Non-invasive mapping of corticofugal fibres from multiple motor areas—relevance to stroke recovery

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Recovery of motor function after subcortical stroke appears to be related to the integrity of descending connections from the ipsilesional cortical motor system, a view supported by the observation of greater than normal movement-related activation in ipsilesional motor regions in chronic subcortical stroke patients. This suggests that damage to the descending output fibres from one region of the cortical motor system may be compensated by activity in areas that retain corticofugal outputs. Though the trajectories of corticofugal fibres from each major component of the motor system through the corona radiata and internal capsule are well described in non-human primates, they have not been described fully in humans. Our study set out to map the trajectories of these connections in a group of healthy volunteers (8 male, 4 female; age range = 31–68 years, median = 48.5 years) and establish whether this knowledge can be used to assess stroke-induced disconnection of the cortical motor system and better interpret functional reorganization of the cortical motor system. We describe the trajectories of the connections from each major component of the motor system to the cerebral peduncle using diffusion-weighted imaging and probabilistic tractography in normal subjects. We observed good reproducibility of these connections over subjects. The comparative topography of these connections revealed many similarities between humans and other primates. We then inferred damage to corticofugal pathways in stroke patients (n = 3) by comparing the overlap between regions of subcortical white matter damage with the trajectories of the connections to each motor area. In a small series of case studies, we found that inferred disconnections could explain enhanced hand-grip-related responses, as assessed with functional MRI, in the ipsilesional motor system. These results confirm that selective disruption of motor corticofugal fibres influences functional reorganization and outcome in individual patients.

Keywords: diffusion tensor; tractography; stroke; motor recovery; functional MRI

Abbreviations: DWI = diffusion-weighted imaging; FA = fractional anisotropy; fMRI = functional MRI; M1 = motor cortex; MNI = Montreal Neurological Institute; NHPT = Nine-Hole Peg Test; PMd = dorsal premotor area; PMv = ventral premotor area; SMA = supplementary motor area


Introduction

Evidence from studies of non-human primates has challenged the traditional view of the primary motor cortex (M1) as the locus of a final common motor output signal. Observation of corticospinal fibre contributions from premotor areas, such as the ventral premotor area (PMv) and the dorsal premotor area (PMd) on the lateral surface of the hemisphere and the supplementary motor area (SMA) on the medial wall, suggests that these areas have the potential to act in parallel to generate output to the spinal cord necessary for movement (Catsman-Berrevoets and Kuypers,
Organization of corticofugal connections

This dispersal of influence on activity of spinal motor-neurons over different components of the cortical motor system is relevant to the mechanisms of recovery of motor function after stroke. Evidence from clinical studies suggests that the integrity of the descending connections from the ipsilesional motor system is critical for restitution of motor function after subcortical stroke (Fries et al., 1993; Werring et al., 1998; Pineiro et al., 2000; Werring et al., 2000). In addition, observation of increased movement-related brain activation in ipsilesional motor regions in patients with chronic subcortical hemiparetic stroke has led to the suggestion that this reflects increased reliance on undamaged ipsilesional corticofugal fibres (Seitz et al., 1998; Marshall et al., 2000; Ward et al., 2003). These findings support the view that damage to the descending output fibres from one motor cortical area can be partially compensated for by activity in another area with intact corticofugal fibres. Recent combined transcranial magnetic stimulation (TMS) and functional MRI (fMRI) findings suggest that damage to primary motor output pathways can lead to recruitment of ipsilesional motor areas such as PMd, PMv and SMA that retain intact, parallel, corticofugal fibres (Ward et al., 2006).

To establish the relationship between increased activation in components of the cortical motor system and the post-stroke integrity of their respective corticofugal outputs, it is necessary to characterize these connections non-invasively. Diffusion tensor imaging allows evaluation of the integrity of the white matter by calculation of fractional anisotropy (FA) (Werring et al., 1998; Werring et al., 2000; Pierpaoli et al., 2001) as well as mapping of probable fibre trajectories, which involves following the estimated fibre orientation of successive voxels to generate streamlines connected to chosen start points (Conturo et al., 1999; Mori et al., 1999). Mapping descending fibres in the cortical motor system is often used to validate tractography algorithms due to the size of the tract and knowledge of its trajectory in non-human primates. Many techniques have been successful in identifying connections from the cortical motor system to the cerebral peduncle (Ciccarelli et al., 2003; Guye et al., 2003; Parker et al., 2003; Johansen-Berg et al., 2004; Holodny et al., 2005; Konishi et al., 2005; Pagani et al., 2005; Parker and Alexander, 2005). The same techniques have been used to investigate how white matter damage affects corticofugal connections (Kunimatsu et al., 2003; Yamada et al., 2004; Konishi et al., 2005; Lee et al., 2005; Pagani et al., 2005). However, previous studies have not attempted to identify the trajectories of corticofugal fibres originating from separately identified components of the cortical motor system nor to estimate the degree of damage to each connection after stroke.

In this study we characterized the trajectories of connections from each major component of the motor system to the cerebral peduncle using information derived from probabilistic tractography in a group of healthy volunteers. We used methods that estimate the orientation of crossing fibres to avoid the limitations of single tensor tractography (Alexander et al., 2002; Tuch et al., 2002; Parker and Alexander, 2003; Tournier et al., 2004; Parker and Alexander, 2005). We then assessed the extent of white matter damage in patients with subcortical stroke and mapped the areas of damage onto the probable corticofugal fibre trajectories to infer quantitatively the disconnection of segregated motor areas. Finally, we considered how this inferred damage related to functional outcome and motor activation patterns measured with fMRI in these patients.

