A debilitating consequence of complete spinal cord injury (SCI) is the loss of motor control. Although the goal of most SCI treatments is to re-establish neural connections, a potential complication in restoring motor function is that SCI may result in anatomical and functional changes in brain areas controlling motor output. Some animal investigations show cell death in the primary motor cortex following SCI, but similar anatomical changes in humans are not yet established. The aim of this investigation was to use voxel-based morphometry (VBM) and diffusion tensor imaging (DTI) to determine if SCI in humans results in anatomical changes within motor cortices and descending motor pathways. Using VBM, we found significantly lower gray matter volume in complete SCI subjects compared with controls in the primary motor cortex, the medial prefrontal, and adjacent anterior cingulate cortices. DTI analysis revealed structural abnormalities in the same areas with reduced gray matter volume and in the superior cerebellar cortex. In addition, tractography revealed structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects. In conclusion, human subjects with structural abnormalities in the corticospinal and corticopontine tracts of the SCI subjects.
hypothesize that SCI will be associated with significant decrease in gray matter volume and increased water movement with the primary motor cortex and that these changes will be greatest in the region of primary motor cortex that innervates the paralyzed part of the body (the paracentral lobule). Furthermore, we hypothesize that the descending corticospinal tract fibers will display changes in their diffusion properties indicative of significant neural loss.

Methods

Subjects

Fifteen male subjects with SCI (mean age 41 ± 3 [standard error of the mean]; range 26–57) and 27 male control subjects without SCI (mean age 37 ± 3; range 23–60) were recruited. All SCI subjects had complete (American Spinal Injury Association Impairment Classification A) SCIs between T1 and T10 and were 24–590 months post-SCI (mean 150 ± 33 months) (Table 1). Informed written consent was obtained for all procedures, and the study was approved by Institutional Human Research Ethics Committees of Northern Sydney Central Coast Area Health, the University of Sydney and the University of New South Wales.

MRI Acquisition

Subjects lay supine on the bed of a 3T MRI scanner (Philips, Acheiva, the Netherlands) with their head immobilized in a head coil. For each subject, TR-weighted and DTI scans were acquired, with repeated acquisitions to improve signal-to-noise ratios. Three high-resolution 3-dimensional TR-weighted anatomical image sets, covering the entire brain, were collected (turbo field echo; time echo = 2.5 ms, time repetition [TR] = 5600 ms, flip angle = 8°, voxel size 0.8 × 0.8 × 0.8 mm). The DTI data were acquired with a whole-brain single-shot, spin-echo echo-planar pulse sequence (TR = 8788 ms; flip angle = 90°, 112 × 112 matrix size, 224 × 224 mm field of view, 2.5 mm slice thickness, 55 axial slices), with 4 image sets collected for each subject. For each slice, diffusion gradients were applied along 32 independent orientations with b = 1000 s/mm² after the acquisition of b = 0 s/mm² (b0) images.

Analysis

VBM

Using Statistical Parametric Mapping (SPM; Friston et al. 1995), the 3 TR-weighted images from each subject were coregistered and averaged. The averaged image was bias corrected using the SPM5 unified diffusion gradients were applied along 32 independent orientations with b = 1000 s/mm² after the acquisition of b = 0 s/mm² (b0) images.

DTI—Whole-Brain Analysis

DTI data were processed using SPM5 and Matlab-based custom software, including the SPM "Diffusion toolbox." The 4 DTI sets were realigned and averaged to improve the signal-to-noise ratio. Using diffusion-weighted images collected from 32 directions and b0 images, the diffusion tensor was then calculated from the averaged images using a linear model (Basser and Pierpaoli 1996). Once the elements of diffusion tensor were calculated, FA maps were derived and the images spatially normalized and smoothed (full width at half maximum = 10 mm). A voxel-by-voxel analysis was performed to search for significant differences in FA values between control and SCI patients (ANCOVA, corrected P < 0.05, age as a nuisance variable). Significant FA differences were overlaid onto an individual's TR-weighted anatomical image for visualization.

Results

VBM analysis revealed significantly lower regional gray matter volume in SCI subjects compared with controls (Fig. 1). These reductions appeared in the region of left primary motor cortex innervating the lower limbs, the left and right medial prefrontal and adjacent perigenual and subgenual anterior cingulate cortices, the left and right anterior temporal cortex, lateral hypothalamus, and insular cortex. No region showed greater gray matter volume in SCI subjects compared with controls.

