Clinical significance of the pallidoreticular pathway in patients with carbon monoxide intoxication

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Whereas globus pallidus lesions resulting from carbon monoxide intoxication have been extensively described in the literature, the clinical significance of pallidoreticular lesions has rarely been mentioned. This study incorporated information from functional and structural imaging to explore the correlations of pallidoreticular lesions with parkinsonian features and neuro-behavioural performance. Twenty-five patients (11 males) with globus pallidus lesions after carbon monoxide intoxication and 25 age- and sex-matched controls were enrolled for detailed neurological examinations, cognitive testing, susceptibility weighted imaging, diffusion tensor imaging and 99mTc-TRODAT-1 single photon emission computed tomography. The post-processing analysis of the neuroimaging included voxel-based morphometry to assess the regional atrophy, tract-based spatial statistics related to white matter involvement, tractography to investigate the rostral and caudal projections from the midbrain level and specific uptake ratios of 99mTc-TRODAT-1 for presynaptic dopaminergic transporter activity. In susceptibility weighted imaging, low-intensity pallidoreticular lesions were detected from the minimal-intensity projections, which were visible in only 7.7% of the T1-weighted images and 15.4% of the T2-weighted images, whereas inhomogeneous intensities were detected in the globus pallidus. The patients were further divided into two subgroups based on the presence (n = 13) or absence (n = 12) of pallidoreticular lesions. The patients with pallidoreticular lesions showed increased parkinsonian features, poorer performances on the neuropsychiatric tests, lower 99mTc-TRODAT-1 availability in both the caudate and the putamen and greater atrophy of the thalamus, posterior corpus callosum, cerebral peduncle and white matter surrounding the globus pallidus compared to those without pallidoreticular lesions. The tractography results obtained with seed regions of interest in the substantia nigra showed rostral projections to the supplementary motor cortex and anterior cingulate cortex via the globus pallidus; the two pathways were distinct but ran in parallel, caudal to the level of the globus pallidus. In conclusion, the presence of pallidoreticular lesions after carbon monoxide intoxication indicates a poorer cognitive state, which is associated

with extensive grey and white matter damage in addition to the damage to the nigra-striatal neuronal networks. The presence of parkinsonian features may be related to pallidal and presynaptic dopaminergic dysfunction. The sensitivity for detecting pallidoreticular lesions can be greatly improved by using susceptibility weighted imaging compared with conventional imaging.

Keywords: pallidoreticular pathway; carbon monoxide intoxication; diffusion tensor imaging; parkinsonian symptoms; cognitive deficits

Abbreviations: SPECT = single photon emission computed tomography; 99mTc-TRODAT-1 = 99mTc-[2-[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl)aminomethyl]ethanethiolate(3-)-N2,N2, S2, S2, oxo-[1R-(exo-exo)]

Introduction

Carbon monoxide intoxication secondary to charcoal burning has become the most common method for committing suicide in Taiwan (Kuo et al., 2008). The neuropathology of carbon monoxide intoxication has been thoroughly described in post-mortem studies (Laprese and Fardeau, 1966, 1967, 1971). Although basal ganglia necrosis is frequently mentioned in the carbon monoxide intoxication literature (Prockop and Chichkova, 2007), recent data have produced conflicting estimates for lesion frequency that range from 4 to 88% (Hopkins et al., 2006; Pulsipher et al., 2006). Injuries to the white matter and the globus pallidus have been reported to predict neurological outcomes (Vieregge et al., 1989; Pracyk et al., 1995), but the frequency of globus pallidus lesions appears to be lower than that of white matter lesions (Parkinson et al., 2002). In 73 cases of carbon monoxide intoxication with sequential brain MRI follow-ups, only one case was found with changes to the basal ganglia (Hopkins et al., 2006).

While most of the pathological data from carbon monoxide intoxication studies have emphasized the necrosis or haemorrhaging of the basal ganglion area, a pallidoreticular topography specifically targeting the fibre tract along the pallidum and substantia nigra pars reticulata was first described by Auer and Benveniste (1996). However, the nomenclature describing the pallidoreticular pathway in the carbon monoxide intoxication literature is different from that in other fields in neurological sciences regarding the designation of the projection from the pallidum to the reticular nucleus of the thalamus (Ambadekar et al., 2003). Auer and Benveniste (1996) speculated that these two iron-rich regions display selective tissue vulnerability because of the high affinity of carbon monoxide for haem molecules, resulting in direct neurotoxicity. The brain MRI finding for pallidoreticular lesion in patients with carbon monoxide intoxication was not described until a report by Kawanami et al. (1998), which was followed by results from three other groups (Gandini et al., 2002; Kinoshita et al., 2005; Adam et al., 2008). The clinical presentations of carbon monoxide intoxication with pallidoreticular lesions include parkinsonism (Kawanami et al., 1998; Kinoshita et al., 2005), mild cognitive deficits (Gandini et al., 2002), compulsive behaviour and self-activation deficits (Adam et al., 2008).

Parkinsonian symptoms have frequently been observed in patients following carbon monoxide intoxication (Chang et al., 2010a) and have been shown to be associated with white matter lesions (Sohn et al., 2000). It is known that the dopamine transporter activity distribution in the CNS coincides with the dopaminergic innervations and that there is a high clinical correlation between a decrease in dopamine transporters and parkinsonian symptoms (Huang et al., 2003; Su et al., 2010). 99mTc-[2-[[3-(4-chlorophenyl)-8-methyl-8-azabicyclo[3,2,1]oct-2-yl]methyl](2-mercaptoethyl)aminomethyl]ethanethiolate(3-)-N2,N2, S2, S2, oxo-[1R-(exo-exo)] (99mTc-TRODAT-1) single photon emission computed tomography (SPECT) allows for an in vivo assessment of presynaptic dopaminergic function by measuring regional dopamine transporter activity (Weng et al., 2004). Whether there is a similar linkage between dopaminergic dysfunction and parkinsonian symptoms following carbon monoxide intoxication has not been fully established.

