

# Integrity of White Matter Microstructure in Alcoholics With and Without Korsakoff's Syndrome

Shailendra Segobin,<sup>1,2,3,4</sup> Ludivine Ritz,<sup>1,2,3,4</sup> Coralie Lannuzel,<sup>1,2,3,4</sup>  
Céline Boudehent,<sup>1,2,3,4,5</sup> François Vabret,<sup>1,2,3,4,5</sup> Francis Eustache,<sup>1,2,3,4</sup>  
Hélène Beaunieux,<sup>1,2,3,4</sup> and Anne-Lise Pitel<sup>1,2,3,4\*</sup>

<sup>1</sup>INSERM, Caen, France

<sup>2</sup>Université De Caen Basse-Normandie, Caen, France

<sup>3</sup>Ecole Pratique Des Hautes Etudes, Caen, France

<sup>4</sup>Centre Hospitalier Universitaire, Caen, France

<sup>5</sup>Centre Hospitalier Universitaire, Service D'addictologie, Caen, France

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**Abstract:** Alcohol dependence results in two different clinical forms: “uncomplicated” alcoholism (UA) and Korsakoff's syndrome (KS). Certain brain networks are especially affected in UA and KS: the frontocerebellar circuit (FCC) and the Papez circuit (PC). Our aims were (1) to describe the profile of white matter (WM) microstructure in FCC and PC in the two clinical forms, (2) to identify those UA patients at risk of developing KS using their WM microstructural integrity as a biomarker. Tract-based spatial statistics and nonparametric voxel-based permutation tests were used to compare diffusion tensor imaging (DTI) data in 7 KS, 20 UA, and 14 healthy controls. The two patient groups were also pooled together and compared to controls. k-means classifications were then performed on mean fractional anisotropy values of significant clusters across all subjects for two fiber tracts from the FCC (the middle cerebellar peduncle and superior cerebellar peduncle) and two tracts from the PC (fornix and cingulum). We found graded effects of WM microstructural abnormalities in the PC of UA and KS. UA patients classified at risk of developing KS using fiber tracts of the PC from DTI data also had the lowest scores of episodic memory. That finding suggests that WM microstructure could be used as a biomarker for early detection of UA patients at risk of developing KS. *Hum Brain Mapp* 36:2795–2808, 2015. © 2015 Wiley Periodicals, Inc.

**Key words:** alcoholism; Korsakoff's syndrome; frontocerebellar circuit; Papez's circuit; tract-based spatial statistics; classification

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## INTRODUCTION

The effects of chronic and excessive alcohol consumption on the human brain and cognition result, among others, in two different clinical forms of alcohol dependence, which differ mainly by the extent of brain damage [Pitel et al., 2012]. The more severe clinical form is the Korsakoff's syndrome (KS) [Korsakoff, 1889], which is defined by permanent and debilitating neurological complications that arise from a combination of heavy alcohol

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\*Correspondence to: Anne-Lise Pitel, GIP Cyceron, Bd Becquerel, Caen Cedex 14074, France. E-mail: pitel@cyceron.fr

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consumption and thiamine deficiency. Patients with KS suffer from retrograde [Kopelman, 1989; for review Oscar-Berman, 2012] and anterograde [Fama et al., 2012 for review] amnesia, as well as ataxia [Sullivan et al., 2000], visuospatial deficits [Jacobson et al., 1990], and executive dysfunctions [Oscar-Berman, 2012 for review]. Postmortem [Harper, 2009; Harper and Kril, 1990; Mayes et al., 1988; Victor et al., 1971] and neuroimaging studies [Colchester et al., 2001; Krabbendam et al., 2000; Pitel et al., 2009; Sullivan and Marsh, 2003; Sullivan et al., 1999] revealed structural brain abnormalities in KS patients, especially in the thalamus, cingulate cortex, cerebellum, mammillary bodies, and white matter (WM) tracts of the superior cerebellar vermis [Harper et al., 2003]. Studies using magnetic resonance imaging (MRI) have also shown shrinkage of the frontal and parietal cortices [Christie et al., 1988]. The impact of the pathology on the hippocampus is still under debate with some studies reporting the region as preserved [Colchester et al., 2001; Squire et al., 1990], while others found it damaged [Sullivan and Marsh, 2003; Visser et al., 1999].

The other clinical form of alcohol-dependence refers to patients often considered as “Uncomplicated Alcoholics” (UA) [Pitel et al., 2009, 2012; for review Oscar-Berman et al., 2014; Zahr, 2014]. Those patients, equally coined as “detoxified alcoholics” [Chanraud et al., 2007 for example], or “non-Korsakoff alcoholics” [Parsons, 1998 for example] are those without ostensible and severe neurological complications or liver dysfunctions [Alexander-Kaufman et al., 2006; Harper, 2007; Harper and Matsumoto, 2005; Matsumoto, 2009; for review Oscar-Berman et al., 2014; Zahr, 2014]. This clinical form is characterized by its heterogeneity but is known to result in mild-to-moderate cognitive deficits [Parsons and Nixon, 1998; Sullivan et al., 2000] and brain damage [Chanraud et al., 2007; Rosenbloom et al., 2003]. The neuropsychological profile includes impairment of working memory and executive functions such as planning, organization, categorization, flexibility, inhibition, and deduction of rules [Ambrose et al., 2001; Ihara et al., 2000; Noël et al., 2001; Pitel et al., 2007]. Episodic memory is also affected in UA with both encoding and retrieval processes being impaired [Noël et al., 2012; Pitel et al., 2007]. Neuroimaging investigations revealed gray matter volume losses in the frontal, parietal, and medial temporal lobes, the cerebellar cortex, cerebellar vermis, as well as subcortical structures including the thalamus and the caudate nucleus [Chanraud et al., 2007; Pfefferbaum et al., 1992; Shear et al., 1996; Sullivan, 2003; Sullivan et al., 2003]. Neuropathological studies reported a disruption of the cytoskeleton and WM shrinkage especially in the corpus callosum, superior frontal cortex, anterior superior cerebellar vermis, and the limbic system [Chanraud et al., 2009; Harris et al., 2008; Pfefferbaum et al., 2006, 2009]. While postmortem examination of the human brain did not indicate demyelination because of the inherent rapid disintegration of cellular membranes following death,

animal studies have shown thinning of myelin sheaths [Phillips et al., 1991]. Differences in microstructural integrity have also been found in vivo in diffusion tensor imaging (DTI) studies of alcoholic patients having lower fractional anisotropy (FA) in the genu of the corpus callosum in men and the centrum semiovale in women [Pfefferbaum and Sullivan, 2005]. When WM fiber tracts were defined a priori, lower FA values were revealed in the mesencephalic and pontine region, superior longitudinal fasciculus, external capsule, fornix, and cingulum [Chanraud et al., 2009; Harris et al., 2008; Pfefferbaum et al., 2006, 2009].