Whole-brain analysis of DTI indices revealed decreased FA and increased MD in SCI patients in the region immediately lateral to and extending into the left and right primary motor and sensory cortices supplying the lower limbs and in the superior cerebellar cortex (Figs. 2 and 3). In addition, lower FA values in SCI subjects occurred in regions associated with the left and right corticospinal tracts, that is, the corona radiata.

Table 1

<table>
<thead>
<tr>
<th>SCI subject’s characteristics</th>
<th>Age</th>
<th>Time since injury (months)</th>
<th>Neurological level of SCI (based on sensory loss)</th>
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<td>1</td>
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<td>252</td>
<td>T8</td>
</tr>
<tr>
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<td>26</td>
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<td>T10</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
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<tr>
<td>14</td>
<td>50</td>
<td>33</td>
<td>T9</td>
</tr>
</tbody>
</table>

SCI subject’s characteristics
posterior limb of internal capsule, ventral pons, and pyramids. Significantly lower FA values in SCI subjects also occurred bilaterally in the medial prefrontal and adjacent perigenual and subgenual anterior cingulate cortices and in the primary somatosensory and precuneus cortices. No region showed greater FA in SCI patients compared with controls.

Tractography identified the location of the corticospinal and corticopontine tracts from the cortex, through the internal capsule, midbrain, and pons (Fig. 5). The tractography differentiated the 2 fiber tracts at each brain level. The visualization revealed motor pathways originating in the primary motor cortex and also in the primary somatosensory and supplementary motor cortices.

Group analysis of the average FA and MD values in each individual’s corticospinal and corticopontine tracts revealed significantly lower FA and axial diffusivity values in SCI subjects compared with controls in both the corticospinal and corticopontine tracts (Fig. 6). In addition, the corticospinal tract displayed significantly lower MD values in SCI subjects, whereas the corticopontine tract did not. No significant correlation was found between tract properties and age or time since injury (“corticospinal tract”: FA vs. age: $R^2=0.14$, $P=0.20$; MD vs. age: $R^2=0.05$, $P=0.82$; FA vs. time since injury: $R^2=0.04$, $P=0.51$; MD vs. time since injury: $R^2=0.004$, $P=0.40$ and “corticopontine tract”: FA vs. age: $R^2=0.02$, $P=0.68$; MD vs. age: $R^2=0.02$, $P=0.65$; FA vs. time since injury: $R^2=0.05$, $P=0.47$; MD vs. time since injury: $R^2=0.05$, $P=0.40$).

Discussion

Complete SCI results in anatomical changes in the human motor cortex and in the descending pathways from the motor cortex. Decreases in gray matter volume and increases in MD occurred in the region of primary motor cortex that projects to the region of the spinal cord innervating the paralyzed lower limb muscles as well as in the superior cerebellar cortex. In addition, decreases in FA (indicative of fiber loss and/or demyelination) occurred in the corticospinal and corticopontine tracts of SCI subjects. Anatomical changes were also found in areas of the brain not directly involved in motor control, that is the medial prefrontal and anterior cingulate cortices.

Over 150 years ago, it was shown that following injury of a peripheral nerve, axons distal to the injury undergo progressive retrograde degeneration, a process termed Wallerian degeneration (Waller 1850). Although recent evidence suggests that Wallerian degeneration does indeed occur in the spinal cord of SCI subjects (Buss et al. 2004), the issue of whether SCI results in significant central changes including the loss of descending M1 neurons remains controversial. Some studies report very little change in M1 neuron numbers following disruption of the corticospinal tract (Tower 1940; Lassek 1942). For example, a recent study by Wannier et al. (2005) found that following disruption of the corticospinal tract in 2 macaque monkeys, no change in M1 pyramidal cell numbers occurred. However, the authors reported that the somata of the surviving M1 pyramidal...
neurons shrank significantly compared with those in the opposite M1 region, a result consistent with the M1 volumetric changes reported in our investigation.