Material and methods

Subjects

Twelve right-handed healthy volunteers with no history of neurological abnormality were studied (8 male, 4 female; age range = 31–68 years, median = 48.5 years). In addition, three stroke patients were recruited from the outpatient services at the National Hospital for Neurology and Neurosurgery, London. All patients had suffered from first-ever stroke with subcortical white-matter damage >20 months previously, resulting in weakness of at least the wrist and finger extensors and interosseous muscles (motor scores <4+ on the Medical Research Council (MRC) scale), for at least 48 h after onset of symptoms. All patients were scored on the following outcome measures: (i) Barthel activities of daily living (ADL) index; (ii) motricity index (MI) for upper limb function; (iii) Nine-Hole Peg Test (NHPT); (iv) grip strength; and (v) time to complete a ten-m walk (Table 1). Maximum grip strength was measured with the same manipulandum used for MRI scanning. Grip strength was calculated as the maximum grip strength for the affected hand divided by that of the unaffected hand, expressed as a percentage (Sunderland et al., 1989). NHPT score represents pegs/second for the affected hand divided by pegs/second for the unaffected hand, expressed as a percentage.

Written consent was obtained from all participants in accordance with the Declaration of Helsinki. The study was approved by the Joint Ethics Committee of the Institute of Neurology (UCL) and National Hospital for Neurology and Neurosurgery (UCL Hospitals NHS Foundation Trust), London.

MRI protocol

All scans were performed on a 3 T Siemens Allegra MRI System (Siemens, Erlangen, Germany) using a standard transmit/receive head coil. Three data sets were acquired: diffusion-weighted echo-planar images, motor event-related fMRI and a T1-weighted structural scan.

A single-shot diffusion-weighted echo-planar imaging sequence with a double spin-echo module to reduce the effect of eddy currents was used (Reese et al., 2003). Each data volume consisted of 60 axial slices 2.3 mm thickness, with no interslice gaps, and an acquisition matrix of 96 × 96 and a field of view of 220 mm, resulting in 2.3 mm³ isotropic voxels. Interleaved slice sampling was chosen to avoid cross-talk between adjacent slices. Each diffusion-weighted imaging (DWI) data set consisted of 64 high diffusion-weighted images (b = 1000 mm² s⁻¹), with diffusion gradients applied along 64 optimized diffusion directions (Jones et al., 1999) and 7 additional images with minimal diffusion-weighting (b = 100 mm² s⁻¹). An effective b = 0 image was calculated by
extrapolation from the seven minimally diffusion-weighted images. Data acquisition was cardiac gated to reduce motion artefacts due to pulsation of the cerebrospinal fluid (Wheeler-Kingshott et al., 2002); diffusion data acquisition time was on average 25 min, depending on heart rate.

MRI data were acquired in three sessions during which subjects performed a dynamic isometric hand-grip task using either their right or left hands only, or either hand in a randomized counterbalanced order. Hand grips were performed using an MRI-compatible manipulandum as described previously (Ward et al., 2003). The dynamic change in grip force was projected in real time onto a screen as a column whose height varied linearly with change in voltage measured from the manipulandum and hence force. Before scanning, but whilst lying in the scanner, subjects were asked to grip the manipulandum with maximum force to generate a maximum voluntary contraction (MVC). These measurements were made separately for each hand. The target force for each hand was set at 20% of the corresponding MVC for each subject and was indicated by a horizontal bar on the screen. Each hand grip was cued by the appearance of an arrow at the bottom of the display. The arrow cued the hand required to make the grip and only the grip force level for the cued hand was presented on the screen. The volunteers were instructed to make a single brief hand grip, to be continued until the column representing force applied came into contact with the horizontal bar on the screen, at which point the grip could be released.

Single-hand grip sessions consisted of 24 cued events (hand grips) and 24 null events, whereas in the session in which subjects made hand grips with either hand there were 24 hand grips with each hand and 24 null events. These events were presented in a randomized and counterbalanced order with a cue onset asynchrony of 5.7 s. Before scanning, subjects were trained until comfortable with the task. Participants held the manipulandum in both hands during each session, enabling identification of any mirror movements.

The functional data acquired during motor task performance consisted of T2*-weighted MRI transverse echo-planar images [echo time (TE) = 30 ms] with blood oxygenation level-dependent (BOLD) contrast. Each echo-planar image comprised 48 contiguous axial slices (2 mm thick with an inter-slice gap of 1, 3 mm in-plane resolution) positioned to cover the whole cerebrum with an effective repetition time (TR) of 3.12 s per volume. The single-hand grip sessions consisted of 105 volumes acquired continuously; the session in which grips of either hand were cued comprised 140 volumes. The first 6 volumes were discarded to allow for T1 equilibration effects.

In addition, a T1-weighted structural image was acquired using an optimized 3D MDEFT sequence with an isotropic spatial resolution of 1 mm (Deichmann et al., 2000) and special RF pulses to compensate for the transmit coil’s inhomogeneities (Deichmann et al., 2000).

Pre-processing of DWI data

The diffusion tensor eigenvalues and eigenvectors were calculated from the DWI data set for each control subject and patient using a conventional single tensor analysis, and FA maps were generated (Pierpaoli and Basser, 1996).

