COGNITIVE AND BEHAVIOURAL ASPECTS

Broad Disruption of Brain White Matter Microstructure and Relationship with Neuropsychological Performance in Male Patients with Severe Alcohol Dependence

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Abstract — Aims: In the last years, refined magnetic resonance diffusion tensor imaging (DTI) methods have become available to study microstructural alterations in the human brain. We investigated to what extent white matter tissue abnormalities are present in male patients after chronic, excessive alcohol consumption and if these alterations are correlated with measures of alcohol consumption and neuropsychological performance. Methods: Twenty-four detoxified adult male patients with severe alcohol dependence and 23 healthy male control subjects were included in the study. Neuropsychological tests were assessed for executive function, attention, memory and visuospatial function. DTI was acquired and preprocessing of the data was performed using tract-based spatial statistics. Group differences of fractional anisotropy (FA) as well as correlation analyses with neuropsychological measures and drinking history were calculated. Results: Performance in alcoholic patients was significantly poorer in tests of non-verbal reasoning and attention. In detoxified alcoholic patients, lower FA was primarily found in the body of the corpus callosum, but these findings did not correlate directly with behavioral measures. However, executive and psychomotor performance (Trail-Making Test) correlated significantly with FA in right anterior cingulate and left motor areas. Conclusion: These findings provide further evidence for reduced integrity of interhemispheric connections in male patients with severe alcohol dependence, and neuropsychological performance was in part correlated with FA.

INTRODUCTION

There is much evidence from post-mortem, animal and neuroimaging studies that chronic alcohol consumption affects brain morphology and function (Daurignac et al., 2005; Sullivan and Pfefferbaum, 2005; Feht et al., 2008). Magnetic resonance imaging (MRI) studies have demonstrated gray matter (GM) volume reductions primarily in the frontal lobe in patients with alcohol dependence (for review: Moselhy et al., 2001). However, GM atrophy has been shown also in other brain areas (Channaud et al., 2007; Mechtcheriakov et al., 2007). White matter (WM) atrophy in patients with alcohol dependence has primarily been demonstrated in the corpus callosum (Pfefferbaum et al., 1996; Hommer et al., 1996), but also in the pons and in temporal WM (Sullivan et al., 1996; Pfefferbaum et al., 2002). Though, it has been shown that the structural loss is at least partly reversible during abstinence from alcohol (Cardenas et al., 2007).

During the last years, diffusion tensor imaging (DTI) became available to investigate human brain microstructure, i.e. the integrity of WM fiber tracts. Fractional anisotropy (FA) provides an index of directional selectivity of water diffusion (Beaulieu, 2002). In brain WM, myelination properties, fiber organization, axonal diameter, fiber density and the ratio of intracellular/extracellular space contribute to differences in FA (Beaulieu, 2002; Schmithorst et al., 2002). Using DTI, microstructural properties of brain regions in alcoholic patients have been investigated: A recent voxel- and region-based DTI analysis in 15 abstinent long-term chronic alcoholic men demonstrated lower FA predominantly in the right frontal WM, cingulum bundle and superior longitudinal fasciculus (SLF) compared with 15 control subjects (Harris et al., 2008). Another study investigated genu and splenium of the corpus callosum and the centrum semiovale in 15 alcoholic men and 31 control men and demonstrated lower FA and higher diffusivity in all these regions in the patient group (Pfefferbaum and Sullivan, 2005). The same authors described lower FA in the centrum semiovale and in the genu of the corpus callosum in 12 alcoholic women compared with 18 control women (Pfefferbaum and Sullivan, 2002).

It is well known that alcoholism affects behavior in different ways; several studies investigated neuropsychological impairments in alcohol-dependent patients (for review: Scheurich, 2005; Oscar-Berman and Marinkovic, 2007). Deficits were primarily reported in cognitive performance, including particularly executive functions (Moriyama et al., 2002; Zinn et al., 2004; Rupp et al., 2006; Liappas et al., 2007), memory and visuospatial functions (Fama et al., 2004), and motor control (Sullivan et al., 2000; Sullivan, 2003).