MRI is widely used to investigate brain modifications after disease processes. Susceptibility weighted imaging is a heavy T2*-weighted, gradient-recalled, 3D, fast, low-angle shot sequence with full-flow compensation in all three directions. In conventional MRI, the phase information is purposely discarded, and only the magnitude is presented. In contrast, susceptibility weighted imaging uses the phase information to increase the sensitivity of diagnosis. Studies have shown that susceptibility weighted imaging can be used to evaluate the course and pattern of mineralization in the grey matter (Saleh et al., 2003; Gupta et al., 2010). Susceptibility weighted imaging has been shown to be highly sensitive to iron in the form of haemosiderin, ferritin and deoxyhaemoglobin (Barnes and Haacke, 2009; Ayaz et al., 2010; Haacke et al., 2010). This property allows for the improved detection of microbleeds and demyelinating processes with haemorrhagic transformations. Efforts have been made to use the phase changes to discriminate the different contents of the brain deposit. For example, paramagnetic material appears dark in both the magnitude and the normalized negative phase of the susceptibility weighted image. Similarly, diamagnetic substance could be visualized in the normalized positive phase images (Haacke et al., 2009).

Through assessing tissue microstructure by mapping water proton motions (Basser and Pierpaoli, 1996), current MRI technology has also made possible the in vivo assessment of white matter integrity by using diffusion tensor imaging (Le Bihan, 2003). Fractional anisotropy is a diffusion tensor imaging-derived parameter that quantifies the directionality and coherence of the white matter tracts; it is believed to represent such factors as...
myelination, axonal density and/or integrity (Rorschach et al., 1991; Sykova, 2004). An increased axial diffusivity has been attributed to axonal changes in patients with trauma, while changes in radial diffusivity strongly correlate with myelin abnormalities (Kraus et al., 2007). Tractography is a further development of diffusion tensor imaging, which could enable the tracing of neural fibres (Conturo et al., 1999). This technique could therefore help to shed more light on the linkage between the pallidoreticular pathways and the clinical presentation after carbon monoxide intoxication.

The purpose of this study was to examine the changes in neural network integrity in patients with carbon monoxide intoxication who developed pallidoreticular lesions. By analysing possible damage on three levels: mesencephalic (dopaminergic neuronal damage), basal ganglia (dopaminergic deafferentiation) and cortical regional network connections, we explored the clinical significance of pallidoreticular lesions in patients with carbon monoxide intoxication. The neural substrates responsible for the pallidoreticular lesions from the perspective of local magnetic susceptibility were analysed. The clinical parameters related to the initial hypoxic insults, neuropsychiatric performances, parkinsonian features and severity were further analysed in the study participants and correlated with the imaging findings.

Materials and methods

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital and complied with the ethical standards established in the Declaration of Helsinki. The experiments were undertaken with the written, informed consent of each subject and their caregiver (when appropriate).

Patient enrollment

The neurology clinic at the Kaohsiung Chang Gung Memorial Hospital initiated this study in 2008. The clinical diagnosis of carbon monoxide intoxication was made based on a history of a charcoal-burning suicide attempt and an elevated carboxyhaemoglobin level (>10%) in the emergency room (Chang et al., 2009b, 2010a).

Because it was not possible to combine all the influential factors in the carbon monoxide intoxication group to produce a uniform population, we enrolled patients with globus pallidus interna lesions, as demonstrated by brain CT or MRI (Fig. 1A–C). The exclusion criteria

![Figure 1](https://academic.oup.com/brain/article-abstract/134/12/3632/261349)
included a pre-existing neurological disorder, an agitated mood or a confused state that prevented either a neuropsychiatric interview or neuromaging. After screening the carbon monoxide cohort (Chang et al., 2009b, 2010a; Huang et al., 2011), 25 patients (mean carboxyhaemoglobin 35.2%; range, 10–80%) fulfilled the inclusion and exclusion criteria, agreed to participate in the study and completed it.

In addition, 25 age- and sex-matched healthy subjects were used as controls. The inclusion and exclusion criteria for the control subjects were based on our previous studies (Chang et al., 2009b, 2010a).

Cognitive testing

The cognitive testing was performed 2 h before the brain MRI study. General intellectual function was assessed using the Mini-Mental State Examination (Folstein et al., 1975). Verbal and non-verbal episodic memory was assessed using a modified California Verbal Learning Test-Mental Status (Chang et al., 2010b) and the Rey–Osterrieth Complex Figure Test after a 10-min delay (Boone, 2000). The language screening included the 16-item Boston Naming Test (Kaplan et al., 1983), a three-step comprehension test and semantic verbal fluency tests. The subjects’ visual–spatial abilities were assessed by a modified Rey–Osterrieth Complex Figure Test, pentagons, a transparent cube copy and the number–location test from the Visual Object and Space Perception Battery (Warrington and James, 1991). The ability to perform five arithmetic calculations was evaluated, and frontal lobe function was assessed using the digit-forward and backward-span, design fluency, Stroop Interference (Amieva et al., 2004) and Modified Trails B tests (Reitan, 1995). The severity of parkinsonian features was evaluated using the Unified Parkinson’s Disease Rating Scale motor score. The Neuropsychiatric Inventory was used to assess behavioural symptoms (Cummings et al., 1994).

Magnetic resonance imaging protocols

Magnetic resonance images were acquired using a 3.0-T MRI scanner (Excite, GE Medical Systems). Structural images were acquired for an anatomical reference using the following protocols: (i) a T2-weighted, turbo spin-echo sequence with the repetition time/echo time/number of averages = 4200 ms/101.2 ms/2, a 240 mm × 240 mm field of view, a 320 × 224 matrix and a 5-mm axial slice thickness; and (ii) a T1-weighted, inversion–recovery prepared, 3D, spoiled, gradient-recalled acquisition in a steady-state sequence with repetition time/ inversion time = 8600 ms/450 ms, a 240 mm × 240 mm field of view and a 1-mm slice thickness.

The susceptibility weighted imaging used the following parameters: repetition time/echo time/flip angle = 35 ms/14 ms/40°, 52 slices, thickness = 2 mm, matrix size = 512 × 512 and field of view = 80 mm × 130 mm. The diffusion tensor imaging was acquired using the following parameters: repetition time/echo time/flip angle = 9600 ms/62.7 ms/90°, a 192 mm × 192 mm field of view, a 128 × 128 matrix and a 4-mm axial slice thickness. For whole brain coverage, 40 contiguous axial slices were obtained. The diffusion-weighting gradients were applied in 61 non-collinear directions, optimized by the static electron-repulsion model. The b-value used was 1000 s/mm². One reference image was acquired using the same imaging parameters but without diffusion weighting.

