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Diffusion Tensor Tractography in Mesencephalic Bundles: Relation to Mental Flexibility in Detoxified Alcohol-Dependent Subjects

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Components of the corticocerebellar circuit and the midbrain individually play a central role in addictive processes and have been associated with altered volumes and impairment of cognitive flexibility in alcohol-dependent subjects. The microstructure of white matter bundles composing the corticocerebellar network and passing through the midbrain was studied using diffusion tensor imaging in a group of detoxified alcohol-dependent men ($n = 20$) and a group of healthy men ($n = 24$). The relationship between properties of these white matter bundles and cognitive flexibility performance was investigated in alcohol-dependent subjects. Bundles connecting two regions of interest were analyzed using a fiber-tracking quantitative approach, which provided estimates of the fractional anisotropy and the apparent diffusion coefficient, as well as the number of tracked fibers normalized by the volume of regions of interest. Within the bundles running between the midbrain and pons, a mean of 18% fewer fibers per unit volume were tracked in alcohol-dependent men than in healthy controls. In addition, the normalized number of these fibers correlated with the performance in the Trail-Making Test part-B. Even though the alcohol-dependent subjects were detoxified and apparently neurologically intact, their earlier excessive use of alcohol seems to be associated with altered neural microstructure of mesencephalic white matter bundles, which may contribute to their cognitive flexibility impairment.

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INTRODUCTION

In the corticocerebellar circuit, the midbrain is a core region that contains both top-down and bottom-up fiber bundles (Wakana *et al*, 2004), and it is highly involved in addictive processes (Hyman *et al*, 2006). Also, the pons and the thalamus have been identified as sites of an intermediate step in the feed-forward and feed-back loops of the corticocerebellar circuit, respectively (Schmahmann and Pandya, 1997). Chronic alcohol exposure has been associated with smaller volumes of the thalamus, pons, and cerebellum (Baker *et al*, 1999; Kril *et al*, 1997; Sullivan *et al*, 2000, 2003), as well as with cortical volume alterations (Harper *et al*, 2003).

Alcohol-dependent individuals often express distinct impairments in problem solving, cognitive planning, and working memory (Oscar-Berman *et al*, 1997). Such neuropsychological deficits have classically been associated with impaired function of the frontal lobes, but more recently, also with alterations within the corticocerebellar circuit (Schmahmann and Pandya, 1997; Sullivan, 2003). Previous investigations on alcoholism have provided evidence of an association between white matter structure in the corpus callosum and certain components of executive functions, eg attention skills (Pfefferbaum *et al*, 2000) and inhibitory control (Schulte *et al*, 2006). In our own recent study, decreased white matter volume in the midbrain of alcohol-dependent subjects was related to impaired performance in two executive tasks: the Trail-Making Test part-B (TMT-B) and the WAIS letter–number sequencing test (LNS; Chanraud *et al*, 2007). Both tasks invoke cognitive flexibility,

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a component of executive functions, which is known to influence treatment outcome (Goldman, 1990) and experience-dependent recovery (Roehrich and Goldman, 1993) in chronic alcoholism. However, our earlier study did not identify the specific regions of the midbrain and associated white matter tracts that are involved in the alcohol-dependent subjects' cognitive flexibility impairment.

Over the last decade, diffusion tensor imaging (DTI) has increasingly been applied to the assessment of white matter microstructure characteristics in normal development as well as various neurological conditions and psychiatric disorders. This noninvasive technique allows imaging of the microstructure of biological tissue (Le Bihan *et al*, 2001). In addition to quantitative fiber tracking (Johansen-Berg and Behrens, 2006; Sullivan *et al*, 2006), DTI can measure the apparent diffusion coefficient (ADC; a direction-independent measure of average diffusivity that reflects water motility in a voxel; see Le Bihan *et al*, 2001) and fractional anisotropy (FA; an estimate of highly restricted diffusion in a linear framework; see Pfefferbaum *et al*, 2006) in the white matter tissue.

In order to explore the yet largely unknown relationship between white matter characteristics in the corticocerebellar circuit and cognitive flexibility in alcohol-dependent subjects, we performed a tractography analysis of our DTI data (Chanraud *et al*, 2007). We hypothesized that microstructural alterations in white matter fiber bundles linking the midbrain to the pons and/or to the thalamus would be related to the impaired cognitive flexibility of these subjects, but not to other types of cognitive deficits (eg semantic memory) present in alcohol-dependent subjects.

MATERIALS AND METHODS

Subjects

DTI images from 20 alcohol-dependent subjects (DSM-IV criteria) and 24 healthy subjects were selected from a larger sample (Chanraud *et al*, 2007) on the basis of the DTI image availability. Data from one alcohol-dependent subject were excluded from the analysis to enable matching of groups for age and educational level (Table 1).