To date, there is only little evidence about the correlation of microstructural changes and neuropsychological performance in alcoholic patients. Harris et al (2008) failed to show a correlation between FA and working memory in several regions-of-interest. Recently, a correlation of lower FA in frontal and limbic fiber tracts with lower visuospatial memory performance was shown adopting DTI and fiber tracking methodology (Yeh et al., 2009). Furthermore, findings about correlations between impaired WM integrity and measures of alcohol consumption are inconsistent: a correlation between alcohol consumption in the last year before abstinence and DTI measures in the corpus callosum and the internal capsule has been reported recently (Yeh et al., 2009). Greater lifetime alcohol consumption correlated significantly in alcoholic men with lower FA in several WM tracts including particularly the internal capsule and forceps.
In the present study, we adopted magnetic resonance DTI to investigate the regional distribution of microstructural WM abnormalities in a large sample of male patients with particularly severe chronic alcoholism after withdrawal therapy. In addition, we assessed to what extent these regional alterations directly correlate with measures of alcohol consumption, neuropsychological performance and performance in tests of executive function. We hypothesized impaired neuropsychological performance particularly in visuospatial and executive tests as well as lower mean FA particularly in frontal and interhemispheric WM tracts in alcoholic patients.

MATERIALS AND METHODS

Subjects
We investigated 24 detoxified adult male patients with severe alcohol dependence (mean age 48.5 years, range 32–63 years; Table 1). All patients were recruited during inpatient alcohol withdrawal therapy at the Department of Psychiatry and Psychotherapy of the University Medical Center Mainz (Germany). Neuroradiological and neuropsychological investigations were performed 13 ± 4 days (range 6–20 days) after completion of the withdrawal pharmacotherapy. We included only male patients because gender differences in the effect of alcohol on the brain have been described (Homer et al., 2001; Pfefferbaum et al., 2001). We did not include patients with other severe psychiatric or medical illnesses at the time of the study.

In addition, 23 healthy male control subjects (mean age 47.4 years, range 31–62 years; Table 1) were recruited via local newspaper announcements. Control subjects were only enrolled in the study if there was no evidence for any medical or neurological illness and if there was no history for any other psychiatric DSM-IV axis I or axis II disorder. Approval by a clinically experienced psychiatrist (M.L. and C.F.) in addition to a standardized diagnostic interview (Wittchen et al., 1997). Only subjects without harmful use of alcohol and with low alcohol consumption (not >20 g/day) were included.

All participants were Caucasian and right-handed. Written informed consent was obtained from all study participants in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of the Johannes Gutenberg University in Mainz (Germany).

Clinical measures and measures of alcohol consumption and drinking history
The diagnosis of alcohol dependence was based on the DSM-IV criteria (American Psychiatric Association, 1994) by a clinically experienced psychiatrist (M.L. and C.F.). Detailed data on the different symptoms of alcohol dependence, such as age of onset, as well as the occurrence of lifetime complications, such as withdrawal seizures or a withdrawal delirium, were obtained by using the Composite International Diagnostic Interview (Wittchen et al., 1997). Recent alcohol consumption within the last 30 days was obtained using the timeline followback method (Sobell et al., 1986). Lifetime drinking history was assessed adopting several estimates of drinking severity (Skinner, 1979; Scheurich et al., 2005) including years of drinking and years of heavy drinking as assessed with the German version of the European Addiction Severity Index (Scheurich et al., 2000). Details of the patients’ characteristics are given in Table 1.

Neuropsychological tests
Twenty patients and 21 control subjects underwent a neuropsychological test battery including primarily tests for executive function, attention and different aspects of cognitive performance which have previously been validated by us in alcoholic patients (Scheurich et al., 2004; Scheurich and Brokate, 2009): The Wisconsin Card Sorting Test (WCST) was conducted to test executive and psychomotor functioning (Heaton et al., 1993). The Trail-Making Test (TMT; Reitan, 1992) was assessed to measure visual-motor processing and selective attention (TMT-A), and additionally executive function (TMT-B). Results for TMT-A and -B are reported as the number of seconds required to complete the task. Verbal memory and learning were tested by the Auditory-Verbal Learning Test (AVLT; Schmidt, 1996). The test battery for attentional performance (TAP; version 1.7) was performed to measure attention as an elementary precondition for the overall performance (Zimmermann and Fimm, 1994). For combined testing of attentional bias and executive function, we used a modified Stroop task with alcohol- and drinking-related words (Stetter et al., 1995). Reaction time (RT) and interference time (IT, subtraction of the mean RT for the alcohol stimulus from the mean RT for the neutral stimulus) in the Stroop task have been reported to correspond to the total decision-making time and have widely been used as measures for total information processing speed and attentional bias (Ryan, 2002). Finally, two subtests of the Achievement Measure System [Leistungsprüfsystem (LPS)], which is a common standardized German test to measure general intelligence (Horn, 1983) were adopted: Subtest 4 of the LPS for non-verbal reasoning and Subtest 9 for the