In both UA and KS, two brain networks and associated cognitive functions are predominantly affected: the frontocerebellar circuit (FCC) [Chanraud et al., 2010a,b] and the Papez circuit (PC) [Aggleton, 2012; Parsons, 1998]. The FCC, identified in non-human primates using viral transneuronal tracing technology [Kelly and Strick, 2003], consists of two distinct, parallel closed-loops within the corticothalamo-cerebellar circuitry. The first one underlies executive functions and includes Brodmann areas 9 and 46 of the dorsolateral prefrontal cortex, which receives input from the cerebellar crus I and II through the thalamus, and projects back to the cerebellum through the pons. The second one contributes to motor functions and encompasses the motor cortex, which receives input from lobules IV–VI of the cerebellar vermis through the thalamus and feeds back to the cerebellum via the pons [Chanraud et al., 2010a,b]. DTI technique and tractography analyses [Oishi et al., 2011] have shown that the superior cerebellar peduncle connects the cerebellum to the thalamus, which is then connected to the dorsolateral prefrontal cortex through the anterior limb of the internal capsule and the anterior corona radiata. The feedback loop goes from the dorsolateral prefrontal cortex back to the pons via cortico-pontine tracts and back to the cerebellum through the middle cerebellar peduncle. The PC [Aggleton and Brown, 1999; Papez, 1937], involved in episodic memory, includes the hippocampal formation, which connects to the mammillary bodies via the fornix. The thalamus then receives information from the mammillary bodies via the mammillothalamic tract. The anterior limb of the internal capsule connects the thalamus to the cingulate gyrus, which is in turn connected back to the hippocampus through the cingulum bundle.

The PC and the FCC are affected in both clinical forms, but not to the same extent, hence, the difference of clinical severity between UA and KS patients. In fact, the comparison between these two clinical forms of alcohol-dependence revealed patterns of differences and similarities in the profiles of cognitive impairments and brain structural deficits. Neuropsychological and neuroimaging studies have shown that impairment of the FCC is generally comparable in both UA and KS, whereas graded effects are observed for the PC [Pitel et al., 2008, 2012]. More precisely, it has been shown that while deficits in working memory and executive functions did not differ

significantly between UA and KS patients, deficits in episodic memory are more severe in KS patients compared to UA [Butters and Brandt, 1985; Fama et al., 2012; Pitel et al., 2008]. Volumetric analyses of gray matter [Harper, 2009; Harper et al., 2003; Pitel et al., 2009, 2012; Sullivan and Pfefferbaum, 2009] have also indicated that nodes belonging to the PC including the medial thalami and mammillary bodies were more severely affected in KS compared to UA patients. Similar degrees of shrinkage have been observed in both patient groups in some of the nodes of the FCC including the frontal cortex but not in the pons and cerebellum, for which graded effects were observed [Sullivan and Pfefferbaum, 2009]. The few studies that have compared UA and KS patients regarding WM volumes indicated that the cerebellar WM [Kril et al., 1997], corpus callosum, and thalamic radiations were more severely damaged in KS than in UA [Harper et al., 2003; Pitel et al., 2012].

The comparison of neuropsychological functioning between UA and KS patients gave rise to the hypothesis that the effects of chronic alcohol consumption lie along a continuum from mild-to-moderate impairments in UA to severe ones in patients with KS [Butters and Brandt, 1985; Parsons, 1998; Ryback, 1971]. The existence of a continuum between these two clinical forms reflects the heterogeneity within the UA group with some patients having preserved results similar to those of healthy controls (HC), while others have severe deficits close to those of KS patients [Pitel et al., 2008]. The latter ones are at risk to develop severe alcohol-related neurological complications but they often go undiagnosed [Pitel et al., 2011] and therefore do not receive appropriate treatment. Early identification of these patients would help clinicians in optimizing treatment outcome. Clinical, neuropsychological, and macrostructural brain biomarkers of risks for KS have been proposed. Previous studies have suggested that a subgroup of UA patients with episodic memory impairments [Pitel et al., 2008] and thalamic shrinkage [Pitel et al., 2012] close to those of KS patients can be identified. This subgroup of patients may even be clinically defined by the presence of signs of Wernicke's encephalopathy [Pitel et al., 2011]. How WM microstructure can also be used as a biomarker of KS has never been explored. The WM microstructure and structural connectivity has never been investigated in KS and, therefore, the integrity of the fibers bundles has never been quantitatively compared between UA and KS. Comparisons of WM have so far been limited to neuropathological investigations [Harper, 2009] and one voxel-based morphometry (VBM) study of WM volumes [Pitel et al., 2012]. Those studies gave first insights regarding WM volumes but did not provide a clear picture of the differences in the microstructure of WM bundles. The latter is better represented by observing the differences in FA values of WM fiber tracts, obtained through DTI studies, under the assumption that FA is a structural biomarker that depicts WM disruption involving myelin, cytoskeleton, and the axons' microtubule system [Pfefferbaum et al.,

2006]. We should, however, bear in mind that in absence of sound methodological procedures, measurements of FA values could turn out to be artefactual instead of reflecting impairments due to factors inherent to alcohol-dependence. One example is the "correspondence problem" [Smith et al., 2006] following poor spatial normalization. A preserved WM tract would be observed as impaired if what are being effectively measured are FA values in crossing fibers which are inherently lower.

The first objective of the present study was, therefore, to describe the WM microstructure in UA and KS compared to HC using a voxel-wise approach. We hypothesize graded effects of compromised WM integrity in the bundles of the PC ( $KS < UA < HC$ ) but not in those of the FCC ( $[KS = UA] < HC$ ). Since the UA group is classically heterogeneous, the second objective was to identify UA patients at risk of developing KS through the analysis of WM integrity across the tracts belonging to the FCC and PC.

## MATERIALS AND METHODS

### Participants

Twenty-seven patients (22 men, 5 women) with alcohol-dependence (DSM IV criteria) [American Psychiatric Association, 1994] and 14 healthy subjects (9 men, 5 women) were included in the study. To be included, all participants had to be between 18- and 70-year old, and to have French as their native language. No participant had a comorbid psychiatric disorder (no other axis 1 of the DSM IV as evaluated by MINI 500) [American Psychiatric Association, 1994], was under psychotropic medication, had a history of serious chronic pathology (diabetes, hepatitis, HIV, endocrinal disorder, as revealed by participants' blood tests), neurological problems (traumatic head injury causing loss of consciousness for >30 min, epilepsy, stroke, etc.) that might have affected cognitive function. No participant fulfilled the DSM-IV criteria for abuse of another substance over the last 3 months, nor filled the DSM-IV criteria for dependence of another substance (except tobacco). They had not taken any other psychoactive substance for more than 5 times over the last month (except alcohol for the patients) and had not participated in any neuropsychological study or had any neuropsychological evaluation during the previous year. All participants gave their informed consent to the study, which was approved by the local ethics committee. Their demographical details are summarized in Table 1. All patients were recruited for this study while being inpatients at Caen University Hospital. The study was carried out in line with the Declaration of Helsinki [1964].