All study participants were Caucasian men, between 25 and 65 years of age, fluent in French, and right handed, as assessed by Annett's questionnaire (Annett, 1967). Patients were recruited on admission to detoxification or day-care units in two addiction departments of the Paul Brousse and Emile Roux hospitals in the Paris area (Assistance Publique, Hôpitaux de Paris, France).

All subjects were evaluated using the Alcohol Use Disorders Identification Test (AUDIT; Reinert and Allen, 2002) and the Social Adjustment Scale Self Report (Weissman and Bothwell, 1976; see Table 1).

Alcohol-dependent patients were interviewed and clinically examined by a senior psychiatrist (CM, JLM, or HJA). The medical consequences of chronic alcoholism on other organs may confound the association between brain microstructure and behavioral measures. Thus, as brain changes maybe, in part, secondary to liver damage in alcohol-dependent patients with alcoholic cirrhosis (Arria *et al*, 1991), we selected detoxified drinkers with moderate alcoholism, ie with no clinical evidence of brain dysfunction or medical conditions considered to be clinical indicators of severe alcoholism (eg alcohol-induced dementia or chronic liver disease). The inclusion criteria were (1) fewer than three periods of withdrawal because more than two periods of withdrawal may be associated with greater cognitive impairment in alcohol-dependent subjects (Duka *et al*, 2003), (2) detoxification for at least 3 weeks and abstinence, as assessed by normal levels of γ-glutamyl-transferase and carbohydrate-deficient transferrin (see Table 2), and (3) no use of sedative medications for at least 7 days preceding the study (before participating in this study, the patients had been treated during withdrawal with decreasing doses of lorazepam and vitamins B1 and B6). The exclusion criteria included (1) signs or symptoms of malnutrition, (2) signs of liver dysfunction: aspartate aminotransferase/alanine aminotransferase ratio greater than two (Cohen and

Table 1 Demographic Data and Neuropsychological Test Results of the Study Subjects

	Alcohol-dependent subjects ($n=20$)	Healthy subjects ($n=24$)	Statistical significance*
Age (years)	49 ± 7	46 ± 6	0.15
Body mass index (kg/m^2)	24.2 ± 4.4	24.7 ± 3.4	0.6
Education (years)	8.1 ± 3.2	8.7 ± 3.4	0.32
AUDIT	31.9 ± 4.2	2 ± 1.8	0.00
Social adjustment scale score	1.7 ± 0.4	1.5 ± 0.2	0.12
Tobacco consumption (pack years of active smoking)	34 ± 16	18 ± 13	0.09
Neuropsychological tests			
Letter–number sequencing	8.7 ± 3.8	11.3 ± 3.1	0.02
TMT-B (s)	132 ± 69.8	80.3 ± 43.2	0.01
TMT-A (s)	40.5 ± 11.2	31.2 ± 7.6	0.11
WAIS information	8.3 ± 3.6	11.8 ± 3.4	<0.001

Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; TMT-A, Trail-Making Test Part-A; TMT-B, Trail-Making Test Part-B; WAIS, Wechsler Adult Intelligence Third Revision.
Data are presented as mean ± SD.
*p-Values for between-group comparisons performed using two-sample t-test (demographic data) or Mann–Whitney U-test (neuropsychological tests).

Table 2 Characteristics of the Alcohol-Dependent Subjects

Patients' characteristics	Value	Laboratory norm
Alcohol consumption[a]	23.8 ± 12.7	
Duration of dependence (years)	7.95 ± 6.4	
Abstinence (weeks)	29 ± 34	
Age (years) at first drinking	20.9 ± 6.7	
Age (years) at the onset of dependence	39.8 ± 10.6	
Biological variables:		
γ-Glutamyl-transferase	53.16 ± 6.98	<53
Alanine aminotransferase (U/l)	26.53 ± 15.42	<38
Aspartate aminotransferase (U/l)	27.95 ± 15.19	<40
Aspartate aminotransferase/alanine aminotransferase	1.09 ± 0.24	
Carbohydrate-deficient transferrin	1.97 ± 0.25	<2.6

[a]Consumption was defined as drinks per day during 3 months preceding detoxification, where one drink was considered to contain approximately 10 g of ethanol (standardization of beer, wine, and spirits).

Kaplan, 1979), and (3) a score >20 on Hamilton Anxiety Scale or a score >10 on Hamilton Depression Scale.

Healthy comparison subjects were recruited from the community. The inclusion criteria were alcohol consumption of less than two standard units (where one unit equals 10 g of ethanol) of alcohol per week and a score ⩽5 on the AUDIT scale.

All subjects underwent a thorough medical, psychiatric, and neurological examination by a senior psychiatrist. Exclusion criteria for all subjects included any kind of neurological symptoms (cerebellar, sensory, or motor dysfunctions), history of psychiatric disorder (other than alcohol-dependence for patients), medical conditions that may alter cerebral function (ie cardiovascular, endocrinological, autoimmune, or oncological diseases), and brain trauma (seizures, degenerative disease, previous head injury with loss of consciousness). In addition to these examinations, the presence of visible abnormalities on T_2 images (assessed by a radiologist), pace maker, bypass surgery or metallic implants that would preclude magnetic resonance imaging (MRI) scan, or substance abuse (other than alcohol and tobacco) resulted to the exclusion of the subject.