Table 1. Demographic and neuropsychological data

<table>
<thead>
<tr>
<th>Alcohol patients</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>24</td>
<td>23</td>
</tr>
<tr>
<td>Age (years)</td>
<td>48.5 ± 8.6</td>
<td>47.4 ± 7.2</td>
</tr>
<tr>
<td>Alcohol consumption 30 days (g)</td>
<td>5847 ± 6289</td>
<td>173 ± 248</td>
</tr>
<tr>
<td>Years of consumption</td>
<td>21.0 ± 10.6</td>
<td>—</td>
</tr>
<tr>
<td>Years of heavy consumption</td>
<td>14.1 ± 10.2</td>
<td>—</td>
</tr>
<tr>
<td>LPS 4 (non-verbal reasoning)</td>
<td>22.8 ± 3.9</td>
<td>25.7 ± 4.6</td>
</tr>
<tr>
<td>LPS 9 (spatial imagination)</td>
<td>21.7 ± 8.3</td>
<td>24.1 ± 6.6</td>
</tr>
<tr>
<td>RT Stroop task stimulus (ms)</td>
<td>1189 ± 229</td>
<td>1029 ± 149</td>
</tr>
<tr>
<td>IT Stroop task</td>
<td>—9 ± 64</td>
<td>2 ± 37</td>
</tr>
<tr>
<td>TAP (ms)</td>
<td>232.7 ± 49.5</td>
<td>236.8 ± 37.9</td>
</tr>
<tr>
<td>TMT-A (s)</td>
<td>31.0 ± 13.6</td>
<td>24.2 ± 8.5</td>
</tr>
<tr>
<td>TMT-B (s)</td>
<td>103.9 ± 81.7</td>
<td>82.6 ± 40.7</td>
</tr>
<tr>
<td>AVLT (delayed recall right)</td>
<td>9.2 ± 4.0</td>
<td>9.14 ± 3.2</td>
</tr>
<tr>
<td>WCST (number of errors)</td>
<td>44.6 ± 26.9</td>
<td>33.1 ± 22.1</td>
</tr>
</tbody>
</table>

AVLT, Auditory-Verbal Learning Test; IT, interference time; LPS, Leistungsprüfsystem; RT, reaction time; SD, standard deviation; TAP, test battery for attentional performance; TMT, Trail-Making Test; WCST, Wisconsin Card Sorting Test.
assessment of spatial imagination. It is important to mention that the patients did not receive any sedating medication at the time of the neuropsychological investigations.

**Imaging data acquisition**

MRI scanning was performed with a 1.5 T Siemens Sonata® system at the Institute of Neuroradiology of the University Medical Center Mainz (Germany). An eight-channel head coil was used, and head movements were minimized by tightly fixing the head with cushions during the scanning procedure. After a localization sequence for orientation, echo-planar-imaging sequences were acquired in six non-collinear diffusion-sensitizing gradient directions with diffusion sensitivity \( b = 1000 \text{ mm}^2/\text{s} \) and one acquisition without diffusion encoding (\( b = 0 \text{ mm}^2/\text{s} \)). At the time of the initiation of the study in 2004, it was still standard to acquire DTI data sets in six non-collinear diffusion-sensitizing gradient directions. Slices were positioned along the anterior commissure—posterior commissure line. The diffusion acquisition parameters were as follows: the acquisition matrix was 128 × 128 with a resolution of 1.5 × 1.5 × 3.0 mm³. The whole brain was covered without inter-slice gap. Repetition time was 8000 ms, echo time was 90 ms. A total of 10 acquisitions were performed.