In contrast, numerous primate investigations report corticospinal neuron death following pyramidotomy or cervical cord lesions (Levine and Bradford 1938; Pernet and Hepp-Reymond 1975). For example, Pernet and Hepp-Reymond (1975) showed that following unilateral pyramidotomy, approximately 70% of pyramidal cells in the contralateral motor cortex were lost. Recent rodent studies have also demonstrated that thoracic corticospinal tract transection results in programmed death (apoptosis) of a significant proportion of M1 corticospinal neurons (up to 40%) (Hains et al. 2003) and that few corticospinal neurons survive 10 weeks after a thoracic SCI (Feringa and Vahlsing 1985). In addition to M1 neuronal loss, some animal studies have reported that SCI evokes morphometric changes within descending motor control systems (Feringa and Vahlsing 1985; Ganchrow and Bernstein 1985; Tetzlaff et al. 1994; Hains et al. 2003), including changes in M1 synaptic spine density (Kim et al. 2006).

The results from our investigations support the view that SCI results in significant anatomical changes within the human brain and in particular the motor system. These changes include a decrease in gray matter volume, a decrease in FA, and an increase in MD in the region of M1, specifically in the area that innervates the lower (paralyzed) body (Schott 1993). In addition, a significant decrease in FA occurred in the region of the primary somatosensory cortex and a decrease in FA and increase in MD occurred in the superior cerebellar cortex. These cerebellar changes are surprising given that unlike the primary motor and sensory cortices, which have direct connections with the injured region, that is, the spinal cord; the bulk of superior cerebellar cortex connections are with the brainstem and cerebral cortex. This indicates that secondary changes that do not result directly from the injury itself may result in long-term anatomical changes.

Previous studies using VBM have found it difficult to detect changes in gray matter volume in M1 following SCI (Crawley et al. 2004; Jurkiewicz et al. 2006). Although there was a small (3.8%) difference in gray matter volume in the study by Crawley et al., this was insufficient to reach statistical significance but is nevertheless in line with the results of the present study. The more robust findings from the current study are likely due to the selection of only clinically complete, male paraplegic SCI subjects in contrast to previous studies that have included subjects with both complete and incomplete injuries. Although the narrow selection criteria was, in our opinion, critical for the detection of group differences, it may have limited our ability to detect significant correlations between the degree of anatomical change and injury level, that is, we found no significant correlation between injury level and M1 anatomy. Given the large cortical representation which would be lost in all complete thoracic SCI subjects (i.e., the sensory and motor representations to the lower limbs), the additional

Figure 2. Regions showing significantly lower FA ($P < 0.05$; higher values indicate greater axonal integrity or more fibres) in SCI versus control subjects overlaid on a single subject’s T1-weighted anatomical scan. The location of each slice in Montreal Neurological Institute space is shown at the lower right of each section: ACC, anterior cingulate cortex; IC, internal capsule; M1, primary motor cortex; mPFC, medial prefrontal cortex.
loss from segments within the thoracic region (which have relatively sparse cortical representations) would likely not produce a large difference in the overall cortical loss. We suspect that the absence of a significant correlation reflects the lack of sensitivity of the MRI techniques in determining such fine differences.

In addition to changes in the cerebral and cerebellar cortices, we found that SCI results in significant changes in the anatomy of the medial prefrontal and anterior cingulate cortices. These brain regions are known to be critical for the processing of emotional relevant information and in modulating attentional states. It has been shown by Damasio and colleagues that peripheral arousal levels may influence one's emotional state, and it has been suggested that the decoupling of the brain from the body, as happens following SCI, may modify emotional experience (Damasio 1994). Indeed, a recent fMRI investigation has revealed reduced activity in the subgenual anterior cingulate cortex during emotional processing in SCI subjects (Nicotra et al. 2006). It may be that changes in the anatomy of the prefrontal/anterior cingulate cortices are reflected in changes in emotional processing in SCI subjects, although the precise nature of these anatomical changes cannot be determined using MRI techniques.