Of those 27 patients, seven (six men, one woman) filled the DSM IV criteria for persisting amnesic disorder [American Psychiatric Association, 1994] and were, therefore, diagnosed as KS patients. All KS patients had a history of heavy drinking (longer than 20 years), a Mini-Mental State

**TABLE 1. Demographical, clinical, and neuropsychological description of the alcoholics with and without Korsakoff's syndrome and control participants**

Variable	HC(n = 14)	UA(n = 20)	KS(n = 7)	Between-group comparisons
Age (years)	45.4 ± 6.9 [31–55]	45.2 ± 8.1 [34–63]	55.3 ± 7.8 [44–67]	HC = UA; HC < KS UA < KS
Education (years)	11.5 ± 2.5 [7–15]	11.9 ± 1.7 [9–15]	9.4 ± 2.8 [6–15]	HC = UA; HC = KS UA < KS
Alcohol Use Disorders Test (AUDIT)	3.2 ± 1.4 [0–5]	29.2 ± 7.5 [9–39]	16.6 ± 14.0 [1–37]	HC < UA; HC < KS UA < KS
Beck Depression Index (BDI)	3.6 ± 3.2 [0–9]	13.2 ± 7.5 [2–27]	9.0 ± 10.0 [0–29]	HC < UA; HC = KS UA = KS
Mini Mental State (MMS) (/30)	28.4 ± 1.1 [27–30]	27.4 ± 2.2 [21–30]	22.3 ± 3.3 [18–27]	HC = UA; HC < KS UA < KS
MATTIS Total score	141 ± 2.0 [136–144]	136 ± 6.7 [119–143]	114 ± 12.3 [95–132]	HC < UA; HC < KS UA < KS
STAI <sup>a</sup> A	27.2 ± 7.2 [20–47]	31.6 ± 11.0 [20–59]	33.4 ± 6.4 [25–42]	HC = UA; HC = KS UA = KS
STAI <sup>a</sup> B	33.4 ± 7.0 [23–47]	44.4 ± 12.3 [28–66]	42.0 ± 11.1 [26–56]	HC < UA; HC = KS UA = KS
Fagerstrom <sup>b</sup>	0.7 ± 1.8 [0–6]	4.8 ± 3.6 [0–14]	N/A	HC < UA
Abstinence before inclusion (days)	N/A	2.4 ± 3.1 [1–13]	53.7 ± 29.4 [1–100]	UA < SK
Alcohol Use (years)	N/A	29.6 ± 9.3 [18–51]	NR	NA
Alcohol misuse (years)	N/A	18.3 ± 8.7 [2–30]	NR	NA
Alcohol dependence (years)	N/A	9.5 ± 6.7 [1–26]	NR	NA
Number of previous detoxifications	N/A	3.3 ± 2.6 [0–11]	NR	NA

HC = Healthy Controls; UA = Uncomplicated Alcoholics; KS = Alcoholics with Korsakoff's Syndrome.

NR = data not reported since it was not possible to obtain complete and accurate information regarding alcohol history in all KS patients.

Mean ± standard deviation and range [minimum – maximum] are reported.

<sup>a</sup>Mann–Whitney *U* tests *P* < 0.05.

<sup>a</sup>State-Trait Anxiety Inventory for adults, Y-A for “state anxiety” and Y-B for “trait anxiety” [Spielberger et al., 1983].

<sup>b</sup>Fagerstrom [Heatherton et al., 1991].

(MMS) [Folstein et al., 1975] score of at least 20, were abstinent for at least 7 days and were diagnosed with severely impaired episodic memory as revealed by a neuropsychological examination. The consequences of their memory impairments were such that none of the KS were able to go back to their previous jobs and all of them lived in sheltered accommodation or were inpatients waiting for a place in an institution. It was difficult to obtain accurate information about their alcohol intake due to their amnesia. The background information for the KS came mainly from family members and medical records. For each KS patient, the selection was made according to a codified procedure in a French officially registered center for addiction. The case of each patient was examined by a multidisciplinary team made up of specialists in cognitive neuropsychology and behavioral neurology. Clinical and neuroimaging investigations ruled out other possible causes of memory impairments (particularly focal brain damage).

The 20 alcoholic patients without KS were considered as UA patients. They were recruited by clinicians while being inpatients for alcohol-dependence at Caen University Hospital. Although patients were early in abstinence (2.4 ± 3.1 days of sobriety prior to inclusion), none of them presented physical symptoms of alcohol withdrawal as assessed by the Cushman's scale [Cushman et al., 1985] at inclusion. They were interviewed with the Alcohol Use Disorders Identification Test (AUDIT) [Gache et al., 2005] and a modified version of the semistructured lifetime drinking history [Pfefferbaum et al., 1988]. Measures included the duration of alcohol use (in years), alcohol misuse (in years), alcohol dependence (in years), number of withdrawals, and daily alcohol consumption prior to treatment (in units, a standard drink corresponding to a beverage containing 10 g of pure alcohol).

The control group (HC) was recruited locally mainly by word of mouth and to match the demographics of the UA

**TABLE 2. Neuropsychological description of the alcoholics with and without Korsakoff's syndrome and controls**

Cognitive functions	Tasks	HC	UA	KS	Tests*
Intellectual abilities	Information	9.1 ± 3.0	6.04 ± 3.0	5.2 ± 3.1	HC>UA>KS
(WAIS III subtests) <sup>a</sup>	Matrix reasoning	10.4 ± 2.2	7.71 ± 2.26	5.0 ± 4.4	HC>UA>KS
Episodic memory	FSCRT sum of 3 free recalls	34.0 ± 4.9	28.5 ± 7.8	5.4 ± 2.9	HC>UA>KS
Working memory	Forward visuospatial span	8.5 ± 2.3	5.8 ± 1.5	5.1 ± 0.7	HC>UA>KS
(MEM-III subtests) <sup>a</sup>	Backward visuospatial span	8.7 ± 2.5	5.3 ± 1.3	3.9 ± 0.7	HC>UA; HC>KS

HC = Healthy Controls; UA = Uncomplicated Alcoholics; KS = Alcoholics with Korsakoff's Syndrome. (Mean ± standard deviation) and range [minimum – maximum] are reported.

Mann–Whitney *U* tests  $P < 0.05$ .