Therefore, the set of exclusion criteria led up to select alcohol-dependent patients without apparent neurological conditions.

Subjects were fully informed of the nature of the investigation and they provided written consent for their involvement. The study was carried out in accordance with the Declaration of Helsinki. The study protocol was approved by the C.P.P. Île de France 7 ethics committee.

Image Acquisition

All subjects underwent volumetric MRI and DTI of the brain, using a 1.5 T Signa imager (General Electric Healthcare, Milwaukee, WI). Volumetric imaging was performed using a standard 3D T_1-weighted inversion recovery fast-spoiled gradient-recalled sequence with the following parameters: axial orientation, field of view = 24 cm, matrix = 256 × 192 interpolated to 256 × 256, 124 slice locations, 0.9375 × 0.9375 mm^2 in-plane resolution, slice thickness = 1.3 mm, TE = 2 ms, TR = 10 ms, TI = 600 ms, flip angle = 10°, read bandwidth = 12.5 kHz.

Diffusion-weighted images were acquired with an echo-planar imaging sequence (TE = 70 ms, TR = 8300 ms, b-value = 700 s/mm^2, 56 slices of 2.4 mm, axial orientation, in-plane resolution = 1.875 × 1.875 mm, matrix 128 × 128). DTI acquisition included five supplementary T_2 images without diffusion weighting ($b = 0$). Diffusion gradients were applied along 36 orientations. The acquisition process was designed to allow reconstruction of the diffusion tensor, even if subjects had moved during the scanning procedure (Dubois *et al*, 2006b). The number of orientations used to reconstruct the diffusion tensor was similar in the patient and control groups (34.6 ± 2.7 and 35.3 ± 1.9, respectively; $t = -1.053$, $p = 0.3$).

Data Processing and ROI Drawing for White Matter Tracking

The tracking algorithm was initialized using a 'two-region of interest' approach (Conturo *et al*, 1999), where we focused on the fiber bundles passing through specific regions and connecting the midbrain to the pons and to the thalamus.

First, echo-planar distortion in the diffusion images was corrected, then diffusion data were processed and regions of interest (ROIs) were drawn using Brainvisa software (http://brainvisa.info/). Thereafter, the diffusion tensor was assessed and the ADC and FA for each voxel were calculated on the basis of the diffusion tensor.

ROIs were manually defined by SC, who was blind to the subject's diagnosis, using the method described by Sullivan *et al* (2004). The pons and the thalamus (left and right separately) were traced in the sagittal plane with correction in the axial plane, whereas the midbrain was traced in an axial plane with correction in the sagittal plane. Each region was delineated using first a midplane, and then in five successive slices above and below that plane, or on the left and on the right from that plane, depending of its orientation. Then, corrections were made to ensure that two ROIs were separated from each other by a distance of at least five axial slices (Figure 1), as a result the midbrain was covered by seven successive axial slices. ROIs were drawn on individual T_1 realigned anterior commissure—posterior commissure scans, rather than diffusion images, to avoid using the dependent variables (FA and ADC maps) for data preprocessing and analyses (Kanaan *et al*, 2006). An isotropic morphological erosion of 0.5 mm was applied to ROI masks, to prevent interference from other brainstem bundles in the tractography process. ROIs were then individually registered on diffusion images (t2-diffusion weighted, FA and ADC images) with an affine transformation minimizing mutual information implemented in Brainvisa software (Houenou *et al*, 2007; Dubois *et al*, 2006a). The locations of the transformed ROIs in native diffusion space were verified visually.