FA values contain information on both the direction and ease of water movement. When a water molecule lies between parallel axons, then its movement is restricted to 2 directions parallel to the axis of those axons. In this situation, the FA value of that brain region approaches 1. Conversely, in brain areas where water movement is unrestricted in any direction (e.g., as in the ventricles), FA approaches 0. Given this, if a brain region has a lower FA value, then water can move more freely in multiple directions, and this generally indicates fewer axons or demyelination. In contrast, MD is a measure of the ease of water movement, and so as MD increases, the ease of water movement also increases. Thus MD is affected by cellular size, shape, and integrity (Pierpaoli et al. 1996). MD declines with increasing tissue barriers, such as cell membranes and myelin sheaths (Basser and Pierpaoli 1996) and increases in lesions with edema, demyelination, and axonal loss (Stevenson et al. 2000; Iannucci et al. 2001). Thus, the decreased FA and
increased MD in M1 and cerebellum in SCI subjects indicate that there are fewer barriers to water movement indicating a loss of similarly orientated fibres (axons and/or dendrites) and possibly cell death in people with SCI.

Consistent with anatomical changes in M1 of SCI subjects, we found water diffusion changes which were indicative of fiber loss in 2 major descending motor pathways—the corticospinal and corticopontine tracts. The anatomy of these 2 descending motor tracts in lower mammals has been well established, and recent DTI and lesion studies have elucidated the precise trajectory patterns of these pathways in humans (Kuypers 1981; Okada et al. 2006; Zarei et al. 2007). It is known that the corticospinal tract fibres pass from the cortex through the ipsilateral posterior limb of the internal capsule, the ipsilateral cerebral peduncle, and enter the ipsilateral pyramid in the ventral medulla. In humans, the vast majorities of these fibres then decussate and travel to the contralateral ventral and intermediate regions of the spinal gray matter via the lateral funiculus of the spinal cord. Although brain regions such as secondary motor, cingulate, and even somatosensory cortices contribute significant numbers of neurons to the corticospinal tract (see Fig. 5), the corticospinal tract provides an important output pathway of M1 (see Dum and Strick 1991; Galea and Darian-Smith 1994; Strick et al. 1998). The integrity of the corticospinal tract is critical for the performance of fine, skillful movements, not only those involving the fingers during manipulative behaviors (Lawrence and Hopkins 1976) but also those involving the foot (Porter and Lemon 1993; Courtine et al. 2005). In monkeys, bilateral pyramidotomy results in the animal retaining the ability to walk, run, climb, use their feet and hands to grip cage bars, and hold food; however, the ability to perform individual finger movements and the precision grip is lost (Kuypers 1981).

Although corticospinal tract neurons send collaterals to the pontine nuclei (Ugolini and Kuypers 1986), the corticopontine tract neurons do not innervate motor neurons in the ventral horn of the spinal cord but instead synapse on ipsilateral pontine nuclei (Kuypers 1981; Schmahmann et al. 2004). The projection from M1 traverses the posterior limb of the internal capsule and is topographically organized. The pontine nuclei project to the contralateral cerebellar cortex via the middle cerebellar peduncle. Although limitations of tractography preclude our demonstration of the crossed connections between the pons and cerebellum, this cortico–ponto–cerebellar pathway is quantitatively the most important route by which the cerebral cortex can influence the cerebellar cortex. It carries information that the contralateral cerebellum uses to correct for errors during movements and participates in movement planning and initiation. Given their functions, we speculate that the observed changes in the anatomy of the corticospinal and corticopontine tracts would, on their own, have a detrimental effect on fine movement as well as movement initiation and...
planning. Hence, it is possible that their occurrence following human SCI would hinder the capacity for full motor recovery following SCI.

As mentioned above, the change in the corticopontine tract is surprising given that it is not interrupted by the SCI. As corticospinal tract neurons also send collaterals to the pontine nuclei, it is possible that water movement changes in the corticospinal tract reflect changes in the property of some corticospinal tract neurons. Furthermore, current spatial resolution limitations of DTI may have hindered our ability to precisely separate the corticospinal and corticopontine tracts. However, in contrast to the changes in the corticospinal tract, the corticopontine tract displayed lower FA and axial diffusivity values, no change in MD, and a slight increase (although not significant) in radial diffusivity in SCI subjects. It is thought that fiber loss per se is not associated with significant decreases in MD or axial diffusivity (Pierpaoli et al. 2001). It has been shown histologically that Wallerian degeneration is associated with gliosis, astrocytic scarring, and an increase in extracellular matrix, and it is these changes that are thought to be responsible for the reported decreases in MD and axial diffusivity that accompany Wallerian degeneration (Buss et al. 2004). These diffusivity changes are similar to those reported associated with Wallerian degeneration and may indicate a retrograde degenerative process with Wallerian features. In contrast, the diffusion properties of the corticopontine tract in SCI subjects is not reflective of Wallerian degeneration and may result from a different degenerative process that does not involve gliosis or an increase in the extracellular matrix. Indeed, it has recently been suggested that there may be a direct relationship between the death of corticospinal tract neurons and oligodendrocytes following SCI, and the death of these oligodendrocytes may cause further demyelination of other shared fibres (Hains et al. 2003). In any case, our results suggest that even if a fiber tract is not directly traumatized by an injury, it can still display significant changes in its diffusion properties that are indicative of neural loss.