**Tract-based spatial statistics preprocessing analyses**

Original MR diffusion images were registered in DICOM format and converted to ANALYZE format using MRICro software (University of Nottingham, UK). The scans were inspected visually for gross artifacts, and all 47 MRI data sets were included in the study. DTI data processing was performed using FSL 3.3 software (FMRIB’s software library, www.fmrib.ox.ac.uk/fsl). All DTI data were corrected for eddy currents and head motion by registering each data set to the \( b = 0 \) image with affine transformation. FA maps were calculated using FSL DTIFit.

Tract-based spatial statistics (TBSS) was performed using the TBSS 1.0 tool that is part of FSL 3.3 and is described in detail elsewhere (Smith et al., 2006, 2007). In brief, a common registration target was first identified and all subjects’ FA images were aligned to this target. Then the entire aligned data set was affine-transformed into 1 × 1 × 1 mm³ Montréal Neurological Institute (MNI) 152 space. A mean FA image of all images and a skeletonized mean FA image was then created. Each subject’s aligned FA image was then projected onto the skeleton, by filling the skeleton with FA values from the nearest relevant tract centre for each voxel (Smith et al., 2006). The described procedure was then carried out for the patients and control subjects separately: A mean FA image was created for the patient group and for the control subjects. Therefore, we obtained a mean FA template for alcoholic patients and control subjects, respectively (Fig. 1). The obtained FA maps were then smoothed with a 6 mm isotropic full width at half maximum Gaussian kernel to improve signal-to-noise ratio and normalization.

**Group differences in FA**

Voxel-wise contrast analysis was then done to compare both groups using general linear model standard two sample unpaired \( t \)-test implemented in SPM5 (statistical parametric mapping) software. Following established procedures, contrast maps were thresholded at a \( P < 0.05 \), corrected for multiple comparisons on the voxel level [family wise error (FWE)]. FA templates were then overlaid with the statistically significant SPM clusters using MRICro software for graphical presentation in neurological convention. The MRI atlas of human WM (Mori et al., 2005) was used for the identification of WM structures. The MNI coordinates and \( t \)-statistic of the peak voxel, the cluster size and the corresponding anatomical structures were determined (Mori et al., 2005). The FA value of the most significant voxel from the group contrast analysis was taken from each subject’s data set and used to depict data in a scatterplot (Fig. 3).

**Correlation of FA with measures of alcohol consumption and neuropsychological performance**

Within the patient group, we performed additional correlation analyses of FA values with measures of alcohol consumption and drinking history. We therefore used the following parameters: total years of drinking, years of heavy drinking and amount of alcohol consumption in the last 30 days before the beginning of the withdrawal therapy. The latter measure was adopted because previous studies have shown that the amount of alcohol consumption prior to detoxification treatment shows stronger association with the cognitive status than the total lifetime consumption (Scheurich and Brokate, 2009). Correlation analyses with measures of executive function and cognitive performance were carried out separately in the groups of patients and control subjects. We therefore used the following measures as described above in detail: WCST, TMT-A, TMT-B, AVLT, TAP, RT in the Stroop task, LPS Subtests 4 and 9.

For this purpose, voxel-wise correlation analyses were performed using SPM5 to test for significant correlation of FA and behavioral measures. Results of the correlation analyses were thresholded at a \( P < 0.05 \), corrected for multiple comparisons [false detection rate (FDR)]. FA templates were then overlaid with the statistically significant SPM clusters using MRICro software. MNI coordinates...
Regression analysis of peak voxel FA and measures of neuropsychological performance

An additional regression analysis between peak voxel FA value and measures of neuropsychological performance was then performed in the patient group to investigate if FA in the most affected region in the corpus callosum is directly correlated with deficits in executive function and cognitive performance in alcoholic patients. Pearson’s correlation coefficients and P-values were calculated for FA values and test performance measures for each peak voxel using SPSS14 software (SPSS, Chicago, IL, USA).