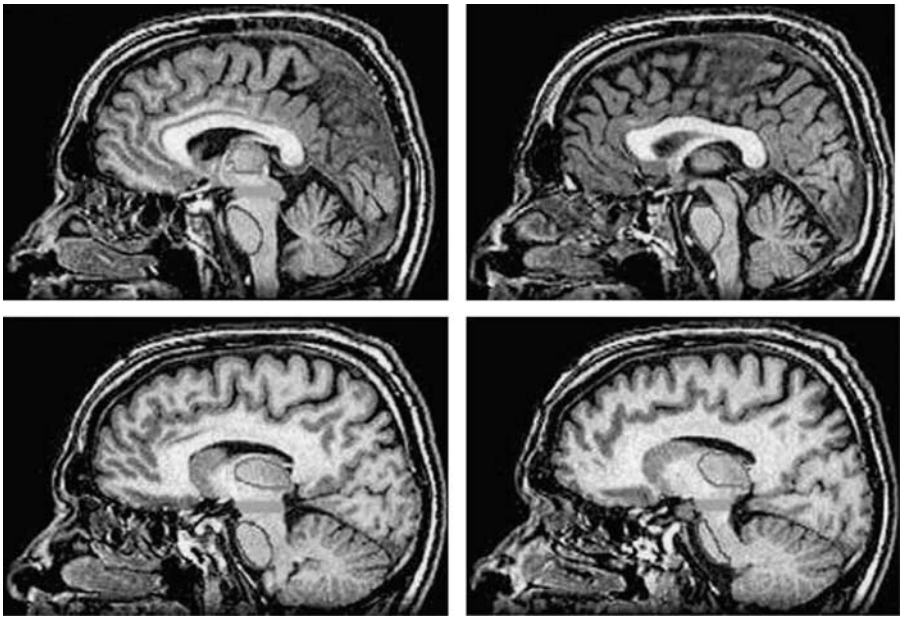

Figure 1 T_1 sagittal slices from a healthy man. Outlines, from the top to the bottom, of the thalamus, the midbrain and the pons.

Bundle Tracking

Tractography algorithms use DTI data for the virtual reconstruction of white matter fibers, by following the continuous path of greatest water diffusivity from a predefined brain region (ROI/seed points) through the white matter (Behrens *et al*, 2003; Conturo *et al*, 1999; Poupon *et al*, 2001).

The fiber components of bundles emerging from ROIs were reconstructed using a voxel-by-voxel regularized streamline algorithm (Perrin *et al*, 2005), which resembles diffusion tensor deflection (Lazar *et al*, 2003). This approach was supposed to minimize the disturbances caused by simple crossing configurations during tractography. The direction of the tract in each voxel was determined using two parameters: the largest tensor direction eigenvector (weighted by the coefficient α that corresponds to local anisotropy) and inertia (weighted by $1-\alpha$), ie the incident direction of the tract that represents the propagation direction from the previous reconstruction step. The algorithm proceeded toward the chosen direction (depending on the weighting and thus on local anisotropy) in half-voxel steps. The propagation mask excluded voxels likely to belong to the gray matter ($FA < 0.2$). A maximum curvature angle of 20° was used to avoid high curvature of the trajectory. Only fibers passing from midbrain to pons or from midbrain to thalamus were analyzed.

For each reconstructed fiber bundle, we counted the number of tracked fibers and determined mean FA and mean ADC along these bundles. In this article, the term 'tracked fibers' refers to the number of fibers generated by the algorithm in fiber bundles connecting predefined brain regions, rather than the actual number of fibers in anatomical fiber bundles (for further explanation of this difference, see Discussion).

In order to avoid the possible confounding effect on tractography of interindividual differences in ROI volume, the number of fibers reconstructed between two ROIs were normalized by the volumes of both regions (ROI1 $\times$ ROI2; cf Houenou *et al*, 2007).

Neuropsychological Tests

All participants underwent the Mini Mental State Examination (Folstein *et al*, 1975) and a battery of neuropsychological tests on the day of the MRI acquisition or within the following 3 days (Chanraud *et al*, 2007). We selected cognitive flexibility tasks in which alcohol-dependent subjects are known to perform worse than healthy subjects and which have been shown to be related to midbrain white matter volume (Chanraud *et al*, 2007): the TMT-B and the WAIS LNS (see Table 1). The TMT-B performance was measured as completion time and LNS performance as reproduced digits.

(1) We used the subjects' performance in a semantic memory task (WAIS-information) to evaluate the specificity of TMT-B and LNS to midbrain–pons/midbrain–thalamus bundle characteristics, as the corticocerebellar circuit is not believed to be directly involved in semantic memory performance (Thompson-Schill, 2003).
(2) We also used the subjects' performance in the TMT-A in statistical analyses as a control for motor skills.

Raw tests scores were converted to *z*-scores by adjusting for age and years of education from the French population data.

Statistical Analyses

Statistical analyses were conducted with SPSS 12.0 software (SPSS Inc., Chicago, IL). The normality of the data was tested with Kolmogorov–Smirnov test. Differences between groups for demographic variables (Table 1) and ROI volumes were analyzed using *t*-tests for independent samples.

In order to limit type 1 error (false positives), the number of variables examined was constrained by *a priori* hypotheses (detailed in Introduction) based on previous findings on the same samples (Chanraud *et al*, 2007), for both the subcortical regions and neuropsychological tests.

As the volumes of the pons and thalamus have been shown to be independently affected in alcohol-dependent subjects (Sullivan, 2003), we performed all statistical analyses separately for the bundles connecting the midbrain to the pons, and for those connecting the midbrain to the thalamus. Between-group differences in the number of tracked fibers/volume, FA, and ADC were assessed using the nonparametric rank-based Mann–Whitney *U*-test.

Associations between bundle features, neuropsychological *z*-scores, brain region volumes, and patients' drinking history variables were assessed with Pearson or Spearman correlations, as appropriate.

For each analysis, we fixed a two-tailed α level at 0.05.