<sup>a</sup>Standard scores.

‡: comparison with KS not done as no data available.

WAIS III Wechsler Adults Intelligence Scale.

FSCRT: Free and Cued Selective Reminding Test.

patients. Inclusion criteria were: a minimum MMS score of 26 or a minimum MATTIS [Mattis, 1976] score of 129, and a maximum Beck Depression Index [Beck et al., 1961] of 29. The maximum score at the AUDIT was six for women and seven for men.

UA and HC were age- and education-matched ( $P = 0.723$  and  $P = 0.76$ , respectively). KS differed from both HC and UA in age, education (years of schooling) and MMSE scores. Age, education, depression (Beck Depression Inventory, and anxiety scores (State-Trait Anxiety Inventory, STAI) for adults with two forms Y-A for “state anxiety” and Y-B for “trait anxiety”) [Spielberger et al., 1983] as well as nicotine dependence level (Fagerstrom Test) [Heatherton et al., 1991] are reported in Table 1.

All participants underwent a neuropsychological examination assessing intellectual abilities (Information and Matrix Reasoning subtests of the WAIS III [Wechsler, 2001a]), global cognitive function (MMSE) [Folstein et al., 1975] and episodic memory (the French version of the Free and Cued Selective Reminding Test [FSCRT]) [Grober and Buschke, 1987; Van der Linden, 2004]. Neuropsychological performances are reported in Table 2.

### DTI Data Acquisition

All participants underwent a DTI sequence on the Philips Achieva 3T MRI scanner (The Netherlands). 70 slices (slice thickness of 2mm, no gap) were acquired axially using a diffusion weighted imaging spin echo sequence (32 directions at  $b = 1,000 \text{ s/mm}^2$ , repetition time = 10,000 ms; echo time = 82 ms; flip angle =  $90^\circ$ , field of view =  $224 \times 224 \text{ mm}^2$ ; matrix =  $112 \times 112$  and in-plane resolution of  $2 \times 2 \text{ mm}^2$ ; one no-diffusion weighted image at  $b = 0 \text{ s/mm}^2$  was also acquired).

### DTI Data Processing

The diffusion-weighted images (DWI) for all subjects were first preprocessed to create FA images using the FSL

Diffusion Toolbox (FDT; <http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT>) that is part of FSL 5.0 toolbox for medical image analysis [Smith et al., 2004]. The FA images were further processed using Tract-Based Spatial Statistics (TBSS) for subsequent voxelwise statistical analysis [Smith et al., 2006]. TBSS presents an improvement on classical voxelwise approaches like VBM. More specifically, it first addresses the “correspondence problem” faced by standard registration algorithms where it is difficult to gauge whether the observed differences are indeed due to differences in tissue volumes/density or are artefactual modifications that result from local misalignment. The issue becomes more pertinent in the case of WM tracts where a higher level of precision is required to ensure that the FA values contained in the voxels come from exactly the same part of WM tract across all subjects. TBSS addresses the problem by tailoring the non-linear registration algorithm to the requirements of the DTI data, followed by projection onto a tract representation that is alignment invariant (referred to as the mean FA skeleton). Such an approach also removes the need for applying a spatial smoothing for which the choice of the smoothing kernel is deemed to be done in an arbitrary manner, and results known to be highly dependent on the kernel size [Jones et al., 2005]. Smoothing increases partial volume effects between tissues such that it is difficult to differentiate between WM differences that are due to the biological mechanism under investigation or an artefactual measurement due to a mixture of tissues.

For each subject, the 32 DWI images were first corrected for distortions due to Eddy currents and aligned to the  $b = 0 \text{ s/mm}^2$  image using rigid-body registration for motion correction [Jenkinson et al., 2002]. FA images were then created by fitting a tensor model to the diffusion images and were further processed using TBSS. Briefly, all subjects' FA data were aligned into MNI space using the nonlinear registration tool (FNIRT), which uses a *b*-spline representation of the registration warp field [Rueckert et al., 1999] resulting in FA maps of matrix size of  $182 \times 218 \times 182$  and voxel size of  $1 \times 1 \times 1 \text{ mm}^3$ . Next, the mean FA image was calculated and thinned to create a

mean FA skeleton, which represents the centers of all tracts common to the group. Each subject's aligned FA image was threshold at 0.3 to exclude low FA values that could be contaminated with partial volume effects from other non-white-matter tissues and to minimize intersubject variability. The resulting image is then projected onto the mean skeleton by filling every voxel of the latter with the maximum FA value that lies perpendicular to the skeleton structure. Voxel-based statistics are performed on these "skeletonized" images.

### Statistical Analyses

#### Comparison of WM integrity in HC, UA patients and KS patients in the whole-brain (voxel-based analysis)

Nonparametric permutation tests [Nichols and Holmes, 2002] were performed between HC and UA; HC and KS; and UA and KS groups. Age was included as a covariate to account for between-group differences (KS being older than the two other groups). For each between-group comparison, 5,000 permutations were done, and the data corrected for multiple comparisons (FWE,  $P < 0.05$ ) using threshold-free cluster enhancement (TFCE) [Smith and Nichols, 2009] for cluster-wise correction. This statistical toolbox is implemented within FSL 5.0. (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise>).

#### Identification of UA patients at risk of developing KS

1. Because of the heterogeneity within the UA group, UA and KS patients were pooled together to form a single group of alcohol-dependant patients (UASK). The UASK group was then compared to the HC group using nonparametric permutation tests (5,000 permutations, FWE  $P < 0.05$ , TFCE).
2. The John Hopkins University International Consortium for Brain Mapping (JHU-ICBM) DTI-81 WM atlas [Oishi et al., 2011], implemented as an atlas tool in FSL 5.0 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases>), was then used to extract and binarise clusters highlighted in the previous between-group analysis (UASK vs HC). Only the WM fiber tracts that belong to the FCC and PC were used to produce "fiber-cluster masks." These "fiber-cluster masks" were then employed to extract the mean FA value within each tract belonging to the FCC and PC for each subject. Focus was laid on a couple of fiber tracts only for each circuit. In that respect, the cingulum and the fornix were chosen for the PC and the middle cerebellar peduncle and superior cerebellar peduncle for the FCC. These are the tracts that are believed to offer less variability in terms of specificity to the PC and FCC, respectively, having a lower involvement in fibers connecting other parts of the brain or involved in other circuitry.