In addition, the DWI data from the 12 control subjects were used to map probable corticofugal connections of the motor system with probabilistic tractography. First, we use the algorithm of Alexander et al. (2002) to identify fibre crossings. The algorithm fits a spherical harmonic model to the apparent diffusion coefficient as a function of gradient direction. The resulting series is truncated at order 0, 2, or 4 using the analysis of variance test for deletion of variables to select the best model in each voxel. The F-statistic thresholds used for model truncation are the same for all individuals in the study under the assumption of similar noise levels in all data sets (Alexander et al., 2002; Parker and Alexander, 2003). Truncation at order 0 indicates isotropic diffusion, whereas truncation at order 2 indicates that the single tensor model is a good approximation. When fourth-order terms are included in the series, the single tensor fit is poor and a two-tensor model is used: the principal directions of the two diffusion tensors provide estimates of the orientations of the crossing fibres, as described in Parker and Alexander (2003).

Generation of transforms between native DWI space and standard MNI space

The transform between native DWI space into standardized space, as defined by the Montreal Neurological Institute (MNI), was estimated by first co-registering the T1 structural scan of each subject with their extrapolated $b = 0$ map. This co-registered structural image was then normalized using an integrated spatial normalization and segmentation routine implemented in SPM5 (Wellcome Department of Imaging Neuroscience—www.fil.ion.ucl.ac.uk/spm), with white and grey matter prior probability maps (Ashburner and Friston, 2005). The estimated normalization parameters were then applied to the FA maps both of the control group and the three stroke patients. All normalized images were re-sampled to 2-mm isotropic voxels. The generation and applications of these transforms to the data sets are detailed in Supplementary Fig. 1.

Tractography seed region selection

The seed regions for tractography were chosen by defining the cross-section of the cerebral peduncle on the $z = –16$ mm plane of the normalized FA map and T1-weighted structural image for
each subject, as shown in Fig. 1A, using MRICro (Rorden and Brett, 2000). The seed region encompassed all white matter voxels in the cross-section as identified on the FA map, corresponding to the crus cerebri, though owing to the resolution of the images some seed voxels may have included parts of the substantia nigra (Duvernoy, 1995). The initial selection of seed points was made in standard MNI space to ensure that the peduncles were defined at an equivalent level across all individuals. The resulting region of interest was then transformed into native space, using the inverse of the normalizing transformation described above.

**Definition of cortical target areas**

The four major cortical areas known to contribute to the descending motor tracts were defined using cortical landmarks on the co-registered T1-weighted structural image of each control subject. M1 was defined as the anterior bank of the central sulcus, the paracentral lobule on the medial convexity and the exposed cortical surface of the precentral gyrus. This was limited by a rostral border formed by a line running from the vertex of the precentral sulcus at the longitudinal cerebral fissure to the vertex of the central sulcus at the level of the junction of the superior frontal sulcus with the precentral sulcus (Geyer et al., 2000). PMd was defined as the posterior bank of the superior precentral sulcus and the remainder of the portion of the precentral gyrus posterior to it, whereas PMv was defined as the posterior bank of the inferior precentral sulcus and the exposed cortical surface of the precentral gyrus posterior to it. The SMA was defined as the medial cortex lying above the cingulate sulcus and caudal to the line drawn through the anterior commissure perpendicular to the anterior commissure-posterior commissure line. The regions defined for a single subject are shown rendered on their T1-weighted structural image in Fig. 1B.

**Fibre tracking**

The Probabilistic Index of Connectivity (PICo) algorithm (Parker and Alexander, 2003, 2005; Parker et al., 2003) was used to trace the tracts connected to the cross-section of the cerebral peduncle of each hemisphere in the native image acquisition space of each subject. This method involves repeated iterations of the streamline process using Monte Carlo methods to exploit the inherent uncertainty in the orientation of the principal diffusion direction(s) defined for each image voxel. The procedure provides maps of the probability of a connection from the seed point to a chosen area (Parker and Alexander, 2003, 2005; Parker et al., 2003). Before tracking, probability density functions (PDFs) are generated from the diffusion tensor(s) for each voxel, which provide voxel-wise estimates of confidence of the fibre tract orientation. For each iteration, a streamline is propagated through the multi-tensor field derived from the PDFs for that iteration. Stopping criteria prevent biologically implausible curvature of streamlines (>180° on the scale of a single voxel) or attempts to transit non-brain voxels.

Here, 1000 streamlines were used to identify the routes of connection from each seed point in the peduncle, through the multi-tensor field, to the cortical motor system areas above. For each of the four cortical areas, maps were formed in which the intensity of each voxel reflected the number of streamlines that crossed that voxel en route to the respective cortical area.

The maps of the total streamline densities between the peduncle and each of the cortical areas were then thresholded to form binary masks showing voxels that had been hit by at least one streamline from the peduncle. The resulting masks were transformed into MNI space using the normalization transforms determined previously. The normalized masks for individual control subjects were then summed to produce trajectory variability maps, in which the voxel intensities ranged from 1 to 12. These values represent the degree of spatial variability and overlap of connections between subjects (Ciccarelli et al., 2003; Pagani et al., 2005; Parker et al., 2005; Ramnani et al., 2005). Finally, the maps generated for the connections from the peduncle in the left and right hemispheres were then combined.

To aid visualization, the four area-specific trajectory variability maps were thresholded so that only voxels that were common to the tracts of eight or more subjects were retained. The ensuing masks were then coded and combined, generating two maps showing the location and overlap of the most common location of tracts connecting the peduncle with (i) M1, PMd and SMA; (ii) PMd, PMv and SMA. The threshold of eight or more subjects, equivalent to two-thirds of the sample, was chosen, as this enabled visualization of the overlap of the most common connections to each of the cortical areas while facilitating the description of the relative locations of these connections. When the threshold is raised to 10 or lowered to 6 or more subjects the same general patterns are still visible as shown in Supplementary Fig. 2.