RESULTS

Drinking history variables for the alcohol-dependent subjects are presented in Table 2. On average, they had their first alcoholic drink in early adulthood and had been alcohol-dependent since approximately 40 years of age. The alcohol-dependent and control groups were similar with respect to mean age, years of education, body mass index, and Social Adjustment Scale Self Report and Mini Mental State Examination scores. At the time of MRI, a half of the alcoholics and 17% of the controls were cigarette smokers.

Between-Group Comparisons

In comparison with healthy subjects, the alcohol-dependent subjects performed worse on TMT-B, LNS, and WAIS-information but not on TMT-A (see Table 1).

Alcohol-dependent subjects had a smaller mean volume of the pons (4784 ± 1104 *vs* $5477 \pm 1074\,mm^3$ in healthy subjects; $t(42) = -2.10$; $p = 0.04$) and there was also a nonsignificant trend toward a lower thalamus volume (9330 ± 1518 *vs* $10\,070 \pm 1391\,mm^3$; $t(42) = -1.66$; $p = 0.1$). No difference was observed in midbrain volume (4363 ± 496 *vs* $4455 \pm 444\,mm^3$; $t(42) = -0.63$; $p = 0.5$).

The main results of bundle tracking are presented in Table 3. Compared to healthy subjects, alcohol-dependent subjects had, on average, 18% fewer fibers per unit volume running between the midbrain and the pons, and the mean ADC along the tracked fiber bundles between these two regions was 3% higher. For the fiber bundles tracked between the midbrain and the thalamus, only ADC differed, being 3% higher in alcohol-dependent subjects than in healthy subjects. A typical example of the tractography output in one alcohol-dependent subject is shown in Figure 2.

Correlation Between Diffusion Indices in Tracked Bundles and Neuropsychological Data

In the group of alcohol-dependent subjects, the normalized number of fibers tracked between the midbrain and the pons was related to the TMT-B *z*-scores ($r = -0.51$; $p = 0.03$; see Figure 3a). The LNS ($r = 0.19$; $p = 0.41$), the WAIS-information ($r = 0.08$; $p = 0.73$), and the TMT-A *z*-scores ($r = -0.29$; $p = 0.235$; see Figure 3b) did not correlate with the characteristics of the midbrain–pons bundles. No correlation was found between the characteristics of the midbrain–thalamus bundles and neuropsychological data.

In the group of healthy subjects, there was no significant association between bundles' properties and TMT-B performance (see Figure 3a) or with other neuropsychological data.

Correlation Between Diffusion Indices in Tracked Bundles, Neuropsychological Data, and Drinking History Variables

Neither neuropsychological performance nor white matter values were related to the patients' drinking history

Table 3 Results of Bundle Tracking

Measures in bundles tracked between two regions	Alcohol-dependent subjects ($n = 20$)	Healthy subjects ($n = 24$)	Statistical significance*
Midbrain-pons			
Number of tracked fibers	1926 ± 1009	2591 ± 1014	0.019
Number of tracked fibers normalized by the volumes of midbrain and pons	$8.85 \times 10^{-5} \pm 3.39 \times 10^{-5}$	$1.02 \times 10^{-4} \pm 4.16 \times 10^{-5}$	0.05
Mean FA	0.54 ± 0.04	0.55 ± 0.05	0.25
Mean ADC ($\times 10^{-10} m^2/s$)	8.94 ± 1.73	8.73 ± 0.44	0.022
Midbrain-thalamus			
Number of tracked fibers	777.55 ± 415.07	797.6 ± 325.57	0.46
Number of tracked fibers normalized by the volumes of midbrain and thalamus	$2.15 \times 10^{-5} \pm 1.16 \times 10^{-5}$	$1.70 \times 10^{-5} \pm 8.31 \times 10^{-6}$	0.25
Mean FA	0.49 ± 0.04	0.48 ± 0.06	0.42
Mean ADC ($\times 10^{-10} m^2/s$)	8.55 ± 0.28	8.25 ± 0.33	0.014

Abbreviations: ADC, apparent diffusion coefficient; FA, fractional anisotropy.
Data are presented as mean ± SD.
*p-Values for between-group comparisons carried out with analysis of covariance (number of tracked fibers) or Mann–Whitney *U*-test (FA and ADC).