99mTc-TRODAT-1 single photon emission computed tomography

For this examination, all the patients were injected intravenously with a single bolus dose of 740 MBq (20 mCi) 99mTc-TRODAT-1. The brain SPECT/CT (Symbia T, SiemensR) images were obtained 4 h later. The SPECT/CT scanner was equipped with low-energy, high-resolution collimators and a dual-slice spiral CT. The acquisition parameters for the SPECT were a 128 × 128 matrix with 60 frames (40 s/frame). The scan parameters for the CT were 130 kV, 17 mAs, 5-mm slices and image reconstruction with a medium-smooth kernel. The SPECT images were attenuation-corrected based on the CT images and scatter-corrected with Flash 3D algorithm (ordered subsets expectation and 3D maximization with resolution correction) with eight subsets and eight iterations.

Processing of susceptibility weighted images

The processing of the susceptibility weighted images followed the procedure from Haacke et al. (2009). In brief, the complex k-space data were filtered through a Hanning window function with a 9 × 9 kernel size. The phase in the filtered data was first unwrapped. The positive phase image was produced by setting the amplitude of the negative phase to 1. The positive part was normalized from 1 to 0. The positive phase image was subsequently multiplied by the original magnitude image. A minimum intensity projection was performed, which was finally overlaid on the susceptibility weighted magnitude image. A negative phase image was calculated in a similar manner but the negative part was normalized from 0 to 1.

Diffusion tensor imaging analysis and tractography

Tract-based spatial statistics were obtained using the functional MRI of the brain software library version 4.0.1 package (http://www.fmrib.ox.ac.uk). Fractional anisotropy images from individual subjects were aligned in a common space using a non-linear registration toolkit (Rueckert et al., 1999) and then projected onto a mean fractional anisotropy skeleton. The comparison of the diffusion indices used permutation-based, non-parametric inference for the cluster size (Nichols and Holmes, 2002) and Randomise 2.0 software. A restrictive statistical threshold was used (the threshold-free, cluster-enhancement threshold with P < 0.05, corrected for multiple comparisons). Abnormal white matter tracts in the maps of the diffusion indices were identified based on an atlas prepared at Johns Hopkins University (Wakana et al., 2004). After using the fractional anisotropy images to achieve non-linear registration and skeleton formation, the projection vector was estimated from each subject onto the mean fractional anisotropy skeleton. The non-linear warps and the skeleton projection were then separately applied to the axial and radial diffusivity images. The resulting statistical maps were thresholded at P < 0.05 and corrected at the cluster level for multiple comparisons. The tractography was performed using constrained-spherical deconvolution, as implemented in the MRTrix software package (Tournier et al., 2007). For procedures of fibre tracts quantification, refer to the ‘Detailed Methods’ in Supplementary Material.
Voxel-based morphometry

Voxel-based morphometry for grey or white matter analysis was processed using the Spatial Parametric Mapping Version 5 system (Wellcome Trust Centre for Neuroimaging) with study-specific templates. This procedure followed the protocol previously used by Chang et al. (2009a, 2010a). The general linear model was used to assess significant differences between groups. Age and gender were considered as covariates of no interest to exclude their possible effects on the regional grey matter or white matter volumes (Good et al., 2001). The significance threshold was set at $P < 0.01$, corrected for multiple comparisons across the entire brain (the false discovery rate) with an extended threshold of 250 voxels and applied to the resulting $t$-statistic maps of the grey matter and white matter.

Semi-quantitative analysis of the 99mTc-TRODAT-1 signals

The reconstructed images of the 99mTc-TRODAT-1 SPECT (Fig. 2A), CT (Fig. 2B) and SPECT/CT fusion images (Fig. 2C) were transferred onto a workstation with imaging fusion software (Siemens Medical Systems). The approach used the information that was mutually available from the CT- and $T_2$-weighted images to perform an initial coregistration (Fig. 2D), which allowed for semi-automatic fusion with a voxel-based algorithm and the application of the resulting transformation coordinates to the SPECT and MRI data. The reconstructed section thickness was 3 mm for the SPECT and MRI images in the transverse plane. The fusion precision was verified both by the fusion programme and by visual delineation of the anatomic structures by a physician who was experienced in nuclear medicine.

Regions of interest (Fig. 2E) were drawn on the caudate and putamen of both hemispheres in the brain MRI (Fig. 2E), and the regions of interest were automatically transferred by the software to the corresponding 99mTc-TRODAT-1 SPECT slices (Fig. 2F) with the values for the radioactivity counts. The occipital cortex was drawn in the same way and served as a background area. The ratio of the specific to non-specific striatal 99mTc-TRODAT-1 binding in each region was calculated by mean region of interest counts/mean occipital cortex counts. The mean striatal 99mTc-TRODAT-1 binding ratio was also calculated by averaging the values from the left and right hemispheres.

Statistical analysis

Categorical variables were compared using the chi-squared test. The Kruskal–Wallis $H$-test was used to compare the neuropsychiatric performances between the groups because these data were not normally distributed. The Spearman correlation was used to explore the relationships between the continuous variables. The statistical analyses were performed using the Statistical Product and Service Solutions software package (version 11.0 for Windows). A $P < 0.05$ (two-tailed) was considered statistically significant.

Results

Globus pallidus lesion characteristics using susceptibility weighted imaging

Figure 1 shows images of the basal ganglion produced using different modalities. The lesion signal intensities in the globus pallidus...
pallidus were low in both the CT (Fig. 1A) and T₁-weighted image (Fig. 1B) but were heterogeneous in the T₂-weighted image (Fig. 1C). In the susceptibility weighted and T₂-weighted images, reduced signal intensity within the globus pallidus was consistent with the location corresponding to low signals in the negative phase image (Fig. 1D), while increased signal intensity corresponded to the positive phase image (Fig. 1E). This result was further confirmed by overlaying either the negative (Fig. 1D) or positive phase onto the susceptibility weighted imaging (Fig. 1E). The open arrows in minimum intensity projection of susceptibility weighted imaging (Fig. 1F) indicate low signal intensity regions that visibly extend from the globus pallidus to the midbrain, which is consistent with a pallidoreticular pattern. Furthermore, signal loss in the corpus callosum (Fig. 1F, black arrow) that was not visible in the conventional T₁- and T₂-weighted images, was observed. No pallidoreticular lesions, as detected on the corresponding CT image, exceeded 100 Hounsfield units.