**Patient versus control group comparisons**

All patient versus control group comparisons were performed with SPM2 (www.fil.ion.ucl.ac.uk/spm).
Fractional anisotropy
The normalized FA maps were smoothed with an isotropic Gaussian kernel of 8-mm full-width half-maximum (FWHM). The patients were compared individually with the group of 12 control subjects on a voxel-wise basis using a one-way ANOVA (analysis of variance). Significant differences in anisotropy were detected at a threshold of $P < 0.001$ (uncorrected) with a minimum cluster size of 10 voxels. These clusters then formed regions of interest that were overlaid onto the trajectory variability maps of the four motor areas. The transaxial slice on which there was the greatest overlap was determined (Pineiro et al., 2000). The number of voxels in the region of overlap was expressed as a proportion of the total voxels forming the tract (at the threshold of eight or more subjects) on that particular transaxial section.

In addition, the outer surfaces of the clusters of reduced FA were defined and the surfaces were overlaid on maps showing overlap of the most common locations of tracts connecting the peduncle with M1, PMd and SMA.

fMRI
All volumes were co-registered to the first volume and then unwarped to allow for echoplanar imaging (EPI) distortions due to head movement during scanning (Andersson et al., 2001). To correct for different acquisition times, the signal measured in each slice was shifted in time relative to the acquisition of the middle slice using sinc interpolation. The resulting volumes were then normalized to a standard EPI template based on the MNI reference brain and re-sampled to 3-mm isotropic voxels. All normalized images were then smoothed with an isotropic 8-mm FWHM Gaussian kernel. The time series in each voxel were high-pass filtered at 1/128 Hz to remove low-frequency confounds and scaled to a grand mean of 100 over voxels and scans within each session.

Statistical analysis was performed in two stages. In the first stage, a single-subject fixed effects model was used. Hand grips of each hand were modelled as delta functions for each of the three sessions. The resulting covariates were each convolved with three basis functions: the canonical haemodynamic response function (HRF) and its temporal and dispersion derivatives (Friston et al., 1998; Henson et al., 2002). These covariates were used in a general linear model together with a single covariate representing the mean (constant) term over scans. Parameter estimates for each covariate were calculated using maximum likelihood. Contrasts of the parameter estimates encoding the effects of all hand grips of each hand were formed. These contrasts selected the effects explained by the canonical HRF and its temporal and dispersion derivatives, pooled over the three sessions. These contrast maps summated the grip-related responses for each subject.

In the final stage of analysis, the contrasts for each subject were entered into second (between-subject) level one-way ANOVAs to compare the fMRI responses to hand grips with the paretic hand in individual patients with responses to movement of the same hand for all control subjects. Linear contrasts were then used to identify voxels that showed greater activation for hand grips in each patient. As we were specifically interested in movement-related signal changes in the cortical motor system, the cortical motor areas defined for each control subject were transformed into standard space to form a search volume for each hemisphere. Voxel-wise $t$-tests were used to identify significant differences in hand-grip-related activation at a threshold of $P < 0.001$ (uncorrected) within each search volume. Additionally, the SPM$|t|$s were thresholded with a family-wise error of $P < 0.05$ (corrected) for more stringent identification of activation differences.

Results
Normal anatomy of the connections between the cerebral peduncle and the cortical motor system
In all subjects, probable connections were successfully tracked from the cerebral peduncle seed points to M1, PMd and SMA, whereas connections to PMv were tracked in 10 of the 12 control subjects. There was good reproducibility of the trajectories between the peduncle and each cortical area across subjects, as demonstrated by the trajectory variability maps shown in Figs 2, 3, 4 and 5. Figure 6 and Supplementary Fig. 3 shows a high degree of overlap between the putative connections from each of the cortical areas, particularly at more inferior levels. The differential topography of connections to each of the cortical areas will now be described. Though the putative routes of the descending connections have been derived from a probabilistic multi-fibre streamline approach initiated in the cerebral peduncles, the anatomy of these connections will be presented in the anterograde direction.

Trajectory of connections between the cerebral peduncle and M1
In the series of axial sections immediately inferior to the cortex, we found that connections between the cerebral peduncle and M1 rapidly form a concentrated bundle as they enter the corona radiata (Fig. 2). On entering the corona radiata they maintain a fixed location in successive inferior sections, suggesting that fibres descending from this region stream vertically through the corona radiata. At the level of the internal capsule, the peduncular–M1 connections are located between the posterior portion of the putamen and the thalamus in the posterior limb of the internal capsule and continue to suggest a vertical course of the related fibres. In both the corona radiata and internal capsule the connections between the cerebral peduncle with M1 are located most posteriorly of all the motor system connections. Connections to M1 are located laterally in the cerebral peduncle, though there is a strong overlap with the connections to PMd, and more medially with the SMA (Fig. 6).

Trajectory of connections between the cerebral peduncle and PMd
As with the peduncular–M1 connections, the concentration of connections immediately inferior to the cortex suggests a rapid aggregation of fibres from the PMd region as they enter the corona radiata. Once in the corona radiata, they also run vertically, located anteriorly to connections originating from M1, though there is a large degree of overlap between connections from these two precentral areas (Fig. 6). As the connections from PMd enter the internal capsule, the most
anterior connections overlap with those of the SMA (Fig. 6) and PMv (Supplementary Fig. 3), whereas the most posterior connections overlap with M1 connections. Connections to PMd are also located laterally in the cerebral peduncle, though there is considerable overlap with the origins of connections reaching each of the motor cortical areas (Fig. 6).