3. For each fiber tract, a k-means clustering classification was then performed on the mean FA values, with the algorithm constrained to separate the 41 participants into two groups. The aim was to find which WM fiber tract enables the identification of some UA patients classified into the same group as KS patients. A reliable classification would include all HC into one group, and all KS patients into the other group with the heterogeneous UA group fitting into either of these. Those UA who will be sorted within the same group as the KS patients will be deemed as UA\_HIGH patients (for high risk of developing KS) while those who will be sorted within the same group as the HC will be labelled UA\_LOW patients (for low risk of developing KS). It is hypothesized that a more robust classification will be obtained when using FA values in the fibers of the PC than in those of the FCC. Comparisons of episodic memory performance among the subsequent subgroups should help in asserting the robustness of the classification step.
4. Between-group comparisons of episodic memory performance:

Episodic memory scores were compared between the groups of HC, UA\_LOW, UA\_HIGH, and KS using nonparametric Mann-Whitney  $U$  tests. The hypothesis is that the scores on the episodic memory test in UA\_LOW patients will differ significantly from the UA\_HIGH and KS patients but not from HC. UA\_HIGH patients are expected to differ significantly from HC and UA\_LOW and KS patients.

## RESULTS

### Comparison of WM Integrity in HC, UA Patients and KS Patients

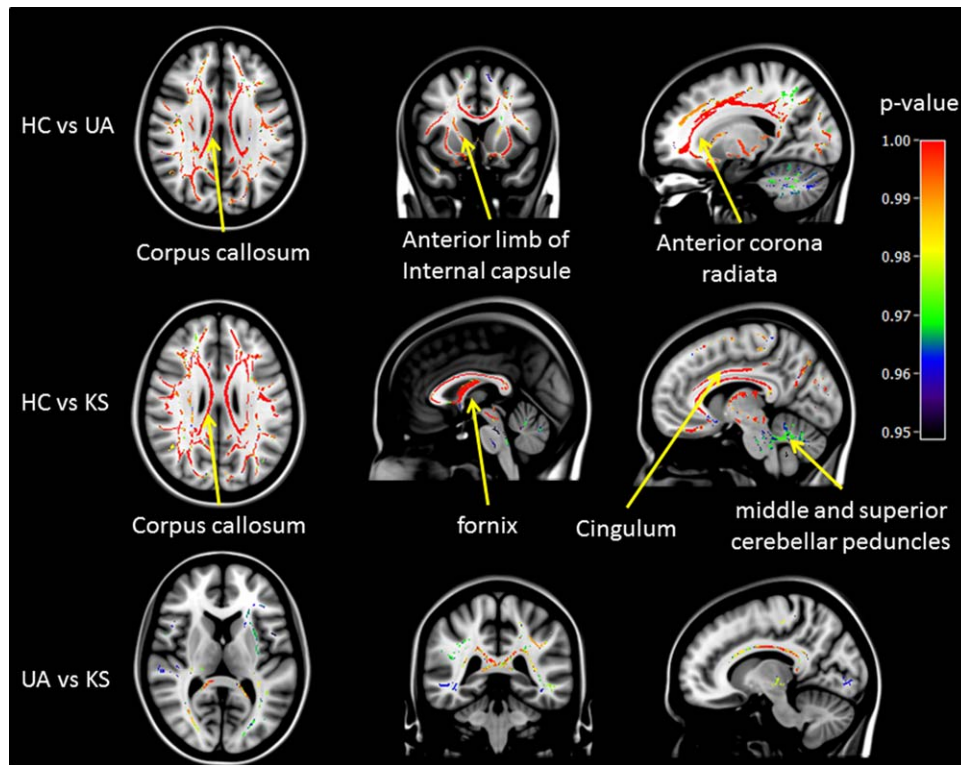
#### UA versus HC

Nonparametric permutation tests (FWE,  $P < 0.05$ ) between UA and HC showed lower FA values in fiber tracts spread across the whole brain including the corpus callosum ( $T_{\max} = 6.01$ ;  $k = 6,258$ ;  $\eta^2 = 0.17$ ), the anterior limb of the internal capsule ( $T_{\max} = 5.86$ ;  $k = 742$ ;  $\eta^2 = 0.10$ ), the anterior corona radiata ( $T_{\max} = 5.52$ ;  $k = 2,215$ ;  $\eta^2 = 0.16$ ), the fornix ( $T_{\max} = 5.91$ ;  $k = 560$ ;  $\eta^2 = 0.15$ ); the cingulum ( $T_{\max} = 4.95$ ;  $k = 821$ ;  $\eta^2 = 0.14$ ); the middle cerebellar peduncle ( $T_{\max} = 5.17$ ;  $k = 1,320$ ;  $\eta^2 = 0.11$ ), and the superior cerebellar peduncle ( $T_{\max} = 4.92$ ;  $k = 252$ ;  $\eta^2 = 0.15$ ) as shown in Figure 1.

Note: Values for effect size ( $\eta^2$ ) have been calculated from  $T$ -scores resulting from the nonparametric permutation tests.

#### KS versus HC

The profile of WM structural impairment in KS versus HC was similar to that between UA and HC. FA values



**Figure 1.**

Voxel by voxel comparisons of FA values between HC and UA (top row), HC and KS (middle row), and UA and KS (bottom row) using nonparametric permutation tests (5,000 permutations, FWE  $P < 0.05$ , TFCE for cluster-wise correction).  $P$ -value maps are shown as (1- $p$ ) images, displayed on a T1-weighted MRI in MNI space.

were lower in KS than in HC in the same tracts as reported above but with higher  $T$  and  $k$  values: corpus callosum ( $T_{\max} = 10.52$ ;  $k = 7,279$ ;  $\eta^2 = 0.26$ ), anterior corona radiata ( $T_{\max} = 7.45$ ;  $k = 2,936$ ;  $\eta^2 = 0.31$ ), anterior limb of the internal capsule ( $T_{\max} = 7.00$ ;  $k = 1,179$ ;  $\eta^2 = 0.23$ ); cingulum ( $T_{\max} = 6.93$ ;  $k = 869$ ;  $\eta^2 = 0.14$ ); the fornix ( $T_{\max} = 6.60$ ;  $k = 693$ ;  $\eta^2 = 0.31$ ); the middle cerebellar peduncle ( $T_{\max} = 6.30$ ;  $k = 1,153$ ;  $\eta^2 = 0.27$ ), and the superior cerebellar peduncle ( $T_{\max} = 7.27$ ;  $k = 270$ ;  $\eta^2 = 0.35$ ).

### UA versus KS

Group differences between UA and KS were found mainly in the corpus callosum ( $T_{\max} = 4.93$ ;  $k = 4,453$ ;  $\eta^2 = 0.139$ ) and anterior corona radiata ( $T_{\max} = 4.9$ ;  $k = 932$ ;  $\eta^2 = 0.13$ ). Other tracts with significant differences were also observed but they either had low  $T$ -values or their cluster sizes were small. For example, anterior limb of the internal capsule ( $T_{\max} = 3.59$ ;  $k = 163$ ;  $\eta^2 = 0.11$ ); cingulum ( $T_{\max} = 3.61$ ;  $k = 103$ ;  $\eta^2 = 0.14$ ), and the fornix ( $T_{\max} = 3.81$ ;  $k = 107$ ;  $\eta^2 = 0.16$ ). There were no significant differences in the middle and superior cerebellar peduncles.