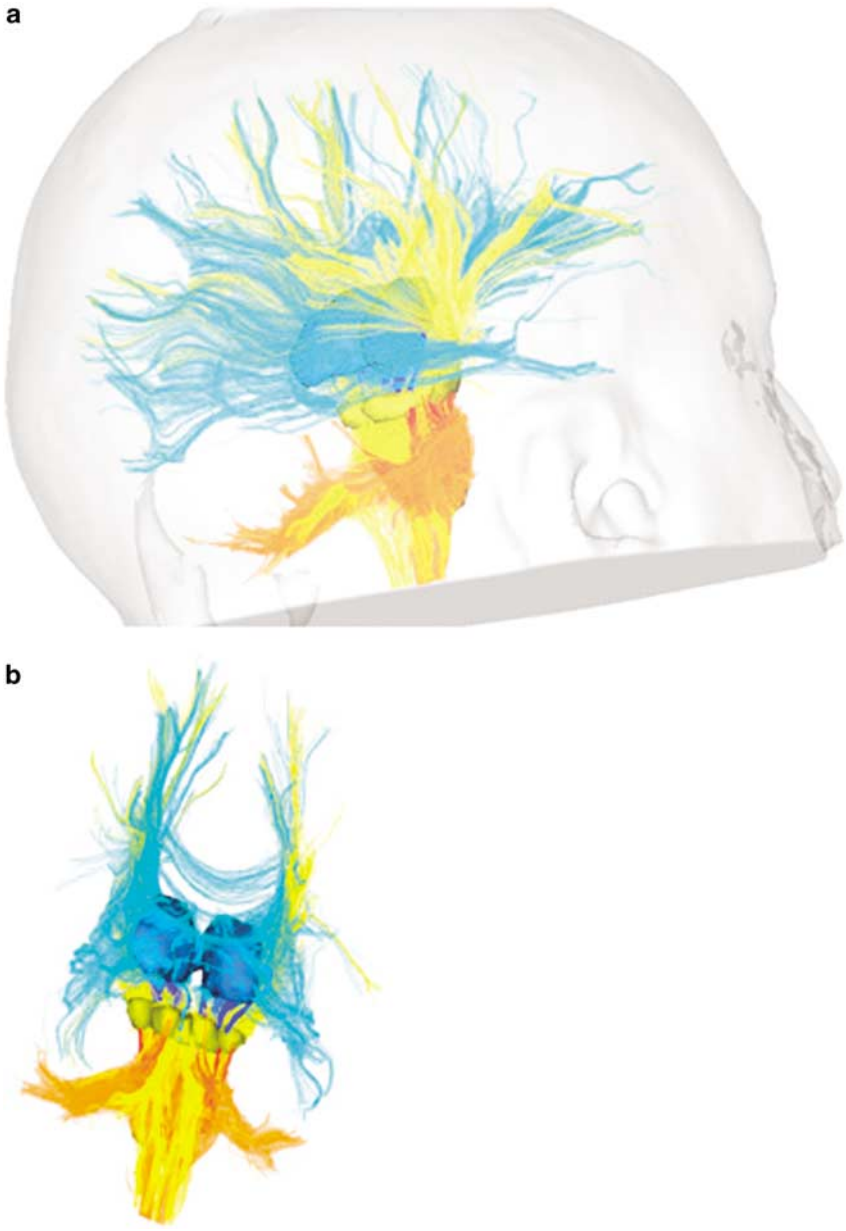

Figure 2 Tractography results in one healthy subject: (a) 3D view, (b) oblique plane. Orange: Pons and reconstructed fiber bundles connecting the pons and the cerebellum. Yellow: Midbrain and reconstructed fiber bundles passing through the midbrain. Light blue: Thalamus and reconstructed fiber bundles passing through the thalamus. Dark blue: Reconstructed fiber bundles connecting the midbrain to the thalamus. Red: Reconstructed fiber bundles connecting the midbrain to the pons.

variables such as: duration of dependence, duration of abstinence, age at first drinking, number of alcohol drinks per day, age of drinking onset, or AUDIT.

DISCUSSION

Alcohol-dependent subjects had fewer tracked white matter fibers per unit volume running between the midbrain and the pons, and a smaller pons volume compared to controls. Furthermore, the normalized number of such fibers was correlated with the alcohol-dependent subjects' performance in the neuropsychological TMT-B test. This is the first report of an alteration of the white matter microstructure between the midbrain and the pons that might have functional consequence for executive skills in alcohol-dependent subjects without manifest neurological complications.

Alcoholism-Associated Changes in White Matter Tracts

Previously reported alcoholism-associated pathologic features include demyelination, loss of myelinated fibers and axonal deletion, which possibly results from regional neuronal loss (Harper *et al*, 2003). The reduction of the alcoholics' brain weight and volume, which is well documented in the literature (Harper and Matsumoto, 2005), is largely due to an overall decrease of white matter volume. According to our current data, moderately excessive use of alcohol may