**Trajectory of connections between the cerebral peduncle and PMv**
Connections between the peduncle and PMv were identified in 10 of the 12 control subjects. The consistency of trajectories within this subset of subjects was high and revealed a continuous tract between the peduncle and the cortex (Fig. 4). However, after thresholding of the variability maps to visualize the voxels through which connections passed in eight or more individuals, a common route was observed between the peduncle and the inferior level of the corona radiata, though not to the cortex (Supplementary Fig. 3). As shown in Fig. 4, there is a rapid medial shift of connections immediately inferior to the PMv. The resulting bundle tends to be located more anteriorly than the majority of PMd fibres, though overlap does occur (Supplementary Fig. 3). The trajectory of peduncle–PMv connections follows a mostly vertical route through the corona radiata and posterior limb of the internal capsule, with an anterior shift relative to the majority of M1 and PMv connections visible at these levels. At the level of the peduncle the PMv connection origins do not extend as laterally as those of M1 and PMd (Supplementary Fig. 3).

**Fig. 2** Trajectory variability maps for the peduncle/M1 connections shown on representative sections of the average of the T1-weighted structural. Colour bar shows number of subjects with connections in each voxel: range = 2–12. The top panel shows sagittal slices $x = -24$ and $x = +24$ either side of coronal slice $y = -18$. The lower two panels show transverse slices. The right side of the brain is depicted on the right side of the transverse and coronal images.

As shown in Fig. 4, there is a rapid medial shift of connections immediately inferior to the PMv. The resulting bundle tends to be located more anteriorly than the majority of PMd fibres, though overlap does occur (Supplementary Fig. 3). The trajectory of peduncle–PMv connections follows a mostly vertical route through the corona radiata and posterior limb of the internal capsule, with an anterior shift relative to the majority of M1 and PMv connections visible at these levels. At the level of the peduncle the PMv connection origins do not extend as laterally as those of M1 and PMd (Supplementary Fig. 3).

**Trajectory of connections between the cerebral peduncle and the SMA**
Connections to the SMA are located progressively more laterally in successive axial slices at the levels below the SMA until the approximate level of the lateral ventricles (Fig. 5).
In addition, the SMA connections shift in a posterior direction on subsequent inferior axial planes from the cortex to the peduncle, suggesting that fibres from the SMA course caudally and inferiorly in the subcortical white matter. The route of the SMA connections passes close to the caudal portion of the head of the caudate in the genu and anterior limb of the internal capsule at its most superior level, before passing over the putamen and shifting to the posterior limb of the internal capsule at more inferior levels. SMA connections tend to be located anterior to those of any of the three precentral regions, though there is some overlap of these connections with those of other non-primary regions, as well as with M1 connections, though only at inferior levels of the internal capsule and the peduncle (Fig. 6). At the level of the cerebral peduncle, the SMA-connected seed points tend to be found in more ventral and medial locations than those of the precentral region.

**Structural consequences of damage to the descending motor fibres**

For each patient, voxel-wise comparison of FA with the control group revealed focal reductions in anisotropy (Table 2). All patients showed relative FA reductions in the internal capsule and/or corona radiata, which extended beyond the margins of the lesion(s) as seen on the corresponding T1-weighted structural image. Figure 7 illustrates the intersection of FA reductions in each patient with the combined trajectory maps, and shows voxels through which streamlines pass to each cortical area for eight or more control subjects. The maximal overlap of damaged white matter on the most common connections to each cortical area is reported for each patient in Table 3.

As shown in Fig. 7A and Table 3, the region of reduced anisotropy in the corona radiata of Patient A intersected mostly with the connections of M1 and PMd. There was...
minimal overlap with SMA projections located rostral to this region of reduced FA. No voxels at the level of this damaged area were common to the PMv trajectories of eight or more subjects. In addition, the other region of significantly reduced anisotropy in Patient A, located at the level of the internal capsule, did not overlap with any motor system connections (Fig. 7A). In Patient B, there was significant overlap between the region of reduced FA and connections to all precentral regions. The highest proportion (80%) of damaged voxels overlapped with connections to PMv and no more than 10% to SMA (Table 3, Fig. 7B). The region of reduced FA in Patient C was located anterior to connections from the precentral components of the motor system; there was minimal overlap with SMA projections (Table 3, Fig. 7C).

In summary, the subcortical lesions (as inferred by analysis of FA) in Patient A interrupted more posterior trajectories connecting to M1 and PMd. Patient B had more extensive damage anteriorly, implying a disconnection of PMv, as well as M1 and PMd. The lesions in Patient C were even more anterior and spared the connections to the motor areas we considered. We therefore predicted that Patients A and B would show abnormal patterns of cortical responses in the ipsilesional motor system and that Patient C would show no abnormalities. This is largely what we observed.

Functional consequences of damage to the descending motor fibres

Comparison of the activations associated with grip of the affected hands in individual stroke patients with activations in the control group revealed only relative increases in task-related activation in the patients (Table 2). In Patient A, there were two clusters of overactivation in the ipsilesional
precentral sulcus in regions corresponding to rostral PMd in the depth of the sulcus, extending into the middle frontal gyrus, and PMv (though this cluster was only significant at uncorrected \( P < 0.001 \) level) (Fig. 8A). In Patient B, peaks of relative overactivation were observed on the ipsilesional precentral gyrus, encompassing portions of PMd and M1 (though not the hand knob region) as shown in Fig. 8B, and contralesional PMv. The activation pattern of Patient C did not significantly differ from those of the control group.