Despite evidence that compensatory structural changes including sprouting can occur in the spinal cord distal to the site of a spinal cord lesion for at least a few weeks after injury (Hill et al. 2001; Weidner et al. 2001), only modest functional improvement in motor control is observed (Blight 1983; Fehlings and Tator 1995). This recovery may be limited because in addition to ongoing degeneration of the transected corticospinal tract, changes are also occurring in fiber tracts above the SCI level. Furthermore, peripheral nerves also undergo significant changes at levels below the SCI. Krenz and Weaver (1998) report time-dependent sprouting of myelinated and unmyelinated primary afferent fibres within the dorsal horn, below the level of a SCI. Lin et al. (2007) has also reported decreases in the excitability of motor axons in the common peroneal and median nerves of SCI subjects, with the changes suggestive of motor axon loss and/or changes in ion channel expression on the axons. In some SCI subjects, motor axons in major nerves were completely inexcitable.

It is likely that the anatomical changes described in the present study are associated with the functional changes that occur in the cortical motor system following SCI. For example, during imagined and attempted foot movements, SCI subjects display a reduced volume and variability of M1 activity and abnormal patterns of motor system activation (Cramer et al. 2005). In addition, M1 displays functional reorganization following SCI with the location of activated M1 maxima during upper limb movements significantly shifted in complete thoracic SCI subjects (Lotze et al. 1999), and SCI subjects display larger areas of M1 activation in tasks involving activation of regions proximal to the lesion (Curt et al. 2002). Based on studies using transcranial magnetic stimulation and fMRI, the degree of this shift may increase with the duration of time since the lesion (Lotze et al. 2006). Furthermore, even if the remaining motor fibres within the spinal cord are repaired after SCI and reinnervate their original targets, permanent deficits in motor control may persist. Despite the potentially deleterious functional and anatomical changes in the primary motor cortex after SCI, there remains coherent volitionally commanded
activity in the primary motor cortex which can be recorded from implanted intracortical electrodes and then processed to drive brain-computer interfaces (Hochberg et al. 2006) or neurorehabilitation devices. Also, remaining pathways are plastic and can potentially improve with specific training (for review, see Edgerton et al. 2004).

Efforts to prevent neuronal death may be critical for complete motor recovery following SCI. Although the necrosis that occurs following SCI is irreversible (as it results from plasma membrane damage and the subsequent leakage of the cell constituents into the extracellular space), apoptosis is potentially preventable as it is initiated by a number of factors including Ca$^{2+}$ and free radicals (Ellis et al. 1991). Indeed, in animals at least some of the apoptosis that occurs within the spinal cord following SCI is mediated by a Fas/Fas ligand system (Yoshino et al. 2004). Furthermore, in animals in which this system is blocked, SCI results in significantly lower levels of spinal cord apoptosis, and the level of motor recovery is greater when compared with animals in which the Fas/Fas ligand system is intact. Although the evidence is limited, intensive rehabilitation, physical therapy, and even motor imagery have been suggested as avenues that may also help prevent and even reverse some of the structural cortical changes and loss of motor function that occurs following SCI (Corbetta et al. 2002; Dobkin et al. 2007). A combination of these techniques may help to limit the loss of descending motor axons that occurs following SCI. Such measures may also improve motor recovery when local neuro-regenerative spinal cord interventions are used. Given the rapid speed of cell death, these measures would ideally be delivered in the very early stages following SCI.

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## Notes

Conflict of Interest: None declared.

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