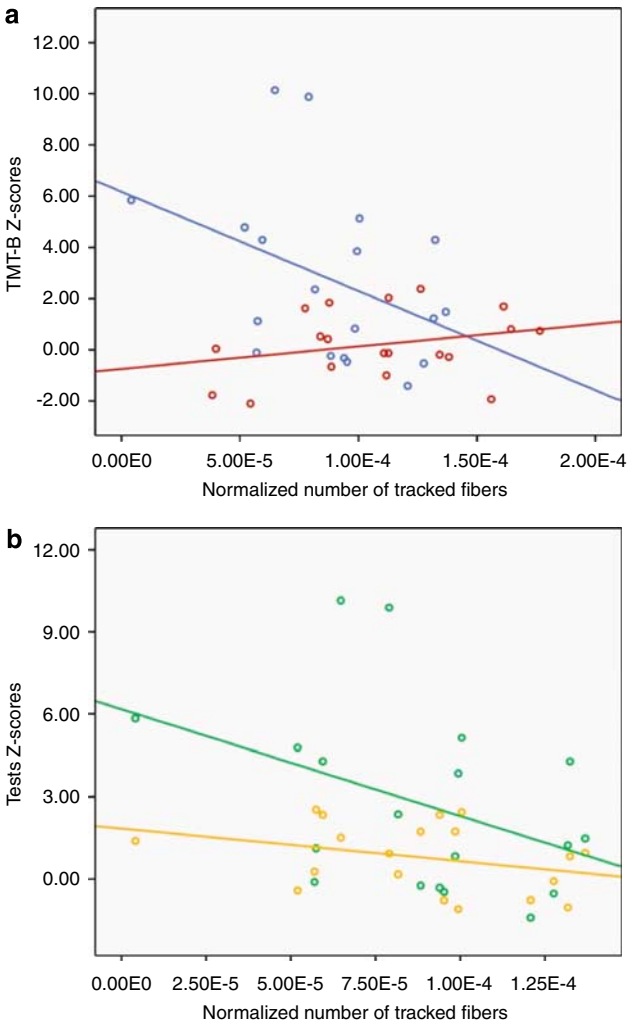

Figure 3 (a) The relationship between the normalized number of tracked midbrain–pons fibers and corrected *z*-scores obtained in the Trail-Making Test part-B (TMT-B) by the alcohol-dependent subjects (in blue; $r^2 = -0.154$) and by the controls (in red; $r^2 = -0.071$). (b) The relationship between the normalized number of tracked midbrain–pons fibers and corrected *z*-scores obtained by the alcohol-dependent subjects in the TMT-A (in orange; $r^2 = -0.107$) and in TMT-B (in green; $r^2 = -0.154$). In both graphs, high *z*-scores are in the direction of impairment.

also be associated with a loss of white matter fibers between the midbrain and the pons.

The abnormally high ADC along both midbrain–pons and midbrain–thalamus tracts suggest cell volume changes arising from tissue degeneration. More specifically, the degradation of cell membranes increases the extracellular space fraction, which allows larger net displacements of water molecules within a given diffusion time, and thus increases ADC (Sotak, 2004). As we did not study the whole brain, we cannot estimate whether this abnormally high ADC in alcoholics is specific to the explored regions, or more diffuse in nature.

Similar FA along the midbrain–pons tract in both alcohol-dependent and healthy subjects may be expected in a region, such as the pons, where FA reduction due to white matter fibers crossing may mask any reduction due to disease (Pierpaoli *et al*, 2001). Furthermore, the small change in ADC and the correlation between the number of tracked fibers and the pons volume are both consistent with a primary damage of the pons, which has caused secondary white matter degeneration (Pierpaoli *et al*, 2001). Although DTI cannot determine the nature of the neural alterations, the location of the detected abnormalities seems to imply a central pontine myelinolysis-like condition (Sullivan and Pfefferbaum, 2001). As already mentioned by Sullivan and Pfefferbaum (2001), this condition may occur with higher incidence in subjects without gross neurological deficits and contribute to their neuropsychological impairment.

Correlation with Neuropsychological Performance

TMT-B evaluates several different processes, such as 'cognitive flexibility' and 'set shifting', as well as 'general attention' (Zakzanis *et al*, 2005). The correlation between the normalized number of tracked midbrain–pons fibers and TMT-B score supports the assumption that microstructural white matter alterations may contribute to the neuropsychological impairment observed in alcohol-dependent subjects. Taken together, our results indicate that fiber bundles linking the midbrain and the pons might be related to executive skills (Schmahmann *et al*, 2004). This interpretation is also in line with our previous finding of the association between midbrain volume and cognitive flexibility in alcohol-dependent subjects (Chanraud *et al*, 2007).

In contrast with TMT-B, we observed no statistically significant correlation between LNS scores and the normalized number of tracked midbrain–pons fibers. One possible explanation for this difference could be that the TMT-B task incorporates an additional motor component, whereas LNS focuses more purely on the use of working memory. It should be noted that the cognitive flexibility deficits of our alcohol-dependent study subjects cannot be explained by markedly impaired motor processing, because they were no slower than control subjects in TMT-A. Our current results thus lend support to the proposition that there are separate pathways within the corticocerebellar circuit underlying the cognitive and motor processes (Kelly and Strick, 2003; Schmahmann, 2004), which are, however, strongly interconnected.