The corresponding peak voxel FA values from the significant clusters of the voxel-wise correlation analyses with neuropsychological parameters were also taken and Pearson’s correlation coefficients were calculated for FA values and test performance measures for each peak voxel. These data were then depicted in scatterplots.

Additional statistical analyses

Demographic data, measures of alcohol consumption and neuropsychological performance data were compared between groups using t-tests for independent samples adopting a significance level of P < 0.05 using SPSS14 software.

RESULTS

Demographic, clinical and neuropsychological data

Age and IQ did not differ between patient and control group (Table 1). Alcoholic patients had an average of 21 years of consumption including 14.1 years of heavy alcohol consumption. RT in the Stroop test as a measure of cognitive flexibility performance was significantly longer in the patient group than in the control group as well for the stimulus category (1189 ± 229 vs. 1029 ± 149 ms) as in the alcohol-related word category (1149 ± 249 vs. 1025 ± 155 ms). IT as a measure of attentional performance did not differ significantly between the patient group (−9 ± 64 ms) and the control group (2 ± 37 ms). The performance in the TAP did not differ between both groups. Non-verbal reasoning performance (LPS 4) was significantly lower in the alcoholic patient group (22.8 ± 3.9) than in the control group (25.7 ± 4.6) while there was no difference in spatial imagination performance (LPS 9). Patients’ visual search and psychomotor performance were poorer in the TMT-A and in the TMT-B, though these differences were not significant. In the remaining neuropsychological tests (WCST, AVLT), the performance in the patient group was also poorer, but without statistical significance (Table 1). As the different tests are not independent (all tasks included a substantial attentional component), we did not apply Bonferroni correction for multiple comparisons.

Group differences in FA

The average FA images for the alcoholic patient and the control group are represented in Fig. 1. Differences of the FA templates between alcoholic patients and control subjects are visible throughout the brain, particularly in the corpus callosum.

Voxel-wise parametric FA contrast analyses between patients and controls revealed statistically significant clusters (P < 0.05, FWE corrected) in several WM structures (Table 2, Fig. 2). In these areas, FA was lower in the patient group than in the control subjects. The most extensive cluster was localized in the posterior body of the corpus callosum (peak voxel MNI 3, −17, 28; t = 8.87; cluster size 24,921 voxels). The peak voxel FA values from all subjects’ data sets are depicted in a scatterplot (Fig. 3). In our analysis, we did not find any voxel with a significant higher FA value in the patient group compared with the control group.

Table 2. Group differences in FA between n = 24 alcohol patients and n = 23 control subjects

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Peak voxel MNI</th>
<th>Peak voxel t-value</th>
<th>Cluster size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body of the corpus callosum</td>
<td>3, −17, 28</td>
<td>8.86</td>
<td>24,921</td>
</tr>
<tr>
<td>R superior cerebellum</td>
<td>16, −66, −16</td>
<td>7.9</td>
<td>3512</td>
</tr>
<tr>
<td>L anterior corona radiate</td>
<td>−30, 29, 21</td>
<td>7.65</td>
<td>1903</td>
</tr>
<tr>
<td>L internal capsule</td>
<td>−21, −16, 3</td>
<td>8.08</td>
<td>1497</td>
</tr>
<tr>
<td>R insula</td>
<td>39, 11, 4</td>
<td>7.62</td>
<td>1160</td>
</tr>
<tr>
<td>R vermis cerebelli</td>
<td>4, −43, −21</td>
<td>8.15</td>
<td>599</td>
</tr>
<tr>
<td>L frontal superior gyrus</td>
<td>−15, 53, 32</td>
<td>7.81</td>
<td>411</td>
</tr>
<tr>
<td>R pallidum</td>
<td>24, −7, 1</td>
<td>7.83</td>
<td>326</td>
</tr>
</tbody>
</table>

Given are significant clusters (P < 0.05, FWE corrected). L, left; MNI, Montreal Neurological Institute; R, right.

