

Microstructural White Matter Alterations in Men With Alcohol Use Disorder and Rats With Excessive Alcohol Consumption During Early Abstinence

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[+ Supplemental content](#)

IMPORTANCE Although the detrimental effects of alcohol on the brain are widely acknowledged, observed structural changes are highly heterogeneous, and diagnostic markers for characterizing alcohol-induced brain damage, especially in early abstinence, are lacking. This heterogeneity, likely contributed to by comorbidity factors in patients with alcohol use disorder (AUD), challenges a direct link of brain alterations to the pathophysiology of alcohol misuse. Translational studies in animal models may help bridge this causal gap.

OBJECTIVE To compare microstructural properties extracted using advanced diffusion tensor imaging (DTI) in the brains of patients with AUD and a well-controlled rat model of excessive alcohol consumption and monitor the progression of these properties during early abstinence.

DESIGN, SETTING, AND PARTICIPANTS This prospective observational study included 2 cohorts of hospitalized patients with AUD ($n = 91$) and Marchigian Sardinian alcohol-preferring (msP) rats ($n = 27$). In humans cross-sectional comparison were performed with control participants (healthy men [$n = 36$]) and longitudinal comparisons between different points after alcohol withdrawal. In rats, longitudinal comparisons were performed in alcohol-exposed ($n = 27$) and alcohol-naïve msP rats ($n = 9$). Human data were collected from March 7, 2013, to August 3, 2016, and analyzed from June 14, 2017, to May 31, 2018; rat data were collected from January 15, 2017, to May 12, 2017, and analyzed from October 11, 2017, to May 28, 2018.

MAIN OUTCOMES AND MEASURES Fractional anisotropy and other DTI measures of white matter properties after long-term alcohol exposure and during early abstinence in both species and clinical and demographic variables and time of abstinence after discharge from hospital in patients.

RESULTS The analysis included 91 men with AUD (mean [SD] age, 46.1 [9.6] years) and 27 male rats in the AUD groups and 36 male controls (mean [SD] age, 41.7 [9.3] years) and 9 male control rats. Comparable DTI alterations were found between alcohol and control groups in both species, with a preferential involvement of the corpus callosum (fractional anisotropy Cohen $d = -0.84$ [$P < .01$] corrected in humans and Cohen $d = -1.17$ [$P < .001$] corrected in rats) and the fornix/fimbria (fractional anisotropy Cohen $d = -0.92$ [$P < .001$] corrected in humans and $d = -1.24$ [$P < .001$] corrected in rats). Changes in DTI were associated with preadmission consumption patterns in patients and progress in humans and rats during 6 weeks of abstinence. Mathematical modeling shows this process to be compatible with a sustained demyelination and/or a glial reaction.

CONCLUSIONS AND RELEVANCE Using a translational DTI approach, comparable white matter alterations were found in patients with AUD and rats with long-term alcohol consumption. In humans and rats, a progression of DTI alterations into early abstinence (2-6 weeks) suggests an underlying process that evolves soon after cessation of alcohol use.

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The harmful use of alcohol is one of the largest risk factors for death, disease, and disability (World Health Organization, 2018).¹ Population studies^{2,3} have suggested that alcohol-induced brain damage has no lower risk level boundaries; early detection of negative alcohol-related effects therefore has high priority.

Alcohol-induced brain damage can be revealed noninvasively through diffusion tensor imaging (DTI).⁴ This technique measures water diffusivity in brain and returns indices of microstructural integrity sensitive to tissue abnormalities occurring after alcohol consumption, even in regions that appear normal according to imaging-based morphometry^{5,6} or after a single acute administration of alcohol.⁷

Despite much congruent evidence of microstructural alterations in alcohol use disorder (AUD),^{6,8-10} linking the changes observed in vivo to the pathophysiology of AUD is challenging, owing to numerous comorbidity factors. In this context, animal models reproducing alcohol-induced brain damage can establish a causal link between the observed changes and alcohol consumption.^{11,12} Recently Cosa et al¹³ reported DTI alterations in rats after voluntary alcohol consumption; however, extensive translational studies comparing rodent and human brain microstructure in AUD are missing.