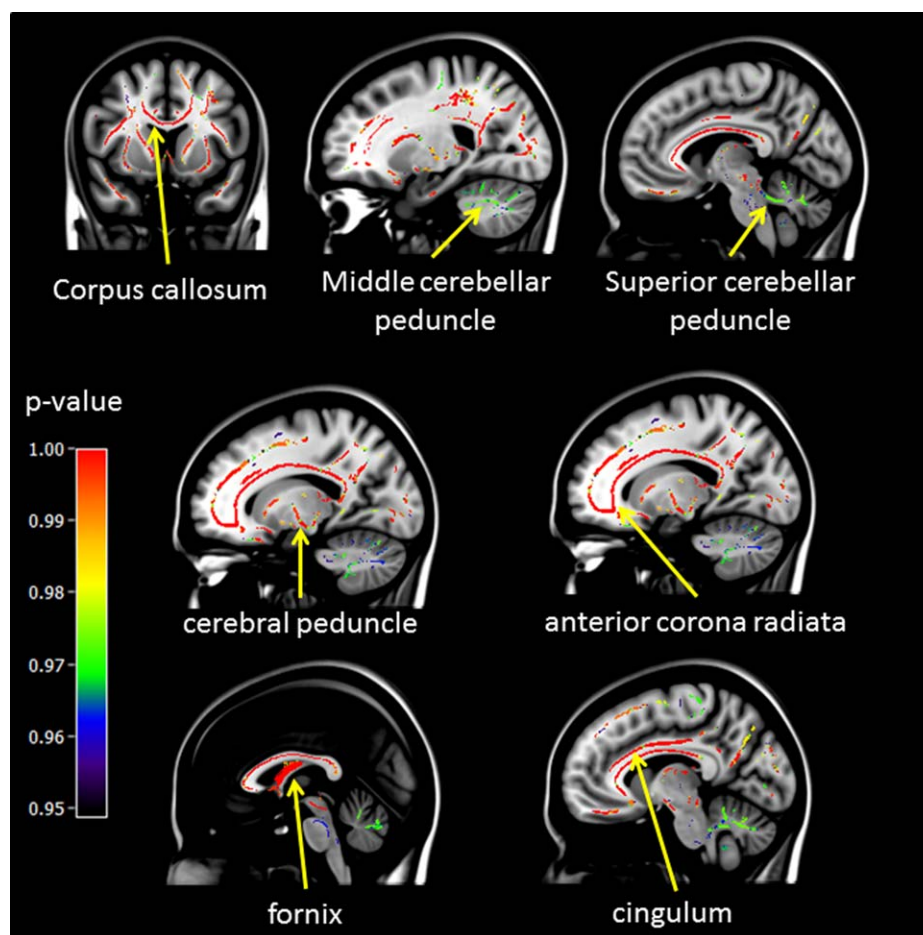
There was no gender effect in the UA and KS groups across all WM fiber tracts. In the control group, differences

between men and women were significant for the cingulum (Mann-Whitney  $U$  test,  $P = 0.014$ ). There was no correlation between FA measures and beck depression index scores or years of education across all tracts for all three groups. There was also no correlation between FA measures and the number of detoxifications in the UA group. The same analysis could not be performed for the KS group since it was not possible to obtain complete and accurate information regarding alcohol history for these patients.

### Identification of UA Patients at Risk of Developing KS

#### Comparison of WM integrity between HC and UASK

The comparison between HC and UASK (UA and SK patients pooled together) showed lower FA values in the patients in all the tracts mentioned in the previous results, including the middle cerebellar peduncle ( $T_{\max} = 5.14$ ;  $k = 1,457$ ;  $\eta^2 = 0.10$ ); the superior cerebellar peduncle ( $T_{\max} = 5.20$ ;  $k = 278$ ;  $\eta^2 = 0.14$ ); the fornix ( $T_{\max} = 7.20$ ;  $k = 609$ ;  $\eta^2 = 0.14$ ), and the cingulum ( $T_{\max} = 5.47$ ;  $k = 840$ ;  $\eta^2 = 0.14$ ). Figure 2 shows the WM tracts of the FCC and



**Figure 2.**

Voxel by voxel comparisons of FA values between HC and UASK using non-parametric permutation tests (5,000 permutations, FWE  $P < 0.05$ , and TFCE for cluster-wise correction).  $P$ -value maps are shown as (1-p) images, displayed on a T1-weighted MRI in MNI space.

PC that are significantly disrupted in the UASK group compared to the HC group.

#### **Identification of UA at risk to develop KS for each fiber tract**

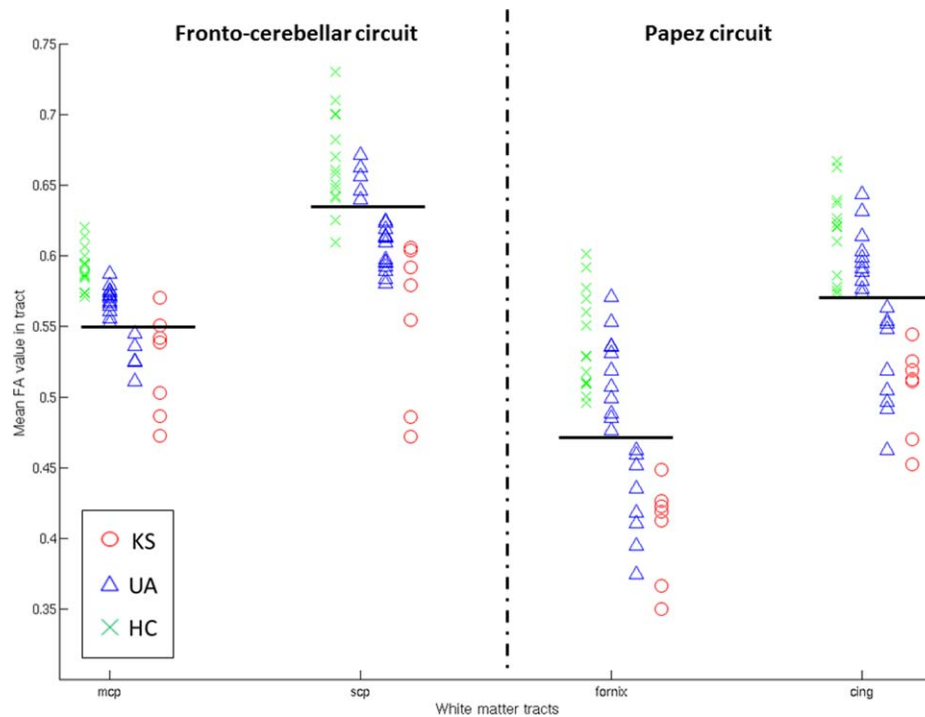
For all participants, mean FA values were extracted from significant fiber-clusters (see above) for the middle and superior cerebellar peduncles, representing tracts from the FCC and the cingulum and fornix, representing tracts from the PC. A  $k$ -means classification was then performed on those tracts. Figure 3 represents the distribution of the mean FA values for each selected tract identified in the WM atlas [Oishi et al., 2011] to be part of the FCC and PC.

