EPSP amplitude (measured from the onset to peak, only from cells with responses), the incidence (proportion of cells with responses) and the product of these measures (connection strength), which provides an estimate of the average effect on the motor neuron pool (including cells without responses).

Statistical comparisons for incidence used Fisher's exact test. Comparisons for amplitude, and amplitude × incidence product, were made using a Monte Carlo resampling method (Press *et al.*, 1989) as follows. For a given muscle group in either control or lesioned animals, experimentally recorded significant EPSPs occurred in M out of N motor neurons. To estimate the distribution of the empirically measured connection strength, we first determined a number m of motor

neurons deemed to have a non-zero EPSP amplitude in the resampled population; this was chosen randomly from a binomial distribution, with success probability M/N. This many EPSP amplitudes were then chosen at random from the M available (sampling with replacement). The mean of these amplitudes was multiplied by m/N, to yield the amplitude × incidence product (connection strength) for the resampled population. This resampling procedure was performed for both control and lesioned motor neuron populations, and the difference in connection strength found. The whole procedure was repeated 1000 times and the differences sorted. The 2.5 and 97.5% points of the difference distribution were determined. If the difference between experimentally determined connection strength in control and lesioned animals fell outside these limits, it was considered to be significant at $P < 0.05$; using the 0.5 and 99.5% points of the distribution yielded a test with $P < 0.01$. A similar procedure was followed for comparisons of amplitude, but without the multiplication of mean amplitude by m/N.

At the end of experimental recordings, animals were perfused through the heart with paraformaldehyde fixative solution. The brainstem was cut in 100 µm sections, which were counterstained with cresyl violet. These sections were used to confirm the extent of the lesions and placement of stimulating electrodes.

Results

Extent of lesions

In these experiments, we aimed to examine plasticity after unilateral lesions of corticospinal tract fibres. Post-mortem histological analysis verified the extent of the lesions; sections from each animal are shown in Fig. 1A. These sections were photographed using dark field illumination, under which axon tracts appear bright. By studying the appearance of landmarks such as the inferior olive, together with the estimated stereotaxic location of the tissue block and the number of the section, we estimated the rostro-caudal location of each section. This is marked on each photograph, using the anterior commissure-based coordinate system adopted by the atlas of Martin and Bowden (1996). The section marked by an asterisk after this coordinate is at the estimated greatest extent of the lesion.

In Monkey M, the lesion had its greatest extent just rostral to the inferior olive. The targeted pyramid was effectively ablated. In addition, the lesion extended into part of the medial lemniscus, trapezoidal body and parts of the gigantocellular reticular formation on the lesioned site. There was also some spread to the medial part of the contralateral pyramid (sections −16.0 and −14.8).

In the other two monkeys, the lesion was more caudal. In Monkey D, as well as the targeted pyramid, the lesion extended into the rostral reticular formation (section −17.4), and there was damage to the most medial parts of the principal, medial and dorsal accessory olivary nuclei. There was also some damage to the medial part of the contralateral pyramid.

In Monkey C, the greatest extent of the lesion was at the level where the olivary subnuclei become simple (without overlapping layers). The lesion extended into the principal and medial accessory olive caudally, but not rostrally. There appeared to be little encroachment into the medullary reticular formation. Once again,
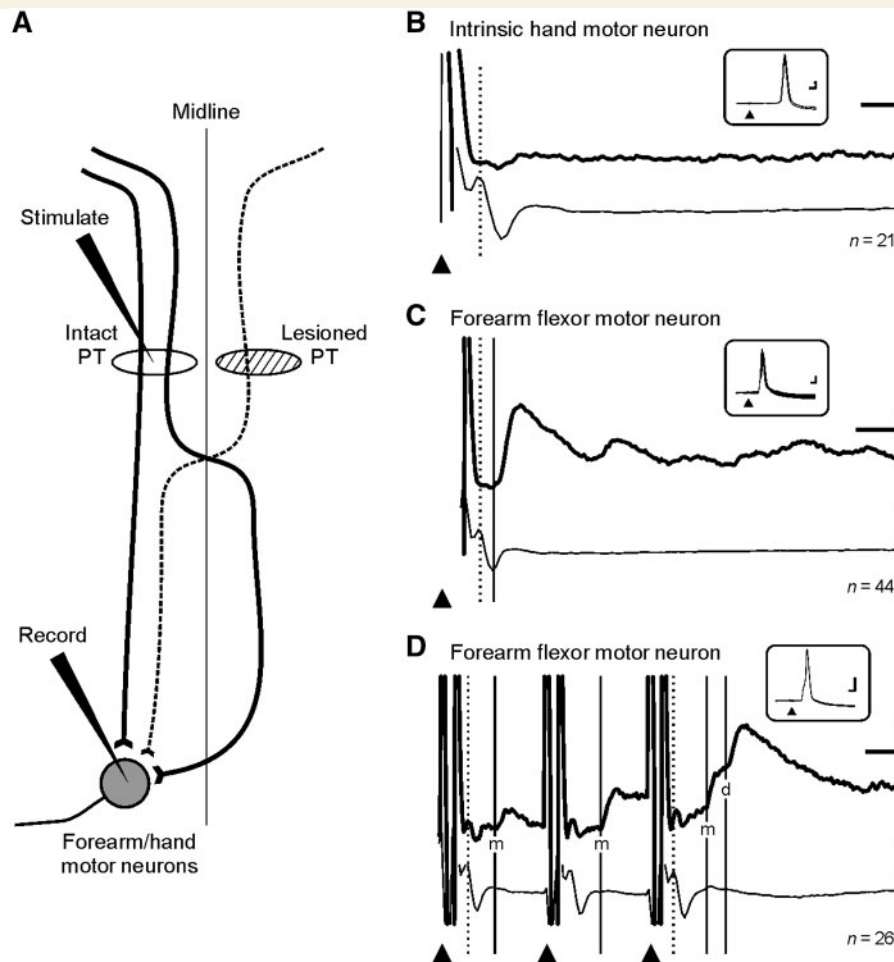
Figure 2 Responses of identified motor neurons to ipsilateral pyramidal tract stimulation. (**A**) Schematic showing the lesioned pyramidal tract (PT, dashed line), and potential pathways descending through the ipsilateral (intact) pyramidal tract that might contribute novel connections. (**B–D**) Each panel shows averaged intracellular recordings from motor neurons (upper thick lines) and epidural recordings from close to the microelectrode penetration point (lower thin lines). Calibration bars are 0.1 mV, 1 ms (thick lines), and 50 μV (thin lines). The arrival of the descending volley and onset of the EPSP (where present) are shown by dashed and solid vertical lines, respectively. Insets show antidromic spikes elicited by stimulation of the most distal peripheral nerve that gave a response (calibration bars 10 mV, 1 ms). Arrowheads mark time of stimulation. Number of stimuli used to compile the averages (*n*) is given below each pair of records. (**B**) Intrinsic hand muscle motor neuron with no synaptic response. (**C**) Forearm flexor motor neuron with monosynaptic EPSP. (**D**) Forearm flexor motor neuron with both mono- (m) and disynaptic (d) EPSPs.