Although relative consensus in literature exists on the white matter alterations in individuals with AUD compared with controls, progression of white matter alterations in early abstinence is more controversial. Although DTI changes can be at least partially reversed after abstinence,⁵ the time scale and extent of this recovery are unclear, especially in early abstinence.¹³⁻¹⁵ Early abstinence is a key phase in the treatment of AUD because patients are more prone to relapse.^{16,17} Translational, longitudinal DTI studies in patients with AUD who are abstinent should help to characterize the course of microstructural changes occurring in this important disease phase.

When interpreting DTI results, changes in imaging parameters in AUD are generally explained as loss of tissue integrity due to demyelination or axonal damage.¹⁸⁻²⁰ However, DTI is sensitive to the compound effect of all the different water pools in the tissue: intra-axonal and extra-axonal space, cell bodies, and glia. Clarifying how a particular change affects this balance would dramatically improve the interpretation of results.

In the present study, we aimed to (1) use DTI to disclose specific patterns of alcohol-related brain changes in treatment-seeking patients with AUD and rats with long-term alcohol consumption; (2) monitor this change longitudinally into early abstinence (up to 6 weeks); and (3) explore the differential contribution of distinct water pools to the DTI signal through simulations and demonstrate that the observed abnormalities may also be explained by fluid accumulation and a glial reaction.

Methods

Human Study

The participants included 127 men enrolled in the following 3 groups (eFigure 1 in the [Supplement](#)): (1) 36 healthy controls; (2) 48 treatment-seeking patients with AUD (cohort A)

Key Points

Question Can commonly observed white matter defects in alcohol use disorder be linked to alcohol using a translational diffusion tensor imaging approach, and can it be used to monitor the progression of defects into early abstinence?

Findings This study of 91 men with alcohol use disorder, 36 healthy male controls, and 27 rats with a high preference for alcohol and 9 control rats found highly similar white matter alterations between species. A similar pattern of progression in diffusion tensor imaging alterations was found in patients and rats during early abstinence (2-6 weeks).

Meaning The reproducible patterns of alterations in humans and rats support an association with alcohol, and the progression of diffusion tensor imaging alterations into early abstinence suggests an underlying process that evolves soon after cessation of alcohol use.

undergoing DTI at 1 week after admission into the clinic and completion of detoxification treatment (TP1h-A) and after 2 to 3 weeks (TP2h-A); and (3) 53 treatment-seeking patients with AUD (cohort B) undergoing DTI after 2 to 3 weeks of admission into the clinics (TP2h-B), 20 of whom underwent scanning again after 4 to 6 weeks of admission (TP3h-B). Cohorts A and B shared 10 patients. The study was conducted at the Central Institute for Mental Health in Mannheim, Germany. The Ethics Committee II of Heidelberg University, Mannheim, Germany, approved the study procedures in accordance with the Declaration of Helsinki.²¹ Participants gave written consent and did not receive any stipend. Inclusion criteria and assessment are reported in the eMethods in the [Supplement](#). Descriptive statistics of demographic data and clinical descriptors appear in the [Table](#).

Animal Study

All animal experiments were approved by the Animal Care and Use Committee of the Instituto de Neurociencias de Alicante, Alicante, Spain, and comply with the Spanish (law 32/2007) and European regulations (EU directive 86/609, EU decree 2001-486, and EU recommendation 2007/526/EC). A total of 36 male rats of the Marchigian Sardinian alcohol-preferring (msP) line²² were used for the animal study. Of these, 27 rats had access to alcohol in a 2-bottle free-choice paradigm for 30 days (eFigure 9 in the [Supplement](#)). Eighteen rats underwent DTI before alcohol access (TP0r-A), after 4 weeks of alcohol access (TP1r-A), and after 6 weeks of abstinence (TP3r-A). Nine rats underwent DTI twice: after 4 weeks of alcohol drinking (TP1r-B) and after 2 weeks of abstinence (TP2r-B). Nine rats were used as age-matched alcohol-naïve controls. The timeline of DTI assessments is shown in eFigure 1 in the [Supplement](#); procedure details including drinking data are described in the eMethods in the [Supplement](#).