Fig. 2. Group differences in FA between n = 24 alcoholic patients and n = 23 control subjects. Significant decreased FA in patients is predominantly located in the body of the corpus callosum (peak voxel MNI 3, −17, 28; t = 8.86). Represented results are thresholded at a P < 0.05 (FWE correction for multiple comparisons) and overlaid on mean FA templates in neurological convention (R = R). Given are the MNI coordinates.
Correlation of FA and measures of alcohol consumption and drinking history

Within the patient group, we performed voxel-by-voxel correlation analyses of FA with the measures of alcohol dependence and drinking history. We did not find any significant ($P < 0.05$, FDR correction) correlation of FA with the following measures: Years of drinking, years of heavy drinking, alcohol consumption in the last 30 days before withdrawal therapy and number of days of abstinence between completion of the withdrawal therapy and investigations of the study.

By the use of a relaxed exploratory significance threshold of $P < 0.001$ without correction for multiple comparisons, we were able to demonstrate negative correlations between FA and measures of drinking history: Significant negative correlation of FA and years of alcohol consumption bilaterally in the internal capsule and significant negative correlation of FA and years of heavy alcohol consumption in the right internal capsule.

Correlation of FA and neuropsychological measures

Additional voxel-by-voxel correlation analyses of FA with neuropsychological test performance (TMT-A, TMT-B, TAP, AVLT, WCST, Stroop task, LPS 4, LPS 9) was performed separately within the two groups (alcoholic patients and healthy control subjects). We were able to demonstrate significant ($P < 0.05$, FDR correction) positive correlations between FA and performance in the TMT-A and TMT-B in the following brain regions (Table 3, Fig. 4): Significant positive correlation of FA and performance in the TMT-A was found in the posterior part of the right anterior cingulate

Table 3. Significant correlation between FA and neuropsychological performance in $n = 20$ alcoholic patients

<table>
<thead>
<tr>
<th>Neuropsychological measure</th>
<th>Brain area</th>
<th>Peak voxel MNI</th>
<th>Peak voxel t-value</th>
<th>Cluster size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT-A</td>
<td>R anterior cingulate</td>
<td>6, 14, 42</td>
<td>8.41</td>
<td>267</td>
</tr>
<tr>
<td>TMT-A</td>
<td>L SLF</td>
<td>−41, 3, 25</td>
<td>6.81</td>
<td>261</td>
</tr>
<tr>
<td>TMT-A</td>
<td>L precentral gyrus</td>
<td>−26, −22, 56</td>
<td>6.59</td>
<td>352</td>
</tr>
<tr>
<td>TMT-B</td>
<td>L precentral gyrus</td>
<td>−26, −23, 58</td>
<td>9.37</td>
<td>277</td>
</tr>
</tbody>
</table>

Given are significant clusters ($P < 0.05$, FDR corrected). L, left; MNI, Montreal Neurological Institute; R, right; SLF, superior longitudinal fascicle; TMT, Trail-Making Test.

Fig. 3. FA in the peak voxel (corpus callosum) of the voxel-based analysis between $n = 24$ alcoholic patients and $n = 23$ control subjects (MNI 3, −17, 28; $t = 8.86$, $P = 2.9 \times 10^{-10}$).

Fig. 4. Correlation analyses of FA with performance in the TMT-A and TMT-B in $n = 24$ alcoholic patients. Significant positive correlation of FA and performance in the TMT-A in the posterior part of the right ACC, in the left SLF and in the left precentral gyrus. Significant positive correlation of FA and performance in the TMT-B in the left precentral gyrus. Given are significant clusters ($P < 0.05$, FDR corrected), overlaid on FA templates in neurological convention (R = R). ACC, anterior cingulate cortex; FA, fractional anisotropy; L, left; PG, precentral gyrus; R, right; SLF, superior longitudinal fasciculus; TMT, Trail-Making Test.
cortex (ACC), in the left SLF and in the left precentral gyrus. Moreover, significant positive correlation of FA and performance in the TMT-B was found in a largely similar cluster in the left precentral gyrus. It has to be mentioned that the results for both TMT-A and -B are reported as the number of seconds required to complete the task; therefore, higher scores indicate greater impairment. ACC, anterior cingulate cortex; FA, fractional anisotropy; L, left; PG, precentral gyrus; R, right; SLF, superior longitudinal fasciculus; TMT, Trail-Making Test.