there was some damage to the medial part of the contralateral pyramid.

These photomicrographs suggest that the lesions interrupted all corticospinal tract fibres from one side; in contrast, the contralateral pyramid was left largely intact. Intraoperative epidural recordings of the antidromic field potentials evoked by stimulation through the pyramidal tract electrodes caudal to the site of the lesion are shown in Fig. 1B. Prior to the lesion (blue traces), a short-latency antidromic field potential was evoked (yellow shading), with onset latency less than 1 ms (range 0.67–0.72 ms). These responses were essentially abolished in all cases after the lesions (red traces). In parallel recordings of responses to stimulation of the right (contralesional) pyramidal tract, we estimated a reduction in the antidromic volley immediately following the lesions of 32, 48 and 0% for Monkeys M, D and C, respectively.

While it is impossible to rule out the existence of a population of surviving fibres on the lesioned side, the evidence of Fig. 1 suggests that this is likely to include only a few fibres, which would not be expected to have a great impact on functional recovery.

Time-course of recovery

Immediately following the lesion surgery, animals showed a flaccid paralysis of the contralateral limbs. Their initial substantial disability necessitated single housing in a small recovery cage, and a high level of care. Although no formal programme of physiotherapy was provided, the limbs on the affected side were regularly mobilized to prevent joint contractures. After 6, 11 and 3 days (Monkeys C, M and D, respectively), the animals' gross motor function was sufficiently well recovered to return safely to a

larger holding cage with high level perches. At 12, 26 and 20 days post-lesion (Monkeys C, M and D, respectively), the animals were able to return to their usual large pens. These contained a range of items to promote locomotion, climbing and foraging. Monkey M was housed with one unlesioned cage mate. Monkeys C and D were housed together, in an enclosure that also included another (unlesioned) monkey. This enriched and social environment provided a powerful and continual stimulus to functional recovery.

By the time of the experimental measurements under terminal anaesthesia, the lesioned animals had recovered to a high level of gross motor function as described previously (Lawrence and Kuypers, 1968b). They were capable of leaping accurately across the holding pen from one perch to another, and could rapidly walk and climb. Monkey M was chair trained, permitting more careful assessment of hand function. This animal was capable of gripping food offered by the experimenter with the contralesional hand, and transporting it to the mouth; however, the grip was weak, and all digits flexed simultaneously, with no evidence of relatively independent finger movements. When food was offered in small circular wells (Kluver board; Lawrence and Kuypers, 1968b), it could not be retrieved. Monkeys C and D had a stronger grip, and could hold on to the bars of their home cages with the contralesional hand almost normally, using this limb for weight support during climbing; hand function was not tested in detail for these animals in an experimental setting. In all aspects, recovery of function was in accord with the extensive previous literature (Tower, 1940; Lawrence and Kuypers, 1968b; Chapman and Wiesendanger, 1982).

Motor neuron recordings

Our sample of motor neurons from the three lesioned animals included 108 forearm flexor motor neurons, 37 forearm extensor motor neurons and 22 motor neurons innervating intrinsic hand muscles. These results were compared with data from six control animals, in which we recorded from 113 forearm flexor motor neurons, 35 forearm extensor and 59 innervating intrinsic hand muscles.