Demographics and neuropsychiatric evaluations in the two subgroups defined by the presence or absence of pallidoreticular lesions

The patients were divided into two subgroups according to the presence of pallidoreticular lesions in the minimum intensity projection of susceptibility weighted imaging. The image criteria for patients with pallidoreticular lesions [pallidoreticular(+), Fig. 3A] were low signals extending from the pallidum to the midbrain. In contrast, the subgroup without pallidoreticular lesions [pallidoreticular(−), Fig. 3B] only had a low signal in the globus pallidus. The demographic data showed no differences between the pallidoreticular(+) (n = 13) and pallidoreticular(−) (n = 12) subgroups with respect to age at examination, gender, carboxy-haemoglobin level, intervals of unconsciousness or the interval between the carbon monoxide intoxication event and the clinical examination (Table 1). The cognitive test scores in the pallidoreticular(−) subgroup were generally higher than those in the pallidoreticular(+) subgroup, most noticeably in the visual–spatial, digit-backward, trail-making and calculation tests (Table 1, P < 0.05 after the Bonferroni correction), but their performances were still significantly poorer than those of the control group (P < 0.05). In the Neuropsychiatric Inventory score comparison, the pallidoreticular (+) subgroup had higher depression and anxiety scores than the pallidoreticular(−) subgroup. The results of the neuropsychiatric tests that are not listed in Table 1 are provided in Supplementary Table 1.

The signals of the pallidoreticular lesions from the phase image were further inspected for magnetic susceptibility. The pallidoreticular lesions appeared in the negative phase imaging (Fig. 3C) rather than the positive phase imaging (Fig. 3D) for all 13 pallidoreticular(+) patients. The sensitivity of the pallidoreticular lesion detection was 1/13 (7.7%) in the corresponding T₁-weighted images and 2/13 (15.4%) in the T₂-weighted images.

Parkinsonian features and their severity in relation to 99mTc-TRODAT-1 ratio

With respect to the parkinsonian features, the pallidoreticular(+) subgroup had a significantly higher frequency of bradykinesia, postural instability, rigidity and gait disturbances but not resting or action tremor compared to the pallidoreticular(−) subgroup.

![Figure 3](https://example.com/figure3.png)

**Figure 3** Image based criteria used in the study, showing subgroup with (A) pallidoreticular lesion (arrows) and (B) without pallidoreticular lesions, based on the minimal intensity projection of susceptibility weighted image. From the susceptibility weighted image phase image, the pallidoreticular lesions appeared only in the negative phase (C, arrows) and were not visible in the positive phase (D).
Table 1 Cognitive and neuropsychiatric performance in individuals with carbon monoxide intoxication and age-matched controls

<table>
<thead>
<tr>
<th></th>
<th>CO intoxication (n = 25)</th>
<th>Without PR lesion (n = 12)</th>
<th>With PR lesion (n = 13)</th>
<th>Controls (n = 25)</th>
</tr>
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<tbody>
<tr>
<td>Age at study (years)</td>
<td>37.4 (10.7)</td>
<td>37.1 (11.2)</td>
<td>39.7 (10.5)</td>
<td>38.4 (9.5)</td>
</tr>
<tr>
<td>Study interval (months)</td>
<td>18.7 (6.6)</td>
<td>19.0 (6.5)</td>
<td>18.4 (6.9)</td>
<td>N.A.</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>11/14</td>
<td>5/7</td>
<td>6/7</td>
<td>11/14</td>
</tr>
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<td>Carboxyhaemoglobin (%) (mean, range)</td>
<td>35.2 (22.9)</td>
<td>28.2, 10–59</td>
<td>37.1, 10–80</td>
<td>NA.</td>
</tr>
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<td>Conscious disturbance period (day)</td>
<td>2.2 (1.7)</td>
<td>1.58 (0.8)</td>
<td>2.77 (2.1)</td>
<td>NA.</td>
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<td>Hyperbaric oxygen therapy (n)</td>
<td>16</td>
<td>8</td>
<td>8</td>
<td>NA.</td>
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<tr>
<td>Mini-Mental State Examination</td>
<td>25.52 (4.7)*</td>
<td>27.67 (3.2)</td>
<td>23.54 (5.1)*</td>
<td>29.28 (0.98)</td>
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<td>Verbal memory: CVLT-MS (9)</td>
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<tr>
<td>30-s free recall</td>
<td>6.4 (2.1)*</td>
<td>6.8 (1.8)*</td>
<td>6.1 (2.4)*</td>
<td>8.7 (0.5)</td>
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<tr>
<td>10-min free recall</td>
<td>5.7 (2.4)*</td>
<td>5.7 (2.7)*</td>
<td>5.7 (2.3)*</td>
<td>8.5 (0.8)</td>
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<td>10-min recognition</td>
<td>7.0 (2.7)*</td>
<td>7.3 (1.7)*</td>
<td>6.7 (3.5)*</td>
<td>9.0 (0.2)</td>
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<td>Visual memory</td>
<td></td>
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<tr>
<td>Modified Rey–Osterrieth recall (17)</td>
<td>9.6 (5.7)*</td>
<td>11.2 (5.7)*</td>
<td>8.2 (5.6)*</td>
<td>14.5 (2.5)</td>
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<tr>
<td>Visuospatial functions</td>
<td></td>
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<tr>
<td>Modified Rey–Osterrieth copy (17)</td>
<td>15.5 (4.0)</td>
<td>17.0 (0.0)</td>
<td>14.1 (5.2)**</td>
<td>17.0 (0.0)</td>
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<td>Pentagon copy (1)</td>
<td>0.8 (0.4)*</td>
<td>1.0 (0.0)</td>
<td>0.6 (0.5)**</td>
<td>1.0 (0.0)</td>
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<tr>
<td>Speech and language ability</td>
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<tr>
<td>Semantic fluency (animal)</td>
<td>14.9 (5.7)*</td>
<td>16.8 (5.5)*</td>
<td>13.2 (5.5)*</td>
<td>21.8 (4.2)</td>
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<td>Boston naming test (16)</td>
<td>14.0 (2.9)*</td>
<td>15.3 (0.9)</td>
<td>12.8 (3.5)**</td>
<td>15.7 (0.6)</td>
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<tr>
<td>Comprehension (4)</td>
<td>3.1 (1.0)*</td>
<td>3.3 (0.8)*</td>
<td>2.9 (1.2)*</td>
<td>3.9 (0.3)</td>
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<tr>
<td>Executive function</td>
<td></td>
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<tr>
<td>Digit backward</td>
<td>4.4 (1.5)*</td>
<td>5.1 (1.2)</td>
<td>3.7 (1.4)**</td>
<td>5.5 (1.6)</td>
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<tr>
<td>Correct line in trail-making (14)</td>
<td>11.5 (3.9)*</td>
<td>13.4 (2.0)*</td>
<td>9.7 (4.4)**</td>
<td>13.5 (2.1)</td>
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<td>Calculation (5)</td>
<td>4.0 (1.2)*</td>
<td>4.6 (0.7)*</td>
<td>3.4 (1.4)**</td>
<td>4.7 (0.9)</td>
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<td>Digit forward</td>
<td>7.73 (1.1)</td>
<td>8 (0.9)</td>
<td>7.54 (1.2)*</td>
<td>8.32 (0.9)</td>
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<td>NPI total score</td>
<td>19.9 (3.6)*</td>
<td>16.8 (165)*</td>
<td>23.5 (17.1)*</td>
<td>1.3 (0.7)</td>
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<td>Significant NPI subdomains</td>
<td></td>
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<tr>
<td>Depression/dysphoria</td>
<td>4.5 (1.1)*</td>
<td>2.6 (4.1)</td>
<td>6.4 (5.5)**</td>
<td>0.3 (1.2)</td>
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<tr>
<td>Anxiety</td>
<td>1.3 (0.6)*</td>
<td>0.8 (2.3)</td>
<td>2.1 (3.5)**</td>
<td>0.0 (0.0)</td>
</tr>
</tbody>
</table>