Our findings, together with those of Sullivan (2003), suggest that some cognitive process impairment in alcohol-dependent subjects could be linked not only to cortical dysfunction but also to modulatory influences from subcortical structures. Alcohol-associated alterations in the corticocerebellar network may have a bottleneck effect on central decision-making processes (Sigman and Dehaene, 2005). In other words, a part of the executive dysfunction in alcohol-dependent subjects could possibly be attributed either to less cortical information or to fewer modulatory signals from subcortical structures arriving at the cerebellum. In addition to cognitive impairment, the disruption of selective neural circuits may also contribute to the complex neurologic symptomatology observed in chronic alcoholism (Koob, 2003). However, it must be emphasized that because our study had a cross-sectional design, we cannot ascribe a causal interpretation of the findings. The structural abnormalities that we observed in our alcohol-dependent subjects may result from long-term alcohol abuse/dependence, or they may reflect a preexisting factor that had

predisposed these subjects to severe alcoholism. In both instances, these neuroanatomic changes may contribute to explain why cognitive behavioral therapy often has a rather limited effect in these patients (Morgenstern and Longabaugh, 2000). Indeed, this kind of therapy is based on changing behaviors in spite of the fact that cognitive impairment limits experience-dependent recovery (Roehrich and Goldman, 1993). Our findings further support the hypothesis that neuroanatomical changes underlying such cognitive impairment could require establishment of compensatory mechanisms (Desmond *et al*, 2003; Pfefferbaum *et al*, 2001).

Methodological Considerations

We used a quantitative fiber-tracking approach to analyze FA, ADC, and the number of tracked fibers per unit volume within the bundles of interest. Fibers reconstructed by tractography algorithms cannot be seen as one-to-one correlates of existing anatomical, histologically determined fiber bundles. Instead, tractography gives a statistical representation of both the voxel-to-voxel coherence of brain matter and the connectivity of white matter tracts, which is, nonetheless, highly representative of the underlying anatomy (Sullivan *et al*, 2006). Tracked fibers were classified using an anatomical white matter atlas (Nieuwenhuys *et al*, 1988) and previous tractography studies (Hagmann *et al*, 2003; Stieltjes *et al*, 2001). The fibers that we tracked between the midbrain and the pons are believed to represent the corticopontine tract, the ascending cholinergic reticular tract (Nieuwenhuys *et al*, 1988), and probably parts of the corticospinal and corticobulbar tracts (Hagmann *et al*, 2003; Stieltjes *et al*, 2001). Similarly, the fibers we tracked between the midbrain and thalamus are believed to represent the superior cerebellar peduncle, the medial lemniscus (Stieltjes *et al*, 2001), and the ascending cholinergic reticular tract.

Limitations

The main limitation of this study arises from the already discussed fact that the number of virtual, reconstructed fibers cannot be directly interpreted as anatomical correlate of real fibers in the respective bundle. The DTI tractography results may be biased by many unspecific factors (Johansen-Berg and Behrens, 2006). Different macro- and microstructural characteristics of fiber bundles, such as myelination, axonal and glial loss, shape, width and curvature of the fiber tract, tissue hydration, and overall brain size can influence the functioning of tractography algorithms (Catani, 2006; Catani *et al*, 2003; Pierpaoli *et al*, 2001). Nevertheless, previous studies have shown that the reconstructed fiber bundle metrics can provide a sufficiently good surrogate marker of real fiber anatomy (Sullivan *et al*, 2006). Our present results are also supported by earlier post-mortem studies, which have demonstrated a loss of white matter fibers—especially those of noradrenergic (Arango *et al*, 1994) and serotoninergic (Halliday *et al*, 1993) neurons—in the brainstem of alcohol-dependent subjects.

Another limitation stems from tobacco use in the alcohol-dependent population, as half of the alcohol-dependent subjects included in this study were smokers. Neuropsychological performance and brain structural differences were reported between smoking and nonsmoking abstinent alcohol-dependent subjects (Meyerhoff *et al*, 2006). However, the interaction of smoking and chronic alcoholism is still unclear. Furthermore, statistical analyses between smoking and nonsmoking alcohol-dependent subjects found no difference in performance or white matter values.

Then, as mentioned above, causal inferences from our data are not possible because of the cross-sectional design.

Finally, our findings and interpretations are drawn from a sample of men. As alcohol-related neuropsychological deficits and brain alterations are gender dependent (Oscar-Berman *et al*, 1997), the questions raised in this study still remain for alcoholic women.

Conclusion

By applying a quantitative fiber-tracking technique to DTI data, we were able to demonstrate in detoxified and apparently neurologically intact alcohol-dependent subjects, marked alterations in mesencephalic white matter bundles, which may underlie their cognitive flexibility impairment. These findings suggest that an early assessment of white matter microstructure and cognitive measures of mental flexibility may provide major insights into capacity to benefit from a heavy emphasis on educational methods early in treatment, even if a patient has a high level of education or intellectual ability. We speculate that future longitudinal DTI studies of abstinent alcoholics might specify which constituents of white matter microstructure exhibit recovery and could thus contribute to restitution of function. Further, considering a link between the microstructural integrity of white matter tracts and neuropsychological performance impairment, the degree of anatomical recovery in this brain region could possibly be estimated with standard neuropsychological tests.

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