Voxel-wise regression analyses of FA with the remaining neuropsychological test scores did not reveal any significant \( (P < 0.05, \text{FDR correction}) \) correlations within the patient group. In the control group, no significant correlations were found between FA and any of the neuropsychological measures. The regression analyses between peak voxel FA values from these significant clusters and TMT performance values are represented in Fig. 5.

In addition, the CC peak voxel FA values of the most significant voxel from the between-group analysis were correlated with measures of neuropsychological performance. Though, we did not find any significant correlation at a \( P < 0.05 \).

**DISCUSSION**

Performance in neuropsychological tests in general was poorer in the detoxified alcoholic patient group, though these differences were only significant for RT in the Stroop task (measure of attentional and executive function) and for non-verbal reasoning performance as measured by the Subtest 4 of the LPS. This is not surprising as several authors described largely normalized neuropsychological performance in alcohol patients after withdrawal therapy and short periods of abstinence (Sharp et al., 1977; Mann et al., 1999; Scheurich and Brokate 2009). In alcoholic patients, abnormal neuropsychological findings have particularly been shown for the Subtest 4 of the LPS (non-verbal reasoning) and for the TMT-B (executive function and psychomotor performance; Mann et al., 1999; Scheurich, 2005; Scheurich and Brokate, 2009).

Consistent with our hypothesis, we were able to detect lower mean FA in detoxified alcoholic patients relative to controls in a number of WM structures. The main finding of this study is that WM integrity of interhemispheric connections (corpus callosum) is particularly compromised in patients with heavy alcohol dependence. Disturbed WM integrity of the corpus callosum has been reported in a number of previous DTI studies in alcoholic patients (Pfefferbaum et al., 2002; Pfefferbaum and Sullivan 2005; Schulte et al., 2005; De Bellis et al. 2008). A possible reason for the notable high statistical significance of our finding (peak voxel \( t \)-value \( = 8.86 \)) is that we only included patients with severe and long-lasting alcohol dependence and a high lifetime alcohol consumption. Moreover, TBSS has been described as a particularly powerful methodology for the investigation of group differences. Adopting the family-wise-error correction for group comparison analyses in our study, which is a fairly conservative measure, there might be false negative findings. Obviously, widespread brain areas in alcoholic patients demonstrated lower FA values in our study, but these differences were not significant at an FWE corrected \( P < 0.05 \). A DTI study in a comparable sample of \( n = 24 \) male alcoholic patients \( n = 24 \) healthy men demonstrated widespread differences in frontal, temporal and parahippocampal areas for mean diffusivity adopting an FDR corrected \( P < 0.001 \) threshold (Chanraud et al., 2009). Though, in contrast to our study, this investigation included also GM and was not restricted to WM (Chanraud et al., 2009). A TBSS study using a largely comparable methodology in a small sample of \( n = 10 \) alcoholic patients demonstrated lower FA in the genu of the corpus callosum and in WM structures in several other brain areas (Yeh et al., 2009). Finally, it is difficult to explain that WM integrity is locally affected to a different extent by chronic alcohol exposure. Tightly packed fibers in the corpus callosum might be particularly susceptible to the toxic effects of alcohol. Interestingly, it has been reported in a number of studies in children with prenatal alcohol exposure that the corpus callosum is a particularly vulnerable region (Roebuck et al., 1998).

Previous studies of correlations between DTI measures of WM integrity and measures of alcohol consumption reported different findings: a recent DTI study using comparable methodology reported a correlation between alcohol consumption in the last year before abstinence and DTI measures in the corpus callosum and the internal capsule (Yeh et al., 2009). Greater lifetime alcohol consumption correlated significantly in alcoholic men with lower FA in several WM tracts including particularly the internal capsule and forceps (Pfefferbaum et al., 2009). We failed to detect a considerable association between FA and measures of alcohol consumption. Though, when using a relaxed significance threshold \( (P < 0.001, \text{uncorrected}) \), we were able to show a negative correlation between FA and years of alcohol consumption as well as with years of heavy consumption in the internal...
capsule. This is currently difficult to explain, but it is largely in line with previous findings (Pfefferbaum et al., 2009; Yeh et al., 2009). One possible explanation for the low statistical significance of this finding is that we only included patients with high lifetime alcohol consumption, which results in a low variation of the measures for alcohol consumption in our sample, thus making it difficult to detect a statically significant correlation. In addition, a structural and functional recovery during abstinence has been described (Gazdzinski et al., 2010; Loeber et al., 2010). Morphometric MRI studies also failed to find a significant correlation between drinking severity and structural brain changes (Cardenas et al., 2007). Though, for our correlation analyses, we did not consider the frequency and duration of abstinence phases. Moreover, several other parameters (age, nutritional deficiencies and abuse on non-alcohol addicting substances) also have a relevant influence on drinking severity (Sullivan and Pfefferbaum, 2005), which makes it difficult to reveal direct correlations with single measures like lifetime alcohol consumption.