Changes in connections from the ipsilateral (intact) pyramidal tract

Figure 2 illustrates some example recordings in the lesioned animals from motor neurons following stimulation of the intact pyramidal tract, ipsilateral to the motor neuron recordings. Any responses observed could be mediated either by ipsilaterally descending corticospinal fibres, or by fibres which decussate at the medulla, but then generate collaterals that recross the midline at the segmental level (Fig. 2A). The most common finding was that illustrated in Fig. 2B. This motor neuron responded antidromically to stimulation of the median nerve at the wrist (inset overlain traces), confirming that it projected to an intrinsic hand muscle. A recording from the dorsal surface of the spinal cord (thin trace) revealed a clear descending volley evoked from pyramidal tract stimulation (dotted line). However, the intracellular recording from the motor neuron (thick trace) contained no synaptic response.

Figure 2C illustrates recordings from a different motor neuron, identified as projecting to a forearm flexor muscle. This cell showed a robust EPSP in response to stimulation of the ipsilateral pyramid. The onset latency of this response (solid vertical line) was 0.37 ms after the arrival of the volley at the segmental level (dotted line), confirming that this response was likely to be mediated monosynaptically.

Figure 2D shows the response of a third motor neuron to a train of three stimuli delivered to the pyramidal tract. Following the first stimulus, a small EPSP was visible, which had a segmental latency within the monosynaptic range (0.8 ms). This potential followed successive stimuli with some potentiation, which has been previously reported for contralateral monosynaptic corticospinal EPSPs (Porter, 1970). In addition, a longer latency EPSP followed the third stimulus. The longer segmental latency (1.1 ms) and the appearance of this potential only after a train of stimuli suggests that it was disynaptically mediated. In lesioned monkeys, monosynaptic responses were seen in only 4/79 forearm flexor and 1/28 forearm extensor motor neurons. Disynaptic responses of this type were seen in 6/79 forearm flexor and 1/28 forearm extensor motor neurons. Interestingly, three of the four forearm flexor motor neurons that had monosynaptic EPSPs were among the six motor neurons in which disynaptic EPSPs were seen. No EPSPs from the ipsilateral pyramidal tract were observed in intrinsic hand muscle motor neurons in any animal.

Figure 3 presents population data on all motor neurons that we recorded in response to ipsilateral pyramidal tract stimulation. Each row of this figure relates to motor neurons projecting to different compartments of the forelimb: forearm flexors (Fig. 3A), forearm extensors (Fig. 3B) and intrinsic hand muscles (Fig. 3C). The blue bars document findings in the control animals, and the red bars in the monkeys following recovery from lesion. Results are presented separately for monosynaptic (filled bars) and disynaptic (open bars) EPSPs.

The left column in Fig. 3 displays the incidence of particular responses. We have previously described the very low incidence of EPSPs in motor neurons from the ipsilateral pyramidal tract in healthy animals (Soteropoulos *et al.*, 2011). In the animals after recovery from lesion inputs from the ipsilateral pyramidal tract were also rare, and were absent from intrinsic hand muscles.