*P < 0.05 between the carbon monoxide group versus the controls.

**P < 0.05 between the carbon monoxide subgroups with or without pallidoreticular lesions; ‘study interval’ indicates the number of months between the carbon monoxide intoxication and the examination; data represent mean (standard deviation); number in parentheses following the task name = the maximum possible score.

CO = carbon monoxide; CVLT-MS = California Verbal Learning Test-Mental Status; NA = not applicable; NPI = Neuropsychiatric Inventory; PR = pallidoreticular lesions.

(Table 2). Although the pallidoreticular(+) subgroup had significantly higher Unified Parkinson’s Disease Rating Scale motor scores and leg agility scores (item 26), the hand movement item was not significantly different from the pallidoreticular(+) subgroup. Ten patients in the pallidoreticular(+) subgroup received levodopa and/or treatment with dopaminergic agonists. Two of these patients displayed a marked clinical improvement at 1-month follow-up, while eight patients showed little or no improvement.

Compared to controls, the 99mTc-TRODAT-1 binding ratio was symmetrically and significantly reduced in the patient group. The degree of reduction (versus controls) was 13.6% in the caudate, 17.9% in the putamen and 15.6% in the striatum (all P < 0.05). In particular, the pallidoreticular(+) subgroup showed a symmetrical significant reduction in 99mTc-TRODAT-1 binding ratios in both the putamen and the caudate compared to the pallidoreticular(+) subgroup and controls. Reductions in the 99mTc-TRODAT-1 binding ratios in the putamen were also detected in the pallidoreticular(+) subgroup compared to controls.

Tractography results for the pallidoreticular lesions

Because the pallidoreticular(+) subgroup was associated with a significantly lower 99mTc-TRODAT-1 binding ratio, which might suggest the presence of striatal–nigral pathology, tractography was performed on the 13 pallidoreticular(+) patients. Seed regions of interest (Fig. 4A, left, yellow areas) were placed bilaterally at the midbrain level within the area of reduced signal intensity in the susceptibility weighted imaging. The middle panel from Fig. 4A shows the tract plotted from the seed regions in the midbrain in one representative patient. The caudal part of the reconstructed fibre projections from a 3D plane is displayed through the medial-mesencephalic region (Fig. 4B and C). The caudal projections are consistent with the pallidoreticular lesion in the corresponding minimum intensity projection of susceptibility weighted imaging (Fig. 4B and C, right, arrows). The rostral projection from the seed region of interest courses through the globus pallidus (Fig. 4D–F) and the frontal periventricular region (Fig. 4G).
Table 2 Parkinsonian features, severity and 99mTc-TRODAT-1 binding ratio in the carbon monoxide subgroup defined by pallidoreticular lesions

<table>
<thead>
<tr>
<th>Parkinsonian features</th>
<th>Without PR lesion (n = 12)</th>
<th>With PR lesion (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinesia (n)</td>
<td>3</td>
<td>9**</td>
</tr>
<tr>
<td>Postural instability (n)</td>
<td>1</td>
<td>6**</td>
</tr>
<tr>
<td>Rigidity (n)</td>
<td>4</td>
<td>11**</td>
</tr>
<tr>
<td>Gait disturbance (n)</td>
<td>2</td>
<td>10**</td>
</tr>
<tr>
<td>Resting tremor; action tremor (n)</td>
<td>0; 1</td>
<td>0; 4</td>
</tr>
<tr>
<td>UPDRS motor score</td>
<td>4.25 ± 2.8</td>
<td>10.38 ± 4.8**</td>
</tr>
<tr>
<td>Rigidity (item 22)</td>
<td>0.42 ± 0.7</td>
<td>1.31 ± 0.8**</td>
</tr>
<tr>
<td>Hand movement (item 24)</td>
<td>0.25 ± 0.5</td>
<td>0.23 ± 0.44</td>
</tr>
<tr>
<td>Leg agility (item 26)</td>
<td>0.58 ± 0.7</td>
<td>1.85 ± 1.3**</td>
</tr>
<tr>
<td>Postural instability (item 30)</td>
<td>0.25 ± 0.6</td>
<td>1.92 ± 1.3**</td>
</tr>
<tr>
<td>Bradykinesia (item 31)</td>
<td>0.25 ± 0.5</td>
<td>0.92 ± 0.8**</td>
</tr>
<tr>
<td>Other movement disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myoclonus (n)</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Dystonia (n)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Chorea (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>99mTc-TRODAT-1 binding ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate (reference: 2.28 ± 0.2)</td>
<td>2.17 ± 0.3</td>
<td>1.81 ± 0.4*</td>
</tr>
<tr>
<td>Putamen (reference: 2.23 ± 0.3)</td>
<td>1.97 ± 0.2</td>
<td>1.69 ± 0.3*</td>
</tr>
<tr>
<td>Striatum (reference: 2.25 ± 0.2)</td>
<td>2.07 ± 0.2</td>
<td>1.75 ± 0.3*</td>
</tr>
<tr>
<td>Mean striatum (reference: 2.25 ± 0.2)</td>
<td>2.07 ± 0.3</td>
<td>1.74 ± 0.3*</td>
</tr>
</tbody>
</table>

The reference values in the 99mTc-TRODAT-1 binding ratio were derived from aged-matched controls.