**Discussion**

**Anatomical findings**

We have demonstrated, in standard anatomical space, the most probable trajectories of the corticofugal connections of the major divisions of the cortical motor system using high-angular resolution DWI and probabilistic tractography in a group of healthy adults. These findings are in agreement with previous clinical and anatomical findings in humans (Englander et al., 1975; Ross, 1980; Fries et al., 1993; Seitz et al., 1998; Pineiro et al., 2000; Wenzelburger et al., 2005) and reveal many similarities between the organization of these connections in humans and other primates, as investigated with neuronal tracer methods (Fries et al., 1993; Morecraft et al., 2002).

The position and degree of overlap of corticofugal fibres from each cortical motor area, particularly in the internal capsule, have been of interest for many years as a means of furthering understanding of the relationship between localized white matter damage and restitution of motor function. We found that the connections from M1 to the cerebral peduncle passed through the posterior limb of the internal capsule in regions corresponding to rostral PMd in the depth of the sulcus, extending into the middle frontal gyrus, and PMv (though this cluster was only significant at uncorrected \( P < 0.001 \) level) (Fig. 8A). In Patient B, peaks of relative overactivation were observed on the ipsilesional precentral gyrus, encompassing portions of PMd and M1 (though not the hand knob region) as shown in Fig. 8B, and contralesional PMv. The activation pattern of Patient C did not significantly differ from those of the control group.
capsule, as previously found in anatomical (Englander et al., 1975; Ross, 1980) and tractography-based (Guye et al., 2003) studies in humans, as well as in tracer studies in non-human primates (Fries et al., 1993; Morecraft et al., 2002). The connections we found linking PMd to the peduncles also ran through the posterior limb, though, in agreement with tracing-derived evidence, these connections tended to be located more anteriorly than those linked to M1 (Morecraft et al., 2002). In contrast to earlier findings in the non-human primates, we did not find evidence of PMv fibres transiting the genu of the internal capsule (Morecraft et al., 2002), though we did find that PMv connections occupied more anterior locations compared with the majority of those linked to PMd. In contrast, connections to the SMA were found to pass through the genu and anterior limb of the internal capsule at superior levels followed by a gradual shift to the posterior limb on more inferior sections—an observation that parallels that of previous tracer studies (Fries et al., 1993; Morecraft et al., 2002).

Correct identification of neuronal pathways with tractography is dependent on accurate classification of the diffusion profile in each voxel of the brain, as this model will determine the possible directions sampled by the tractography algorithm. When tracing descending fibres of the

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**Table 2** Single patient versus control group comparisons

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<th>Patient</th>
<th>Significant decreases in anisotropy</th>
<th>Significant increases in fMRI activation</th>
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</tbody>
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The table shows regions in which there were significant decreases in anisotropy (left) and significant increases in task-related activation between each stroke patient and the control group. Coordinates represent peak voxels within significant clusters. Significance was set at $P < 0.001$ (uncorrected) for clusters of 10 or more voxels for the anisotropy analysis. For the fMRI analysis, significance was defined at $P < 0.05$ corrected in search volume consisting of cortical motor system areas of relevant hemisphere.

†Cluster not significant with correction (FWE, $P < 0.05$)—significant uncorrected $P < 0.001$; L = left; R = right; CR = corona radiata; IC = internal capsule.
motor system, some streamlines would be expected to encounter voxels where corticospinal fibres are crossing other tracts such as the superior longitudinal fasciculus (SLF) or transcallosal fibres. Here, the probability density functions describing the possible diffusion directions in each voxel were based on either a single tensor or bi-tensor model, depending on the best fit to the data (Alexander et al., 2002; Parker and Alexander, 2003). This method

Fig. 7 Sections showing the regions of reduced anisotropy in each patient (black outline) overlaid on the maps of the overlap between the thresholded trajectory variability maps (eight or more subjects) for M1, PMd and SMA shown on the T₁-weighted image for the respective patient. Colour key as for Fig. 6.

Table 3 Details of maximal overlap between lesion and peduncle–motor system connections

<table>
<thead>
<tr>
<th>Patient</th>
<th>Section (z)</th>
<th>In lesion</th>
<th>On section</th>
<th>Prop</th>
<th>Section (z)</th>
<th>In lesion</th>
<th>On section</th>
<th>Prop</th>
<th>Section (z)</th>
<th>In lesion</th>
<th>On section</th>
<th>Prop</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>36</td>
<td>6</td>
<td>21</td>
<td>0.29</td>
<td>34</td>
<td>12</td>
<td>32</td>
<td>0.38</td>
<td>All</td>
<td>–</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>B</td>
<td>28</td>
<td>6</td>
<td>26</td>
<td>0.23</td>
<td>22</td>
<td>10</td>
<td>36</td>
<td>0.28</td>
<td>22</td>
<td>8</td>
<td>10</td>
<td>0.80</td>
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<tr>
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<td>–</td>
<td>0.00</td>
<td>All</td>
<td>0</td>
<td>–</td>
<td>0.00</td>
<td>12</td>
<td>1</td>
<td>29</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The table shows the number of voxels that formed the tract connecting the peduncle to each cortical area in eight or more subjects on the transaxial section on which there was the greatest overlap between the respective tract and the area of reduced anisotropy (‘On section’ values). The number of voxels in the area of overlap is also shown (‘In lesion’ values). The degree of overlap is also expressed as a proportion (‘Prop’) of ‘In lesion’ voxels divided by ‘On section’ voxels. Hence, if ‘Prop’ = 1 on a particular transaxial section then the entire cross-section of the tract to the respective cortical area overlaps with the area of reduced anisotropy. If ‘Prop’ = 0, then there is no overlap between the damaged area and the tract on any transaxial section. The transaxial section where this overlap was greatest for each cortical area and patient is also reported [‘Section (z)’] where z = z-coordinate in MNI space.
has been shown to resolve two fibre populations in the relevant region of the corona radiata likely to contain crossing of the descending fibres and either the SLF or transcallosal fibres (Alexander et al., 2002; Parker and Alexander, 2003). However, using more recently developed methods, a smaller number of voxels in this region have been modelled as the site of crossings of all three fibre populations (Tuch et al., 2003; Parker and Alexander, 2005; Tuch et al., 2005). Such voxels would have been incorrectly modelled in the current study; this would have resulted in poorly informed dispersion of streamlines at these points. This may have resulted in highly variable ‘connections’ in individuals, though it is also conceivable that incorrect pathways may have been reliably identified in the group data, as such crossings may occur in similar locations across individuals. However, as the number of voxels classified as the site of three fibre populations appears fairly low, we may assume that use of this simpler multi-tensor approach would have provided reasonable approximations of the location of the descending connections of the cortical motor system.