With regard to WM tracts within the PC, the  $k$ -means classification conducted on the FA values in the cingulum and fornix showed an expected classification of all HC in one group and all KS patients in another. Some UA

patients were classified in the same group as HC and can be therefore considered as UA\_LOW, while others were classified in the same group as KS and were thus qualified as UA\_HIGH. The same patients were classified as UA\_HIGH for both the cingulum and the fornix, except for two of them (one for cingulum and one for fornix). However, these two patients were those with the lowest FA values in the subsequent UA\_LOW class. Comparisons of mean FA values among the four subgroups for both the cingulum and the fornix (ANOVA Kruskal-Wallis) have shown that the controls did not differ from the UA\_LOW group ( $P = 1$ ). KS differed significantly from HC ( $P < 0.001$ ) and UA\_LOW ( $P = 0.01$ ) but not UA\_HIGH. UA\_HIGH was significantly different from HC ( $P < 0.001$ ), and UA\_LOW ( $P = 0.01$ ).

Regarding the tracts of the FCC, the results of the classification seemed less reliable since each of the two groups identified included a mix of KS patients, UA patients, and





**Figure 3.**

Distribution of the mean FA values for the selected WM tracts within the FCC (mcp = middle cerebellar peduncle, scp = superior cerebellar peduncle) and the PC (fornix and cing = cingulum). Horizontal black lines represent the separation between the two identified clusters. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

HC. Moreover, the number and the identity of the UA patients classified as UA\_HIGH completely differed between the two tracts of the FCC.

#### Comparison of episodic memory performance in the UA\_HIGH and UA\_LOW groups

Since a better classification of patients for UA\_HIGH and UA\_LOW were obtained for the two WM tracts of the PC as opposed to the FCC, we compared episodic memory performances between the two subgroups of UA identified with the classification analysis conducted on FA values in the fornix and cingulum individually. Nonparametric Mann–Whitney *U* tests showed that UA\_LOW did not differ from HC ( $P = 0.37$  for the classification using the fornix and  $P = 0.32$  for the classification using the cingulum). UA\_LOW differed from UA\_HIGH ( $P = 0.038$  for the classification using the fornix and  $P = 0.025$  for the classification using the cingulum). UA\_HIGH differed significantly from HC ( $P = 0.002$  for both fornix and cingulum) and KS ( $P = 0.001$  for classification with the fornix and  $P = 0.002$  for classification with the cingulum). The latter was significantly different to all the other three subgroups for both classifications ( $P < 0.001$ ). These results are illustrated in Figure 4.

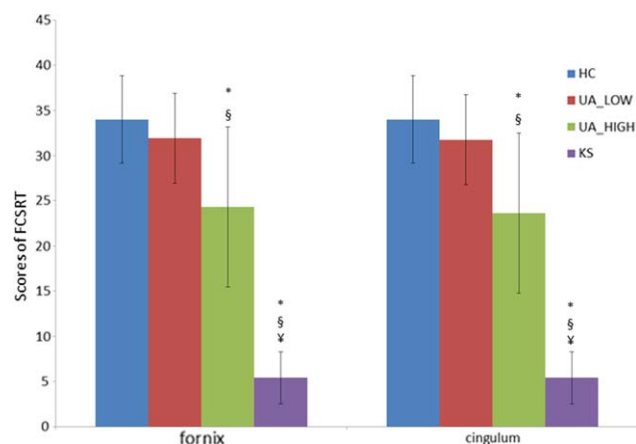
We conducted the same analysis on the performances on matrix reasoning, and forward and backward block

spans but found insignificant differences between the UA subgroups for neither the cingulum nor the fornix (data not shown). Hence, the ability to identify UA patients at risk of developing KS seems to be specific to performances in episodic memory tasks.

Note that we have also tested classification with the corpus callosum but it yielded poor results (data not shown since the corpus callosum is neither part of the FCC, nor the PC).

## DISCUSSION

The first aim of this study was to describe the profile of microstructural WM integrity in UA and KS in the whole brain, at a voxel-level, since most of the previous studies had been carried out using a region of interest approach (with anatomical regions defined a priori). In accordance with neuropathological [Harper et al., 2003; Kril et al., 1997] and neuroimaging studies [Chanraud et al., 2010a,b; Pfefferbaum and Sullivan, 2005; Pfefferbaum et al., 2006, 2009], the present TBSS analysis of FA values in UA revealed widespread compromised WM microstructure including notably fibers of the FCC and the PC. Using a stringent statistical threshold (TFCE and FWE,  $P < 0.05$ ), our voxel-based analyses have successfully replicated previous DTI results in fibers defined a priori [Pfefferbaum et al., 2009] such as the superior longitudinal fasciculus,



**Figure 4.**

Episodic memory performance (sum of the three free-recalls of FCSRT) in the HC, KS and subgroups UA\_LOW and UA\_HIGH. UA\_LOW and UA\_HIGH have been identified using k-means classification conducted on means FA values of significant fiber-clusters in the fornix and the cingulum bundle.

\*: sig different from HC; §: sig different from UA\_LOW; ¥: sig different from UA\_HIGH. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

external capsule, fornix, and cingulum. Moreover, our analyses revealed compromised WM integrity in other fiber tracts such as the internal capsule, cerebral peduncles, corona radiata, and thalamic radiations, which has also been reported in a TBSS study comparing alcoholics who have been abstinent for at least 5 years with HC [Fortier et al., 2014]. Contrary to previous neuropathological [Harper et al., 2003] and neuroimaging studies [Chanraud et al., 2009; Pfefferbaum et al., 2009], we also found microstructural abnormalities in the middle and superior cerebellar peduncles. The use of a voxel-based approach may have enabled the observation of the latter finding since a ROI approach, which would average the FA values within a region defined a priori, would not reveal any localized impairments within those fibers. Thus, WM abnormalities in the middle and superior cerebellar peduncles revealed by TBSS suggest that disruption may be more localized than spread-throughout in those fibers.