The middle column of Fig. 3 shows the average amplitude of the EPSPs that were detected; these values should be interpreted with great caution, given the very small numbers of motor neurons in which any response was seen. Neither incidence nor amplitude adequately assess the total effect that a pathway can have on a motor neuron pool. To produce a better summary measure of overall connection strength, we computed the product of amplitude and incidence (right column of Fig. 3). This effectively measures the mean EPSP size in the population (including as zeros motor neurons in which no response was seen). To aid interpretation of these values, the right axis of these plots expresses the values as a percentage of the input strength from the contralateral pyramidal tract in unlesioned animals. Such a display emphasizes the extremely weak nature of input from the ipsilateral pyramidal tract. This pathway is unlikely to have played a functionally significant role in the recovery of function seen in these experiments.
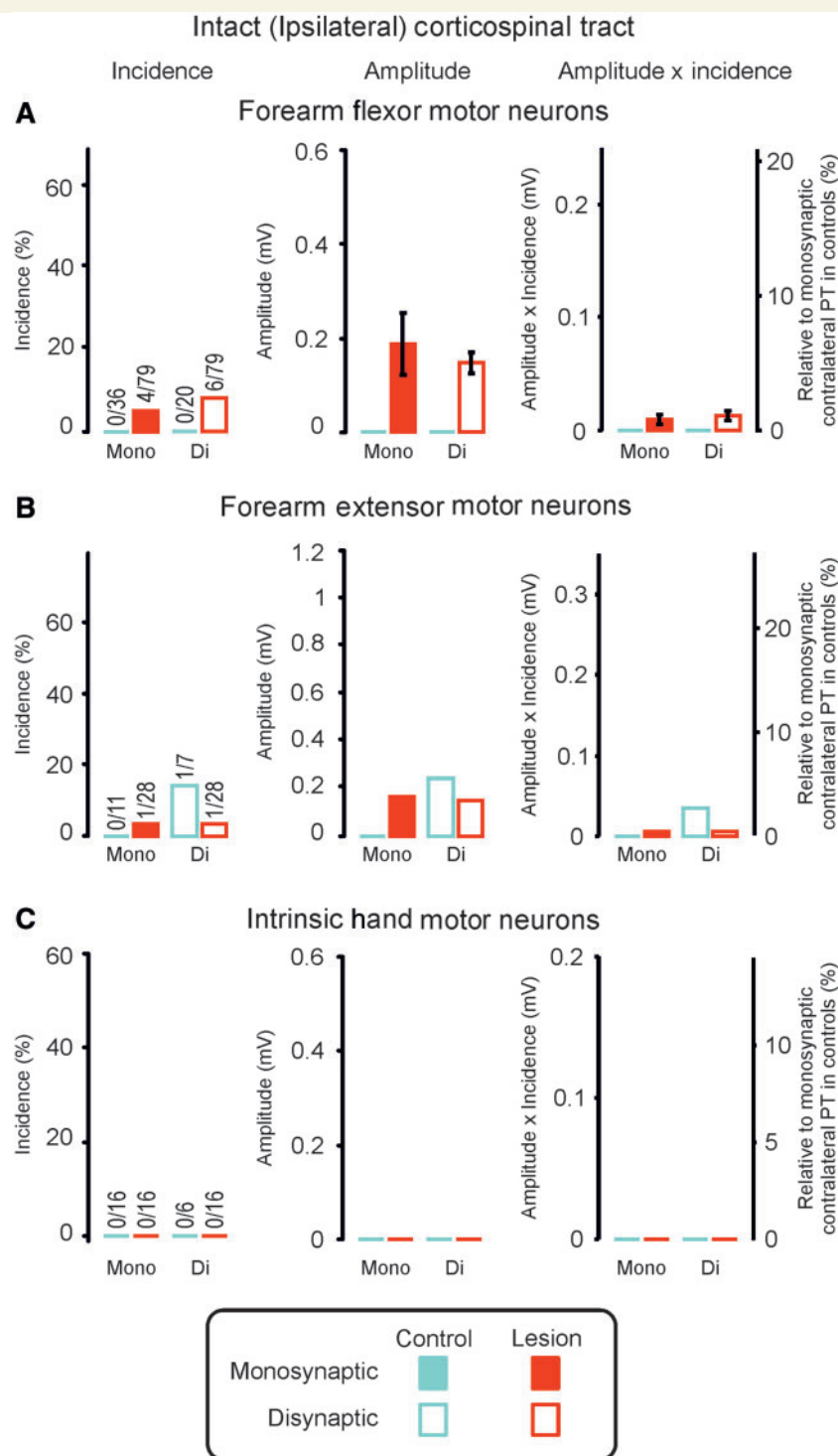
Figure 3 Quantitative measurements of input to motor neurons from the ipsilateral pyramidal tract. Data are presented separately according to the muscle group that the motor neuron innervated: (**A**) forearm flexors; (**B**) forearm extensors; (**C**) intrinsic hand muscles. Blue bars relate to control and red to lesioned animals. Filled bars show monosynaptic and open bars disynaptic responses. The *left* column shows the incidence of EPSPs. The *middle* column shows the mean amplitudes of EPSPs, where present. The *right* column indicates the product of amplitude and incidence, which measures the overall strength of input from a given pathway (connection strength). Statistical tests were performed using Fisher's exact test (incidence) and a Monte Carlo resampling method (amplitude and amplitude × incidence product); there were no significant differences between control and lesioned animals ($P > 0.05$).
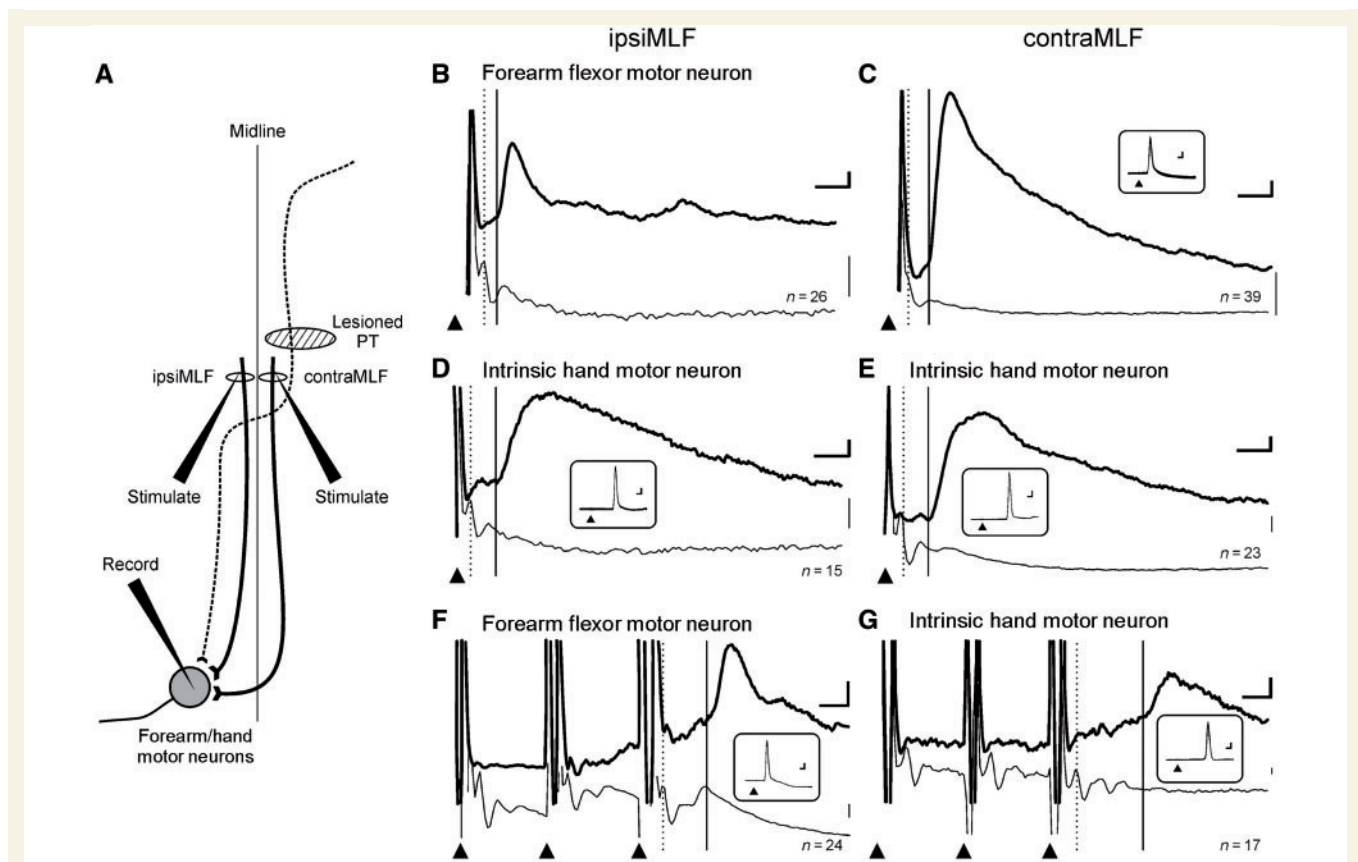
Figure 4 Responses of identified motor neurons to stimulation in the medial longitudinal fasciculus. (**A**) Schematic showing the lesioned pyramidal tract (PT) and pathways descending through the ipsilateral and contralateral medial longitudinal fasciculus (MLF).
(**B–G**) Averaged intracellular potentials from motor neurons, conventions as in Fig. 2. Calibration bars are 0.1 mV, 1 ms (thick lines) and 20 μV (thin lines). The arrival of the descending volley and onset of the EPSP (where present) are shown by dashed and solid vertical lines, respectively. Insets show antidromic spikes elicited by stimulation of the most distal peripheral nerve which gave a response (muscle category indicated above each panel). Calibration bars for the insets 10 mV and 1 ms. Arrowheads mark time of stimulation. Numbers of stimuli used to compile the averages (*n*) is given below each pair of records. (**B**, **D** and **F**) Responses to ipsilateral medial longitudinal fasciculus stimulation. (**C**, **E** and **G**) Responses to contralateral medial longitudinal fasciculus stimulation. Panels **B** and **C** show recordings from the same motor neuron, **D–G** are all from different motor neurons. Synaptic responses in **B–E** were classified as monosynaptic; those in **F** and **G** as disynaptic.