*P < 0.05 with controls.

**P < 0.05 between subgroups with pallidoreticular lesions.

PR = pallidoreticular lesions; UPDRS = Unified Parkinson’s Disease Rating Scale.

Figure 4 Tractography from a single seed located on either side of the midbrain (A) Left: yellow indicates the seed region of interest, showing the fibre tract projecting caudally (B and C, left panel) and rostrally (D–I) overlaid onto the minimal intensity projection of susceptibility weighted images. An overlap was found between the low-signal lesions in the minimal intensity projection of susceptibility weighted images (arrows in A–C) and the fibre tracts (A middle panel; B and C, left panel). (H) Supplementary motor area, arrow; (I) anterior cingulate gyrus, arrow.
and terminates bilaterally in the supplementary motor area (Fig. 4H) and the anterior cingulate cortex (Brodmann area 24, Fig. 4I). Seed region of interests were also applied to the pallidoreticular(−) and control groups in close anatomical proximity to the pallidoreticular lesion in the midbrain. The results showed that both the pallidoreticular(−) and control groups had similar fibres originating from this region of interest located in the pars reticulata. The results from the control group are shown in Supplementary Fig. 1. The quantification of the fibre numbers through the seed region of interest in the control group, pallidoreticular(+) and pallidoreticular(−) subgroups were performed. Of note, the numbers of fibre tracts were comparable [control, range of attempt = 500; pallidoreticular(+) = 500 ± 8; pallidoreticular(−) = 500 ± 8, P > 0.1].

The corticostriatal tract image (Fig. 5A) reveals that the projection from the anterior cingulate cortex (the blue circle in Fig. 5B–D) passes through the frontal periventricular region (Fig. 5B), the anterior globus pallidus (Fig. 5C and D) and the medial mesencephalon (Fig. 5G). The caudal pathway from the supplementary motor area region (the red circle in Fig. 5B–D) projects via the middle corona radiata (Fig. 5B), the posterior globus pallidus (Fig. 5C and D) and the lateral cerebral peduncle regions (Fig. 5H). These fibres again show significant overlap with the pallidoreticular lesion (Fig. 5E, G and H), as observed in the minimum intensity projection of susceptibility weighted imaging (Fig. 5F).

The differences between the two subgroups in the fractional anisotropy

The difference in the fractional anisotropy along the skeletonized white matter tract (black) between the pallidoreticular(+)...
and pallidoreticular(−) subgroups is depicted in Fig. 6. The pallidoreticular(+) subgroup has significantly lower fractional anisotropy (red) in the following regions: the anterior and posterior corpus callosum, the orbital–frontal region, the medial and lateral prefrontal areas, the genu of the internal capsule, the thalamus, the brainstem and the cerebral peduncle. The change was attributed to the increase in both axial and radial diffusivities (Supplementary Fig. 2). No cluster showed a smaller fractional anisotropy in the pallidoreticular(−) subgroup than in the pallidoreticular(+) subgroup.

Correlation between individual fractional anisotropy with clinical parameters and the 99mTc-TRODAT-1 binding ratio

In the patients with carbon monoxide intoxication, we performed a correlation analysis between fractional anisotropy, clinical parameters and the 99mTc-TRODAT-1 binding ratio. The regions of interest in fractional anisotropy were located in the frontal white matter, corpus callosum (anterior and splenium) and substantia nigra. The clinical parameters included the Mini-Mental State Examination, a verbal memory score (10 min recall), a neuropsychiatric inventory total score, a digit backward and calculation score. An inverse correlation was found between the fractional anisotropy value in the substantia nigra and the Neuropsychiatric Inventory total score ($\rho = -0.524$, $P = 0.012$). Because the 99mTc-TRODAT-1 binding ratio is also related to presynaptic dopamine neuron activity in the substantia nigra, we performed a correlation analysis between the ratio in the striatum and fractional anisotropy in the substantia nigra. However, none of them reached the threshold for statistical significance ($P > 0.05$).

The differences between the pallidoreticular subgroups in the grey and white matter volume

Increased atrophy of the white matter (Fig. 7A) and the grey matter (Fig. 7B) was noticed in the pallidoreticular(+) subgroup when compared with the pallidoreticular(−) subgroup. The affected region in the white matter included the bilateral cerebral peduncles, the white matter surrounding the hippocampus, the fornix, the genu of the internal capsule, the bilateral frontal periventricular area and the splenium area of the corpus callosum. The only significantly affected region of the grey matter was in the thalamus. The voxel-based morphometry analysis indicated no increased atrophy in the pallidoreticular(−) subgroup as compared with the pallidoreticular(+) subgroup.

Figure 6  Reduction in fractional anisotropy as identified in tract-based spatial statistics analysis. Comparison between patients with pallidoreticular lesions to those without. The clusters indicated a significant decrease in fractional anisotropy (red) in the subgroup with pallidoreticular lesions, overlaid onto the mean fractional anisotropy skeleton map (black) (all $P < 0.05$, with multiple corrections at the cluster level).
Discussion

This study explored the clinical significance of pallidoreticular lesions in patients with carbon monoxide intoxication via an investigation of the related neural network involvement. There were three major findings. First, using the presence of paramagnetic material, we successfully demonstrated the existence of differences in local susceptibility along the pallidoreticular pathway. Of note, they might serve as MRI markers of poorer cognitive and behavioural scores, higher frequencies of parkinsonian symptoms with higher Unified Parkinson’s Disease Rating Scale motor scores, lower 99mTc-TRODAT-1 binding ratios in the caudate and putamen and more extensive intracerebral damage in patients with carbon monoxide intoxication. Second, the dysfunction in the neural network associated with the pallidoreticular lesion was linked to modulation of the prefrontal lobe with distinct fibre bundles running in parallel. Finally, in addition to the pallidoreticular lesion, other affected regions included the posterior corpus callosum, the orbital-frontal lobe and the thalamus.