We were unable to demonstrate a direct correlation between lower FA in the corpus callosum and neurocognitive performance in detoxified alcoholic patients. One reason may be a lack of power in our study. On the other hand, the lack of direct correlation may be explained by the fact that several brain regions contribute to features of neurocognitive performance and a disruption in one region may (at least in part) be compensated by other intact brain structures. Particularly, visuospatial ability as measured with the TMT-A and LPS 9 in our study is known to depend partly on interhemispheric connectivity and one could expect a correlation of lower FA in the corpus callosum and decline in interhemispheric transfer. Though, we did not use particular measures of interhemispheric integration or transfer time as done by Schulte et al. (2005) in a DTI study of the corpus callosum in 10 alcoholic patients and 11 healthy controls. On the other hand, we were able to demonstrate significant correlations in voxel-wise analyses between FA and the performance in the TMT-A as a measure for psychomotor function and attention, particularly in the posterior part of the right ACC and in the left SLF. Both ACC and SLF are brain structures which have been shown to subserve attention and executive function (Makris et al., 2008). In this context, it should be mentioned that there is a large amount of overlap between neurocognitive domains tested by TMT-A and TMT-B, particularly concerning psychomotor performance (Strauss et al., 2006). This overlap may be reflected by our result of correlations between FA and both TMT-A and -B in a largely similar cluster in the left precentral cortex.

It is difficult to tell what biological processes exactly account for the observed abnormality of FA values in alcoholic patients in our study. To date, the neuroanatomical and physiological correlates of diffusion parameters are not completely understood (Beaulieu, 2002; Versace et al., 2008). Lower FA may correlate with myelination deficits, changes in axonal integrity, lower packing density of fibers or more obliquely oriented fibers (Konrad et al., 2009). In particular, impaired myelination as a consequence of chronic, excessive alcohol consumption has been described previously (Ozer et al., 2000; Lee et al., 2010). Though, based on our results with FA as the only DTI measure in our study, it is not possible to state whether impaired myelination, abnormal axonal integrity or differences in fiber packing are the underlying biological processes of the observed group differences.

Age effects on FA have been previously described in healthy adults (Sullivan and Pfefferbaum, 2006). In our sample, both groups were carefully age-matched to minimize age effects on imaging parameters. Though, it has to be taken into account that an age-alcoholism interaction phenomenon has been established by a number of studies (Pfefferbaum et al., 1988; Pfefferbaum et al., 1992; Pfefferbaum et al., 2006). Investigating the age-related decline, it has been shown that older alcoholic patients had greater brain structural deficits for their age than younger alcoholics (Pfefferbaum et al., 2006). Though, there is evidence that the neuropathology of alcoholism is in part transient, leading to a dynamic course of alcoholism including functional and structural recovery and compensation (Sullivan and Pfefferbaum, 2005). Finally, it has to be considered that lower FA may in part not only be the consequence but also a risk factor for the development of alcohol dependence.

Taken together and in light of previous neuroimaging studies, our findings support the notion of widespread impaired brain WM integrity with a focus in the corpus callosum in detoxified patients with severe alcohol dependence. However, we only found discrete impairments in neuropsychological test performance in these patients compared with healthy control subjects and we were not able to demonstrate a direct correlation between WM alterations in the corpus callosum and neurocognitive performance. Though, we were able to demonstrate that lower FA in the anterior cingulate and motor cortices was correlated with poorer psychomotor and executive performance in patients as a consequence of chronic, excessive alcohol consumption.

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White matter disruption in severe alcohol dependence


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