Changes in connections from medial brainstem pathways

We also assessed inputs to motor neurons from the medial brainstem descending pathways that pass through the medial longitudinal fasciculus (Fig. 4A). In contrast to the results from the ipsilateral pyramidal tract, stimulation of the medial longitudinal fasciculus often elicited robust EPSPs in motor neurons, both in control and lesioned animals. The motor neuron illustrated in Fig. 4B and C, which projected to a forearm flexor muscle and was recorded in a lesioned monkey, received clear EPSPs following stimulation of the medial longitudinal fasciculus both ipsilateral and contralateral to the recording site. The short segmental latency of these effects (0.3 and 0.4 ms, respectively) suggests that they were monosynaptic in origin. Monosynaptic inputs from the medial longitudinal fasciculus were also elicited in motor neurons that projected to intrinsic hand muscles in lesioned animals (two different motor neurons are illustrated in

Fig. 4D and E). In other motor neurons, EPSPs with a longer segmental latency were elicited, which augmented to successive stimuli in a train, suggesting that they were mediated by a disynaptic pathway (Fig. 4F and G).

Several of our records contained evidence for later inhibitory synaptic potentials that followed the earlier excitation. For example, the EPSPs in Fig. 4B, C and F have very rapid falling phases, compared with those in Fig. 4D and E, consistent with the EPSP being curtailed by a superimposed inhibitory synaptic potential. It was not possible to make reliable quantitative measurements of these effects, and beyond reporting their existence we will not consider them further.