The presence of incorrectly modelled voxels containing crossings of the descending fibres, SLF and transcallosal connections would most likely affect the correct identification of connections to the most lateral components of the cortical motor system such as PMv. Streamlines destined for the PMv that pass through such voxels may be diverted to other cortical areas or may not reach the cortex owing to excessive curvature of the streamline route resulting from the incorrect model of the diffusion profile. These scenarios may account for why peduncle–PMv connections were not identified in two of the control subjects, as well as the increased variability in the location of these connections close to the cortex in the other 10 subjects.

Though our findings suggest strong similarities between the organization of the multiple corticofugal pathways in humans and other primates, it must be noted that the proportions of fibres connecting the peduncle to each cortical region will differ between humans and other primates owing to differences in the relative size of these regions; for example, the proportion of prefrontal connections in the peduncle of humans has been shown to exceed that of the macaque in parallel with an increase in grey matter volume of the prefrontal cortex (Ramnani et al., 2005). This observation may explain why PMv connections were not observed to transit the genu of the internal capsule in the current study, in contrast with the results of tracer studies (Fries et al., 1993; Morecraft et al., 2002). As assessed using tractography, the relative contribution of the premotor cortex to the cerebral peduncle is significantly lower in humans compared with macaques, whereas the opposite relation was seen for the contribution of the prefrontal cortex (Ramnani et al., 2005). The shift in the balance between prefrontal and premotor cortices may result in a posterior shift of the anterior limit of the premotor connections in the internal capsule. This suggestion is supported by the trajectory variability map of the peduncle–prefrontal connections that suggest that these fibres pass through the anterior limb and the genu of the internal capsule in humans, though only through the anterior limb in the macaque cases (Ramnani et al., 2005).

Comparison of the trajectories of corticofugal connections described here in humans with invasive tracing data from other primates is also complicated by differences in the techniques used. An important limitation of DWI measurements and the tractography approach is the inability to distinguish afferent and efferent pathways of axonal tracts. Therefore, when using tractography to identify connections between the peduncle and the cortical motor areas we may also be identifying inputs to the cortical areas as well as the descending fibres. However, as such inputs are multi-synaptic, it seems unlikely that such connections are strongly represented in this data, as tracking through grey

Fig. 8 fMRI results for Patients A and B shown on individual patient’s volume-rendered T1-weighted images. (A) Regions of significantly greater left (affected) hand-grip activation for Patient A compared with the control group. Cut-out reveals cluster in right PMd in the depth of the precentral sulcus (x = 33, y = −3, z = +42). (B) Region of significantly greater right (affected) hand-grip activation for Patient B compared with the control group. Cut-out reveals cluster in left precentral gyrus (x = −48, y = −3, z = +51). CS = central sulcus, PCS = precentral sulcus, HK = hand knob region of M1.
matter is subject to high uncertainty due to the lack of directional microstructure. This same argument applies to inclusion of portions of substantia nigra tissue in the tractography seed region. In addition, examination of the connections identified here does not reveal consistent pathways that pass through the subcortical grey matter. Hence, the connections observed with this protocol are most likely to be corticofugal pathways, though in future studies it may be prudent to define subcortical grey matter areas and discard any streamlines that pass through these regions.

Investigations of subcortical routes of corticofugal fibres have employed anterograde tracing techniques that involve injection of tracer compounds into limited portions of each motor cortical area. Both studies limited the initial labelling to specific limb representations—either those of the arm as localized by intracortical mapping (Morecraft et al., 2002), or various combinations of hand, face and foot representations (Fries et al., 1993). Even in the latter case the circumscribed nature of tracer injections results in a narrower definition of the trajectories of corticofugal fibres from each area than that obtained with tractography. Here, tractography identified the route of streamlines from the cerebral peduncle to the total extent of each cortical region, encompassing connections to all representations simultaneously. Therefore, we expect the overlap of fibres to be greater than that in tracer studies. We predict that use of functionally defined representations of arm or hand movements as target cortical areas in future studies will reveal an even more distributed organization of these multiple pathways.

The relative compression and extensive overlap of specific corticofugal pathways at inferior brain levels compared with the more distributed arrangement seen at superior levels, as described in non-human primates using tracing methods (Morecraft et al., 2002), is replicated here using probabilistic tractography. The maps of connectivity generated using tractography are formed by iterations of the streamline process that sample the uncertainty in the orientation of the principal directions of diffusion in each voxel (Behrens et al., 2003; Parker and Alexander, 2003, 2005; Parker et al., 2003; Tournier et al., 2003). Hence, streamlines are dispersed further owing to the propagation of uncertainty as they pass through successive voxels. Stopping criteria are used to prevent biologically implausible curvature of streamlines (>180° on the scale of a single voxel) or attempts to transit non-brain voxels. The continual dispersion of streamlines may result in overestimation of overlap between connections from different cortical motor areas. Our observation of distinct and consistent topographic organization in the internal capsule and corona radiata suggests that the use of probabilistic tractography does not result in gross overestimation of the spread of corticofugal fibres as would be expected with greater dispersion at superior levels. Tractography methods will never achieve the resolution of tracing studies owing to the partial volume effect inherent in the magnetic resonance imaging technique. However, by constructing maps of the most likely routes of connections between brain regions and the peduncle across a group of subjects, we have reduced the influence of low-probability connection routes.