Negative phase imaging suggested a paramagnetic component of the pallidoreticular lesions

Pallidoreticular lesions have been less frequently mentioned in the carbon monoxide intoxication literature than globus pallidus or white matter lesions, possibly due to the low sensitivity of conventional neuroimaging techniques. In our study, only 2 of the 13 pallidoreticular(+) patients had pallidoreticular lesions that were detectable by T2-weighted images. The current study demonstrated the ability of negative phase in susceptibility weighted imaging to discriminate the neural substrate responsible for the pallidoreticular lesions. The negative phase in the susceptibility weighted imaging may have been related to a local inhomogeneity in the magnetic field, which suggests that the pallidoreticular lesions may have been composed mainly of paramagnetic materials. They probably consisted of heterogeneous contents that may have included small amounts of diamagnetic substances, such as crystalline calcium, within the lesion parenchyma, consistent with the report by Adam et al. (2008) of the presence of pathological calcification in carbon monoxide intoxication.

Because no biopsies were available, we cannot conclude from the imaging findings that the pallidoreticular lesions were composed solely of paramagnetic material. The corresponding region in the CT images from the current study did not show signs of calcification, as assessed by the measurement of the CT Hounsfield units. All of the lesions showed a paramagnetic effect in the MRI; however, either the amount of the diamagnetic substances was too small to have an effect or it had become magnetic resonance or CT invisible.

The paramagnetic substances in pallidoreticular lesions might be by-products of haemorrhage

The broad spectrum of neuropathological effects of carbon monoxide intoxication includes petechial haemorrhages in the

Figure 7 Atrophy pattern identified from voxel-based morphometry analysis. The atrophy pattern in (A) the white matter and (B) the grey matter from patients with pallidoreticular lesions, compared to those without. The anatomical reference used Montreal Neurological Institute template.
white matter, laminar necrosis of the cortical regions, pseudocalcification of the blood vessels and the pallidum, multifocal necrosis of the basal ganglion and the loss of Purkinje cells in the cerebellum (Lapresle and Fardeau, 1966, 1967, 1971). Based on a review of the literature, the paramagnetic substances might represent the by-products of haemorrhage, such as ferritin or haem iron. Because of the identical locations of the fibre tracts in the mesencephalon regions of interest and the low signal intensity pallidoreticular lesions in the susceptibility weighted imaging, we believe that the pallidoreticular lesions might be composed of nigrostriatal neuronal projections. The underlying mechanism could be a petechial haemorrhage of the nerve fibres with the subsequent deposition of blood by-products. Alternatively, it could be related to a secondary pathological process, with paramagnetic substances accumulating along the nerve bundle. These hypotheses will require further histological investigation.

**Pallidoreticular pathways modulate presynaptic dopaminergic activity and parkinsonian features**

From our study results, the pallidoreticular(+) subgroup had lower 99mTc-TRODAT-1 ratios in both the caudate and the putamen, as well as a higher percentage of parkinsonian symptoms. Along with the fibre tracing results, this finding may indicate a role for the pallidoreticular pathway in modulating the nigrostriatal dopaminergic activity in carbon monoxide intoxication. Presynaptic nigrostriatal dysfunction is well established as being related to parkinsonian symptoms in idiopathic Parkinson’s disease (Papapetropoulos, 2006; Kagi et al., 2010). In the present study, we reasoned that the symmetric decrease in 99mTc-TRODAT-1 binding ratio could be related to pallidal dysfunction and may be increased by the presence of pallidoreticular lesions.

Pallidal dysfunction can play a major role in causing the parkinsonian features observed in the pallidoreticular(+) subgroup based on the similar degree of reduction in the putamen and caudate binding ratios, the relatively limited response to levodopa or dopaminergic agonists and the lack of correlation between the 99mTc-TRODAT-1 ratio in the striatum and fractional anisotropy in the substantia nigra. The lack of association between the rostral substantia nigra with striatum 99mTc-TRODAT-1 binding observed in this study might also be related to the regions of interest we selected for correlation. It is worth noting in this regard that caudal, rather than rostral, substantia nigra has been shown to have significant sensitivity and specificity for distinguishing patients with Parkinson’s disease from healthy subjects (Vaillancourt et al., 2009). Only one case from the literature of carbon monoxide intoxication reported asymmetric presynaptic dopamine hypofunction in the putamen (Rissanen et al., 2010). The absence of substantia nigra lesions and the association between striatal deficits and parkinsonian symptoms were unique to that case. Because the case (Rissanen et al., 2010) did not include susceptibility weighted imaging, it is unclear whether the patient, whose substantia nigra was analysed by conventional MRI, belongs to the pallidoreticular(+) or the pallidoreticular(−) subgroup. However, it is worth noting that the pallidoreticular(−) subgroup with globus pallidus lesions in the present study also had significant decreases in the 99mTc-TRODAT-1 ratio in the putamen as in the case reported by Rissanen et al. (2010). This observation might also substantiate the role of pallidoreticular lesions in reducing the 99mTc-TRODAT-1 binding ratio in the striatum as observed in our pallidoreticular(+) subgroup.

**Tractography in relation to the clinical manifestations of the pallidoreticular lesions**

In this study, we analysed possible damage on three levels: mesencephalic, basal ganglia and cortical regional network connections. The use of tractography alone was not sufficient to ascertain whether the fibres projecting to the prefrontal regions were directly picked up from the midbrain seed region of interest, a combination of different fibre bundles during tracing or both. Because the seeding region was defined from susceptibility weighted imaging, it is possible that additional fibres could be identified during the tracing process. We interpreted the results of tractography between the globus pallidus and the supplementary motor area or anterior cingulate cortex as the presence of cortical-striatal pathways (Middleton and Strick, 2000; Lehericy et al., 2004) rather than a direct extension of fibres from the midbrain seed region.