Figure 5 presents population data on the medial longitudinal fasciculus responses, in a similar format to Fig. 3. For both forearm flexor and intrinsic hand muscle motor neurons, there were considerable changes in the overall connection strength (incidence × amplitude, right column Fig. 5) from the medial longitudinal fasciculus in the lesioned compared with control
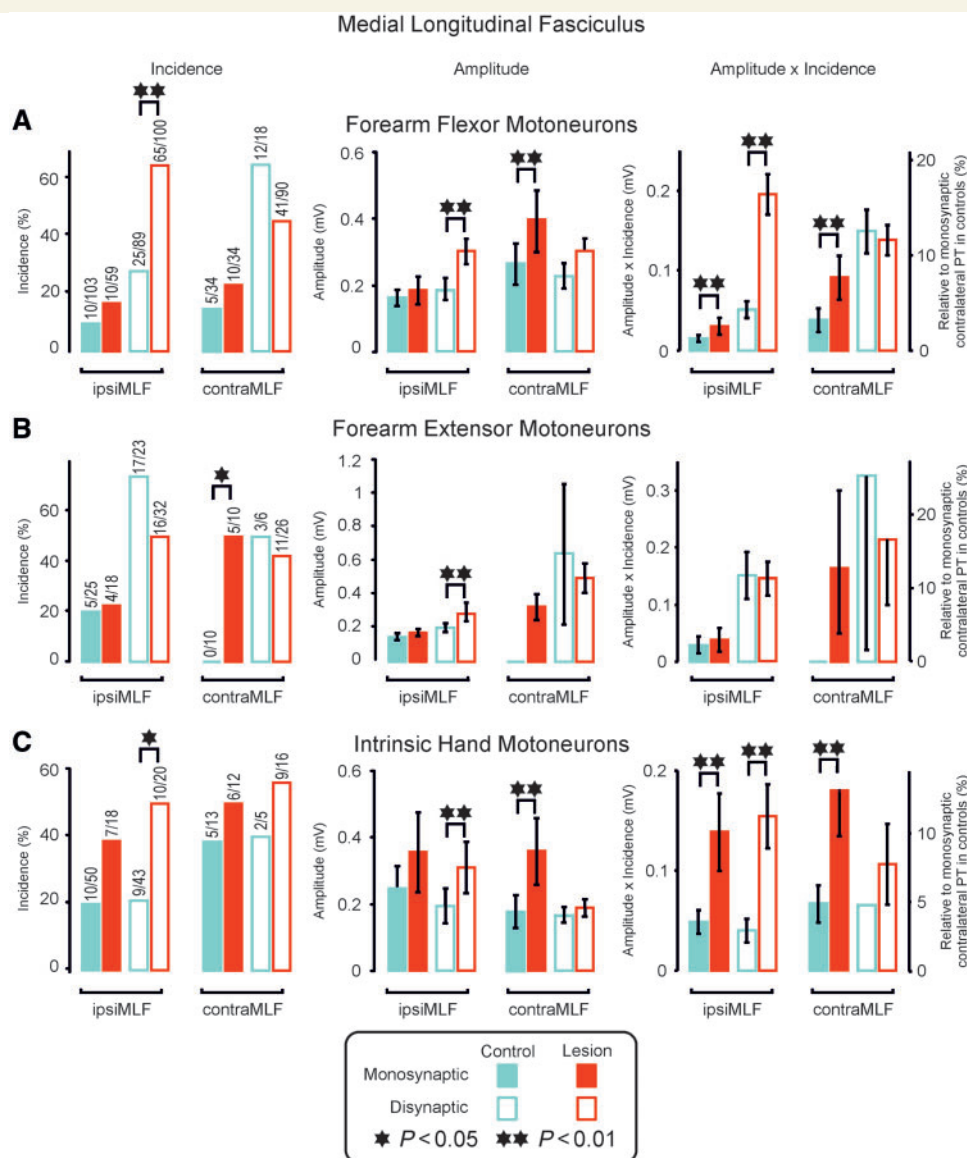
Figure 5 Quantitative measurements of input to motor neurons from the medial longitudinal fasciculus. As in Fig. 3, data are presented separately according to the muscle group which the motor neuron innervated: (**A**) forearm flexors; (**B**) forearm extensors and (**C**) intrinsic hand muscles. Blue bars relate to control and red to lesioned animals. Filled bars show monosynaptic and open bars disynaptic responses. The *left* column shows the incidence of EPSPs. The *middle* column shows the mean amplitudes of EPSPs, where present. The *right* column indicates the product of amplitude and incidence, which measures the overall strength of input from a given pathway (connection strength). Statistical tests were performed using Fisher's exact test (incidence) and a Monte Carlo resampling method (amplitude and amplitude × incidence product). Asterisks mark significant differences between control and lesioned animals (*$P < 0.05$; **$P < 0.01$).

monkeys. In both of these muscle groups, mono- and disynaptic inputs from the ipsilateral medial longitudinal fasciculus strengthened significantly, as did monosynaptic inputs from the contralateral medial longitudinal fasciculus. The amplitude of these inputs was considerable. For example, if we add together all categories (disregarding laterality of stimulation, and the likely number of interposed synapses), the medial longitudinal fasciculus in the lesioned animals provided input to intrinsic hand motor neurons 42% as strong as that from the contralateral pyramidal tract in the controls. Compared with control animals, the total input from the

medial longitudinal fasciculus to intrinsic hand motor neurons increased by 2.5-fold. Changes in medial brainstem pathways are thus likely to play a major role in recovered hand function.

In marked contrast to the substantial changes seen in forearm flexor and intrinsic hand motor neurons, no consistent changes were seen in inputs from the medial longitudinal fasciculus to forearm extensor motor neurons. Although there was an increased incidence of monosynaptic input from the contralateral medial longitudinal fasciculus ($P < 0.05$), and a small increase in the amplitude of disynaptic effects from the ipsilateral medial longitudinal

fasciculus ($P < 0.01$), there were no significant changes in connection strength (amplitude × incidence), which best measures the overall impact of these pathways on the motor neuron pools.

Discussion

Our results provide evidence that connections from medial brainstem pathways undergo functional changes after corticospinal lesions, but we found no evidence for the appearance of significant connections from ipsilateral corticospinal fibres. Importantly, we did not observe a straightforward strengthening of brainstem connections across the board: inputs to forearm flexor and intrinsic hand motor neurons were selectively enhanced, whereas connections to extensors were left apparently unchanged.