### Relating tract damage to functional recovery and activation changes after stroke

Definition of the locations of the most common motor corticofugal fibres in a group of healthy control subjects was used to infer damage to these connections in patients with lesions restricted to the subcortical white matter. Superimposition of regions of reduced anisotropy in individual patients on these trajectory maps revealed varying proportions of damage to corticofugal pathways from each component of the cortical motor system. In the patients we studied, we found that greater damage to the connections is related to increased hand-grip-related activity in the ipsilesional motor system.

Patients B and C had very similar outcome profiles despite considerable disparity in the amount of damage to corticofugal pathways. In contrast to Patient B, the site of lesion in Patient C showed minimal overlap with putative corticofugal fibres from all cortical motor regions. The initial motor function deficits in both patients were also very similar—both showed only minimal motor impairment. In the case of Patient C, the site of lesion suggests that the acute symptoms may have been the result of damage to basal ganglia circuitry rather than to corticofugal fibres. The opposite was the case for Patient B. Increased ipsilesional motor system activity in Patient B but not C suggests that redundancy in the motor corticofugal connections may be responsible; in Patient B, cortical neuronal activity may have increased in motor areas to drive intact corticofugal connections.

In Patients A and B, considerable damage to connections from the precentral gyrus to the peduncle was observed. Patient A, who had the worst initial motor deficit and subsequent recovery, also had the greater damage to connections to PMd and M1, though the difference in M1-related damage was minimal between the two individuals. Increases in hand-grip-related activity were found in ipsilesional PMd in both patients—in the precentral sulcus in Patient A and on the vertex of the precentral gyrus in Patient B, though in this patient the overactivation also included portions of M1 but excluded the hand knob region. Patient A also showed relative overactivation of ipsilesional PMv, though this was only observed at a less stringent statistical threshold. The finding of overactivation of parts of M1 in Patient B, coupled with the lack of significant deactivation within M1 in either Patient A or B, suggests that recovery of function is probably related to intact descending fibres from M1. The importance of M1 fibres for restitution of hand dexterity was recently demonstrated by Wenzelburger et al. (2005); they showed that in patients with capsular stroke greater chronic motor deficits were associated with more posterior lesion locations.
Organization of corticofugal connections

within the posterior limb of the internal capsule and attributed this finding to the greater density of pyramidal fibres from M1 in these posterior locations. However, the over-activations in ipsilesional PMd and PMv support the view that non-primary motor areas also play a role in restitution of normal motor function.

Damage to the connections was quantified by finding the maximum overlap between areas of reduced anisotropy and the locations of tracts connecting the peduncle with each of the cortical motor areas on a single axial section. This is an approximate measure of the interaction between lesion and connections, as the geometry of a lesion relative to the axial sections is not considered and may result in an underestimation of overlap. Previous studies using tractography in stroke patients have used qualitative description of lesion location relative to the corticospinal tract as defined by traced pathways in that individual patient. This approach identified relationships between the overlap of lesion and tract and measures of motor outcome (Kunimatsu et al., 2003; Yamada et al., 2004; Lee et al., 2005; Konishi et al., 2005). However, it may be difficult to assess the full extent of the corticospinal tract with tractography in these patients by virtue of the diffusion properties of their lesions. For example, use of simple streamline-based methods where tracking is terminated when pixels with low FA (defined as FA < 0.3) are encountered may exclude damaged connections from the final traced tract (Yamada et al., 2004; Lee et al., 2005; Konishi et al., 2005). Here, we did not use tractography directly to assess the influence of lesions on corticofugal fibres of the motor system reconstructed in each patient owing to the potential complications in the quantitative interpretation of probabilistic connectivity maps in the presence of reduced FA, as streamlines transiting such areas are subject to greater uncertainty and hence dispersion.

The projections identified here are likely to include corticospinal fibres, corticopontine and corticobulbar connections, though the contributions of these specific projections cannot be segregated with this technique. Corticospinal projections with their direct or multi-synaptic connections to hand motoneurons are important for driving motor output (Porter and Lemon, 1993). Each of the premotor areas investigated have substantial corticospinal projections (Dum and Strick, 1991; He et al., 1993; He et al., 1995; Dum and Strick, 1996). Though the premotor areas are similar to M1 in this respect, it appears that direct projections from SMA to spinal cord motoneurons, for example, are less numerous and less able to generate an excitatory response than those from M1 (Maier et al., 2002).

Clearly, the findings from this small set of patients are not intended to elucidate fully the relationships between amount of fibre damage from motor cortical areas, clinical outcome and changes in motor-related activation patterns seen in any patient with subcortical stroke. Such an analysis will require data from a considerably larger number of patients. Future use of tractography-based inference about damage to corticofugal fibres coupled with functional activation patterns and clinical outcome scores raises the possibility of defining a hierarchy of neural substrates for recovery. Given a lesion in a particular location it may be possible to estimate the initial deficit and potential degree of recovery in patients as a result of altered functional activity in intact frontal motor system components.

Supplementary material

Supplementary material cited in this article is available at Brain online.

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