Our study is the first to evaluate WM microstructural integrity in KS patients. When KS patients were compared to HC, the profile of WM abnormalities was similar to that observed between UA and HC, in agreement with the patterns of GM and WM shrinkage found in a recent VBM study [Pitel et al., 2012]. Compromised WM microstructure in the middle and superior cerebellar peduncles is in-line with previous investigations that have shown significant shrinkage of the cerebellar WM of KS patients [Harper et al., 2003; Kril et al., 1997] and in proteomics studies where changes in the levels of thiamine-dependent enzymes have been observed [Alexander-Kaufman et al., 2006].

Previous neuropsychological and neuroimaging (structural and functional) studies have hypothesized that anterograde amnesia in KS patients is essentially due to a disconnection within the PC [Nahum et al., 2014; Warrington and Weiskrantz, 1982]. More specifically, findings of abnormalities in diencephalic structures, including the thalamus and mammillary bodies, and cortical structures from the frontal and medial temporal lobes have pushed toward the hypothesis of a disruption between nodes belonging to this neural network [Aupée et al., 2001; Kim et al., 2009; Renou et al., 2008]. Our study provides consolidating evidence of WM disruptions in the PC that is linked to episodic memory deficits and therefore amnesia [Kessels and Kopelman, 2012 for review]. Based on the substantial role of the cerebellum in cognitive processes and its connections with cortical areas, damaged FCC has been hypothesized to be involved in working memory and executive dysfunction in KS [Wijnia and Goossens, 2010]. Our data confirm compromised WM integrity in the cerebellum and especially disruption of the middle and superior cerebellar peduncles. In summary, cognitive deficits observed in KS patients, including amnesia, stems from a disconnection of neural networks, which can in turn be due to abnormalities in the nodes of the network(s), disruption of WM tracts linking those nodes, or abnormal synaptic activity between the nodes. While our study confirms the disruption of WM tracts in specific brain networks, and another study showed an absence of atrophy within connected regions [Nahum et al., 2014], it is still difficult to evaluate the cascade of events (neurotransmission dysfunction—local or global network disruption—cellular damage/atrophy) that effectively governs the pathophysiological mechanism of KS when using a cross-sectional paradigm. Longitudinal studies are required to concretely establish this mechanism.

Our direct voxel-based comparison between UA and KS patients showed significant differences mainly in the corpus callosum, which follows previous volumetric studies that has reported further volume loss in the corpus callosum in alcoholic patients with Wernicke's Encephalopathy than those alcoholic patients without [Lee et al., 2005] and the other one between UA and KS [Pitel et al., 2012]. The abnormalities in the microstructural integrity of the corpus callosum have also been hypothesized to be due to thiamine deficiency as observed in a study with rats [He et al., 2007].

Contrary to our initial hypotheses, a clear graded effect of deficits in the PC was not observed. This can be attributed to our sample size and to the heterogeneity of the UA group. As neurological complications from UA to KS lie along a continuum [Ryback, 1971], it is difficult to observe a distinct pattern of microstructural degradation between these 2 groups, justifying the need to divide the UA group into subgroups to better observe their underlying pathological mechanisms. By first combining the UA and KS groups, the statistical power to detect all regions affected in alcohol dependent patients was increased

[Monnig et al., 2013], ensuring that the subsequent classification step did not disregard any WM tracts that are potential structural biomarkers for identifying alcoholics at risk to develop neurological complications such as KS.

The classification step revealed that the use of DTI may be particularly relevant as a structural biomarker toward the early identification of UA patients at risk of developing KS. The early identification is important for clinicians to apply the correct and optimal treatment with the aim of preventing severe, debilitating and irreversible neurological complications. Our analysis provides consolidating evidence of the PC, as opposed to the FCC, being an appropriate neuroanatomical substrate for identifying UA patients that can potentially develop KS [Pitel et al., 2012]. The statistical comparisons of the mean FA values between the subgroups of subjects have confirmed the robustness of the classification step. The fact that there is a clear separation between HC and KS as well as consistent classification of the UA subgroups for the cingulum and fornix complements volumetric findings that have shown graded effects in the mammillary bodies, the hippocampus and the thalamus [Sullivan and Pfefferbaum, 2009]. In a previous study [Pitel et al., 2012], the volume of the thalamus was found to be comparable between some of the UA patients and the KS ones, reinforcing neuropsychological data that have shown the same trend in episodic memory deficits [Pitel et al., 2008]. While the volume of the thalamus was not explored in the present study, we confirm that UA patients classified at risk to develop KS based on WM microstructural abnormalities in the PC had the lowest episodic memory scores. Interestingly, it was also observed that 4 patients out of 10 classified as UA\_LOW filled none of Caine's criteria for Wernicke's encephalopathy [Caine et al., 1997; Pitel et al., 2011], four patients filled one criterion and two filled more than two criteria. For the UA\_HIGH subgroup, two patients filled no criterion, four filled one criterion and four filled more than two criteria. The number of signs of Wernicke's encephalopathy did not differ between the UA\_LOW and UA\_HIGH (Chi-squared test, data not shown). Our refined neuroimaging analyses confirm that a neuropsychological evaluation, especially targeting episodic memory, is highly recommended in clinical settings, where neuroimaging tools are not available, to identify patients at risk of developing KS. Taken together, the analysis of microstructural integrity within the fornix and cingulum, in combination with scores of episodic memory, thalamic volume and signs of Wernicke's encephalopathy, could give a reliable depiction of whether a UA patient is at risk of developing KS. Our study is thus a positive iteration to the heuristic value of the continuity hypothesis [Butters and Brandt, 1985; Parsons, 1998; Pitel et al., 2008; Ryback, 1971]. While this inherent heterogeneity in the UA group enabled us to detect alcoholics at risk to develop neurological complications, the currently used average neuropsychological, structural and functional description of a UA group does not reflect the heterogeneity of individual profiles in clinical settings.

## CONCLUSIONS AND FURTHER WORKS

TBSS has allowed us to describe the profile of WM integrity at a voxel-level in UA and KS patients. While the chronological position of WM disruption is still unknown in the cascade of structural and functional events that govern the pathophysiological mechanism underlying the neurotoxicity of alcohol, we have shown the potential of DTI data to identify UA at risk of developing KS. The method paves the way for more in depth analysis of this subgroup in order to better understand the mechanism underlying these two clinical forms. Multi-modal neuroimaging, combined with biological and neuropsychological analyses will enable researchers to explore the characteristics of these clinical forms in terms of detailed microstructure, regional volume, function, enzyme metabolism, and cognitive deficits. The specificity of the subgroup of patients at risk to develop KS is also likely to be confirmed via longitudinal studies in which the progress of the pathology can be monitored and the efficiency of the treatment can be assessed and optimized.

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