In any study examining functional recovery, it is important to characterize the nature and extent of the lesion that led to the observed neural plasticity. In this study, we made extensive lesions of the corticospinal tract on one side at the level of the medulla. Based on Fig. 1, it would appear that the great majority of fibres on the lesioned side were interrupted as intended, but we cannot rule out the survival of a small population of axons. In particular, smaller calibre slowly conducting axons could escape detection in the dark field images of Fig. 1A, and they do not contribute to the fast antidromic potentials of Fig. 1B. However, it is known that the majority of the corticospinal tract is made up of fine fibres, and that small corticospinal neurons can contribute to cortico-motor neuronal projections (Rathelot and Strick, 2006) and long-latency monosynaptic effects in spinal motor neurons (Maier *et al.*, 1998). It therefore remains possible that surviving corticospinal axons could have contributed to some of the recovery observed, alongside a known contribution from intact pathways that we did not assess (e.g. rubrospinal tract; Belhaj-Saif and Cheney, 2000). However, this does not alter our key findings concerning the absence of connections from fast corticospinal fibres in the ipsilateral pyramid and the strengthened output from the medial brainstem descending tracts.

Direct inputs from ipsilateral pyramidal tract to forearm and intrinsic hand muscle motor neurons are practically non-existent in healthy primates (Soteropoulos *et al.*, 2011). Here, we show that this situation does not significantly change after recovery from a unilateral lesion which removes input from the contralateral pyramidal tract. One potential concern with this finding is that in all three animals the lesion appeared to cross the midline, and there was some damage to the medial part of the ipsilateral pyramidal tract. However, based on the histology of Fig. 1A, this appeared minor, and the great majority of the fibres in this pyramid were unaffected. Although corticofugal fibres from different cortical regions are separated in the internal capsule, they become progressively intermixed such that there is complete overlap in their distributions by the time they reach the cerebral peduncle (Morecraft *et al.*, 2007). A small medial lesion of the pyramidal tract would not thus preferentially affect output from any particular cortical area. Ipsilateral pyramidal tract stimulation led to robust volleys in the cord dorsum recordings (Fig. 2B–D). Given the extremely low incidence of synaptic effects from the ipsilateral pyramidal tract in control animals, even a small increase in

connectivity following lesion should have been detected, yet there were no significant differences. We are forced to conclude that any changes in ipsilateral pyramidal tract input to motor neurons were negligible.

A contribution to functional recovery would be possible if the intact axons from the largely spared ipsilateral tract were to sprout. Recent evidence suggests that this does occur following spinal lesions: Rosenzweig *et al.* (2010) found significant structural plasticity of decussating primate corticospinal axon collaterals following spinal hemisection at C7. Although they focused on plasticity in axons that failed to cross in the medulla but crossed at spinal level, they also reported sprouting in axons that crossed in the medulla, then crossed back to the ipsilateral side intraspinally [see Supplementary Fig. 7 in Rosenzweig *et al.* (2010)]. Our data suggest either that this does not occur following lesions of corticospinal axons at a more rostral brainstem level, or that these sprouted decussating corticospinal collaterals do not contribute to functional mono- or disynaptic connections with motor neurons. As we have previously suggested for the intact animal (Soteropoulos *et al.*, 2011), it is likely that ipsilateral corticospinal pathways play a more complex and indirect role in the modulation and control of spinal circuitry, rather than having a direct influence over forearm and hand motor neuron activity. Such functions could not be investigated by the experiments reported here under terminal anaesthesia.

Dramatic changes in activity and excitability of cerebral cortex on the side opposite cortical lesions have been reported both in man (Cramer *et al.*, 1997; Netz *et al.*, 1997; Marshall *et al.*, 2000; Calautti *et al.*, 2001; Ward *et al.*, 2003a, b) and in macaques (Nishimura *et al.*, 2007; Nishimura and Isa, 2009). In longitudinal studies, these increases were present in the early stages of recovery, but returned toward control levels as recovery progressed (Marshall *et al.*, 2000; Calautti *et al.*, 2001; Ward *et al.*, 2003a; Nishimura *et al.*, 2007). Since all of our lesioned animals were tested at a late phase of recovery, it remains possible that the ipsilateral corticospinal tract had transiently made a contribution to restoration of function that was lost by the time we assessed connection strength. However, ipsilateral responses to magnetic stimulation of the unaffected hemisphere are most consistent with mediation via a cortico-reticulospinal pathway, rather than by ipsilateral corticospinal connections (Benecke *et al.*, 1991). Changes in ipsilateral cortical activation should not therefore be automatically assumed to reflect ipsilateral corticospinal outflow.

The striking feature of our findings is that the connection strength from medial brainstem (probably largely reticulospinal) pathways significantly increased to motor neurons innervating forearm flexor and intrinsic hand muscles, but not to motor neurons innervating forearm extensors. These changes mirror those seen in rubrospinal connectivity after an incomplete unilateral corticospinal lesion (Belhaj-Saif and Cheney, 2000). The rubrospinal tract shows a preference for facilitation of extensor muscles (Mewes and Cheney, 1991). Belhaj-Saif and Cheney (2000) reported an increase in flexor facilitation and a decrease in extensor facilitation after recovery from corticospinal lesion, results that were interpreted as a re-balancing of descending commands following loss of corticospinally mediated flexor facilitation and extensor suppression (Belhaj-Saif and Cheney, 2000).

By examining their published data, it is possible to calculate a product of facilitation incidence and amplitude (similar to our values in Figs 3 and 5), which should portray the overall change in rubrospinal output. We estimate that the amplitude-incidence product was increased by 340% for flexors, but decreased by 8% for extensors in their study.