Adam et al. (2008) noted that motor and behavioural phenotypes after developing pallidoreticular lesions were related to different neural projection networks in the pallidum. Carbon monoxide intoxication with lesions preferentially involving the anterior pallidum would be expected to produce behavioural phenotypes because the prefrontal cortex and basal ganglion loop would be interrupted. Lesions selectively located in the posterior pallidum might lead to parkinsonian symptoms because of the interrupted sensorimotor loops. Because the neuronal network in the striatum might be modulated by the cortical bundles, disconnection from the prefrontal lobe either in the pallidum or at the pallidoreticular lesion may clinically result in behavioural and parkinsonian features, depending on the integrity of the remaining networks.

The tractography study also focused on the section between the pallidum and the substantia nigra pars reticulata, which was found to be damaged. Tractography is possible when the fibres are only damaged rather than entirely lost. Although we cannot rule out the possibility that neuron fibres were lost in this region, both the tract-based spatial statistics findings and the quantification efforts support the view that neuron fibres were only damaged. It should be noted, in any case, that quantification using tractography can be affected by several factors. For example, the integrity of the neuronal fibre can be damaged without a significant numeric reduction. The number of the fibres through the seed region can be affected by interindividual differences in brain size. The spatial resolution of MRI cannot be sufficient to identify all the neuron fibres in the region.
Clinical severity related to fractional anisotropy changes

Compared to the pallidoreticular(−) subgroup, the presence of the pallidoreticular lesions was associated with poorer neuropsychiatric performance in the pallidoreticular(+) subgroup. The changes seen in the pallidoreticular(+) subgroup were linked to cognitive dysfunction as suggested by the inverse correlation between the fractional anisotropy in the substantia nigra and the neuropsychiatric inventory score. The reduction in fractional anisotropy, which was associated with an increase in both axial and radial diffusivity in the cerebrum, also suggested a possible link between the regional disconnection and the poorer cognitive results in the pallidoreticular(+) subgroup. However, we did not find a correlation between individual fractional anisotropy results and the results of cognitive tests. Fractional anisotropy, in general, is a measure for white matter integrity. However, the organization of neurons in the basal ganglia may deviate from this theoretical model. Accordingly, carbon monoxide intoxication affected a more profound region that could also involve grey matter such as thalamus (as shown in this study) and other cortical regions (Hopkins et al., 2006). This in turn may limit the usefulness of using a diffusion parameter (such as fractional anisotropy) in a single region for correlation analyses with clinical parameters in patients with carbon monoxide intoxication.

Globus pallidus lesions were non-homogeneous in terms of magnetic susceptibility

Typical globus pallidus lesions are characterized by well-defined, macroscopic necrosis with asymmetrical extensions to the internal capsule; occasionally, there is small or linear focus of necrosis (Prockop and Chichkova, 2007). The lesions in the globus pallidus have traditionally been considered a hallmark of a hypoxic–ischaemic process, although recent studies have outlined various biochemical mechanisms of carbon monoxide intoxication in addition to hypoxia (Koehler et al., 1983; Sengers and Stadhouders, 1987; Penney, 1988; Young and Caughey, 1990). Our phase-filtered imaging indicated that both diamagnetic and paramagnetic substances existed in the globus pallidus lesions. The phase images indicated a non-homogeneous composition of the globus pallidus lesions, which is in contrast to the pallidoreticular lesion.

Iron deposition in other nerve bundles

A consensus has been growing among many researchers that iron is enriched within oligodendrocytes and myelin in diseased tissue and is associated with the biosynthetic enzymes of myelinationogenesis (Connor et al., 1995; Saleh et al., 2003; Sow et al., 2006). In demyelinating diseases (Tjoa et al., 2005; Brass et al., 2006; Haacke et al., 2009), the mechanism of damage to the brain by iron may be related to oxidative stress induced by the generation of toxic free radicals. Recent studies of diseases related to iron deposition have associated iron deposition with a signal loss in conventional T₂-weighted images. This study also found reduced signal intensities from the corpus callosum in the susceptibility weighted imaging. Although the signals are less prominent compared to those in the globus pallidus, haemorrhages in the corpus callosum have been frequently reported following acute carbon monoxide intoxication (Lapresle and Fardeau, 1967; Pach et al., 1998). This study demonstrated that susceptibility weighted imaging has great potential for visualizing the changes related to iron deposition around the myelin sheath.

Limitations and conclusions

There are several limitations to this study. First, the patients could only be enrolled after carbon monoxide intoxication, and we only selected patients with globus pallidus lesions. Therefore, it was not possible to control for all of the potential confounding factors and obtain a uniform study population. Because the precise duration of exposure might be biased by the drowsy consciousness and retrograde amnesia in most of the study participants, we were unable to analyse the time interval between carbon monoxide intoxication and the measurement of carboxyhaemoglobin. It is thus possible that the worse clinical performances observed in the pallidoreticular(+) subgroup can be ascribed to a greater severity of exposure to carbon monoxide. This possibility is in accordance with the higher carboxyhaemoglobin level observed in the pallidoreticular(+) subgroup, although the difference failed to reach statistical significance. Second, no histological evidence was available in this study because of the cross-sectional nature of studies involving human subjects. The interpretation of the pathological changes in the neuroimaging findings, as reflected by the changes in the phase of the susceptibility weighted image, could not be validated. Nonetheless, the results are consistent with previous pathology studies in which haemorrhages surrounding the vessels or nerve bundles or in the globus pallidus were evident (Lapresle and Fardeau, 1966, 1967, 1971). It is worth noting that a direct link has not been conclusively established between the paramagnetic neural substrate and the microscopic pathology of the pallidoreticular lesion. In this study, we performed tractography on patients with carbon monoxide intoxication. However, the validation and quantification of the identified fibres remain a challenge. The analysis of the diffusion tensor imaging was mainly limited to the white matter. In the future, a generalized diffusion tensor model could extend our understanding of the changes in the grey matter.

In conclusion, we demonstrate a local paramagnetic susceptibility in the pallidoreticular pathways after carbon monoxide intoxication that might be related to a poorer neuropsychiatric performance and higher frequencies of parkinsonian feature related to the extensive neural networks damage. The greater parkinsonian severity observed in the pallidoreticular(+) subgroup may reflect a higher total lesion load due to carbon monoxide intoxication, but does not necessarily imply a direct causal effect of the pallidoreticular lesions in the substantia nigra.
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Supplementary material

Supplementary material is available at Brain online.

References