Similarly, we found a preferential strengthening of medial brainstem inputs to flexor, but not extensor motor neurons. The reticulospinal tract in normal primates shows preferential facilitation of ipsilateral flexors and contralateral extensors (Davidson and Buford, 2006), and after recovery from pyramidal tract lesions, we found significant increase in (facilitatory) connection strength from both ipsilateral and contralateral medial longitudinal fasciculus to flexors, but not to extensors. This does not represent a rebalancing towards a more equal output across the motor groups, but an increased imbalance.

The similarity of the findings across these two very different descending tracts suggests a general tendency towards strengthening descending inputs to flexors over extensors after corticospinal tract lesions, irrespective of the pre-lesion bias which the tract exhibits. We have also demonstrated an increase in inputs to intrinsic hand muscles. It seems likely that these changes in brainstem connectivity not only contribute to the recovery of muscle activity that occurs following pyramidal tract lesions, but also constrain it. Stroke survivors frequently experience an initial paralysis, after which flexor muscles become increasingly active, sometimes developing into an unhelpful spasticity. In contrast, the extensors remain weak; the imbalance can result in a permanently closed hand, which is functionally useless because the fingers cannot extend to allow grasping (Twitchell, 1951). Restoration of the ability to activate extensors voluntarily is often an important aim of physical therapy (Cauraugh et al., 2000), and extensor strength can predict functional outcomes (Fritz et al., 2005).

Following a unilateral lesion of the pyramidal tract at the medulla, there can be considerable recovery of hand function (Schwartzman, 1978). This includes some precision grip movements, although there may be residual slowing in movement initiation and execution (Hepp-Reymond and Wiesendanger, 1972; Hepp-Reymond et al., 1974). In their classic study, Lawrence and Kuypers (1968a) investigated the contributions of brainstem descending pathways to functional recovery after corticospinal tract lesions by making a second lesion after recovery had reached a stable plateau. Lesions of the rubrospinal tract (lateral) had greatest effect on hand function. Lesions of the medial tracts (reticulospinal and vestibulospinal) mainly affected posture and gross movements, while hand function remained. Damage to both corticospinal and rubrospinal tracts had a much greater effect on independent fine hand control, but the hand could still be used to grip the bars of the cage to support climbing and walking (Lawrence and Kuypers, 1968a, p. 21). These data suggested that although the rubrospinal tract makes the greatest contribution to recovery of fine hand function after corticospinal lesion in monkey, some residual grip can be subserved by the medial pathways. The electrophysiological data presented here indicate that the medial brainstem pathways show plasticity, allowing them to contribute to the restoration of hand muscle activity, alongside the

previously reported changes that occur in the rubrospinal tract (Belhaj-Saif and Cheney, 2000). The reticulospinal tract is unlikely to be able to fractionate muscle activity as finely as the rubrospinal or corticospinal tracts. Reticulospinal fibres branch extensively to innervate multiple motor neuron pools: the same axon can even contact motor neurons in both lumbar and cervical segments (Peterson et al., 1975; Matsuyama et al., 1997, 1999) and on both sides of the midline (Edgley et al., 2003; Schepens and Drew, 2006; Davidson et al., 2007). This would explain why Lawrence and Kuypers (1968a) found that only gross hand movements could be generated in animals reliant on medial pathways. Previous work has postulated a role for reticulospinal connections in the recovery of more proximal movements, also with an associated loss of fractionated muscle control (Dewald et al., 1995).

Although in macaque monkeys the rubrospinal tract is an important contributor to recovery, it is unclear how far this can be extended to humans, where the rubrospinal projection is thought to be very weak (Nathan and Smith, 1955; Onodera and Hicks, 2010). It is likely that recovery of function in human patients after corticospinal damage would be even more reliant on reticulospinal pathways than in monkeys following experimental lesions.

There has been recent interest in the C3–C4 propriospinal interneuron system as potentially contributing to functional recovery after corticospinal lesion. These neurons do provide a disynaptic route by which corticospinal neurons can influence forearm motor neurons (Alstermark et al., 1999), although they may normally be subject to high levels of inhibition both in anaesthetized (Maier et al., 1998) and awake (Olivier et al., 2001) animals. After spinal lesions that disrupt the corticospinal tract at the C5 level, independent finger movements can recover, suggesting that propriospinal interneurons can relay sufficiently precise commands to motor neurons to allow fine hand control (Sasaki et al., 2004). In cat, C3–C4 propriospinal interneurons have been shown to receive input from the reticulospinal tract (Illert et al., 1981). In man, these interneurons are inhibited by cutaneous input (Nielsen and Pierrot-Deseilligny, 1991). Mazevet et al. (2003) showed that the extent of inhibition of ongoing EMG by cutaneous inputs was greater in stroke patients than in controls. They interpreted this finding as indicating that a greater proportion of the descending drive to motor neurons came from reticulospinal pathways via propriospinal interneurons. In the present work, some of the disynaptic excitation from the medial longitudinal fasciculus which we measured (Fig. 4F and G) may well have been produced by propriospinal, rather than segmental interneuronal pathways.

Based on these findings, we suggest that brainstem pathways underlie many of the changes seen following severe motor stroke in humans, restoring muscle activity at the expense of imbalance between different muscle groups, and a decreased ability for fractionated output. The reasons for the flexor bias in strengthening of connectivity following corticospinal lesions remain unknown; an understanding of the mechanisms underlying this phenomenon could inform future attempts to manipulate post-lesion plasticity to improve outcomes for patients.

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