Image Processing

Human data were collected from March 7, 2013, to August 3, 2016; rat data were collected from January 15, 2017, to May 12, 2017. The DTI sequences for human and rats and preprocessing and analysis pipelines are reported in the eMethods in the

Table. Demographic and Clinical Data for Healthy Controls and Patients

Characteristic	Patient Group, Mean (SE)			Statistic	P Value
	Control (n = 36)	AUD Cohort A (n = 48)	AUD Cohort B (n = 53)		
Demographical variables					
Age, mean (SE), y	41.7 (1.6) ^a	47.5 (1.4) ^a	45.1 (1.2)	$F_{3,133} = 2.939$.04
Educational attainment, No. of participants ^a					
No graduation	0	1	2	$\chi^2_6 = 19.996$.003
Primary school	5	16	16		
Secondary school	5	13	20		
Attended college or higher	26	18	14		
Substance use patterns, mean (SE)					
Ethanol intake (mean of last 90 d), g/d	6.4 (25.7) ^{b,c}	202.5 (22.0) ^b	196.4 (18.0) ^c	$F_{3,132} = 16.016$	<.001
ADS (total score) ^d	2.1 (0.9) ^{b,c}	15.3 (0.8) ^b	14.2 (0.9) ^c	$F_{3,129} = 41.195$	<.001
Smoking					
No. of participants responding yes/no	4/31 ^{b,c}	31/17 ^b	41/11 ^c	$\chi^2_2 = 40.725$	<.001
Cigarettes smoked per day, mean (SE)	1.3 (0.4)	1.9 (0.2)	1.9 (0.2)	$F_{3,74} = 1.494$.22
FTND total score, mean (SE) ^e	4.8 (1.2)	6.1 (0.4)	6.0 (0.4)	$F_{3,74} = 0.507$.68
Clinical scales, mean (SE)					
OCDS total score ^f	1.5 (1.1) ^{b,c}	16.9 (0.9) ^b	16.4 (1.0) ^c	$F_{3,128} = 47.805$	<.001
STAI trait total score ^g	30.5 (1.8) ^{b,c}	45.9 (1.6) ^{b,h}	40.0 (1.4) ^{c,h}	$F_{3,126} = 14.354$	<.001
BDI total score ⁱ	2.1 (1.5) ^{b,c}	17.2 (1.2) ^{b,h}	11.8 (1.2) ^{c,h}	$F_{3,131} = 20.830$	<.001

Abbreviations: ADS, Alcohol Dependence Scale; AUD, alcohol use disorder; BDI, Beck Depression Inventory; FTND, Fagerström Test for Nicotine Dependence; OCDS, Obsessive-Compulsive Drinking Scale; STAI, State-Trait Anxiety Inventory.

^a Data were missing for 1 participant in the AUD patient cohort B.

^b Significant post hoc differences between the control group and AUD patient cohort A with $P < .05$.

^c Significant post hoc differences between the control group and AUD patient cohort B with $P < .05$.

^d Scores range from 0 to 47, with higher scores indicating higher alcohol dependence severity.

^e Scores range from 0 to 10, with higher scores indicating more intense physical dependence on nicotine.

^f Scores range from 0 to 40, with higher scores indicating more intense subjective alcohol craving.

^g Scores range from 20 to 80, with higher scores indicating higher trait anxiety.

^h Significant post hoc differences between AUD patient cohorts A and B with $P < .05$. Cohorts A and B share 10 patients.

ⁱ Scores range from 0 to 63, with higher scores indicating more intense depressive symptoms.

Supplement. For each participant, the following maps were computed: fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity.

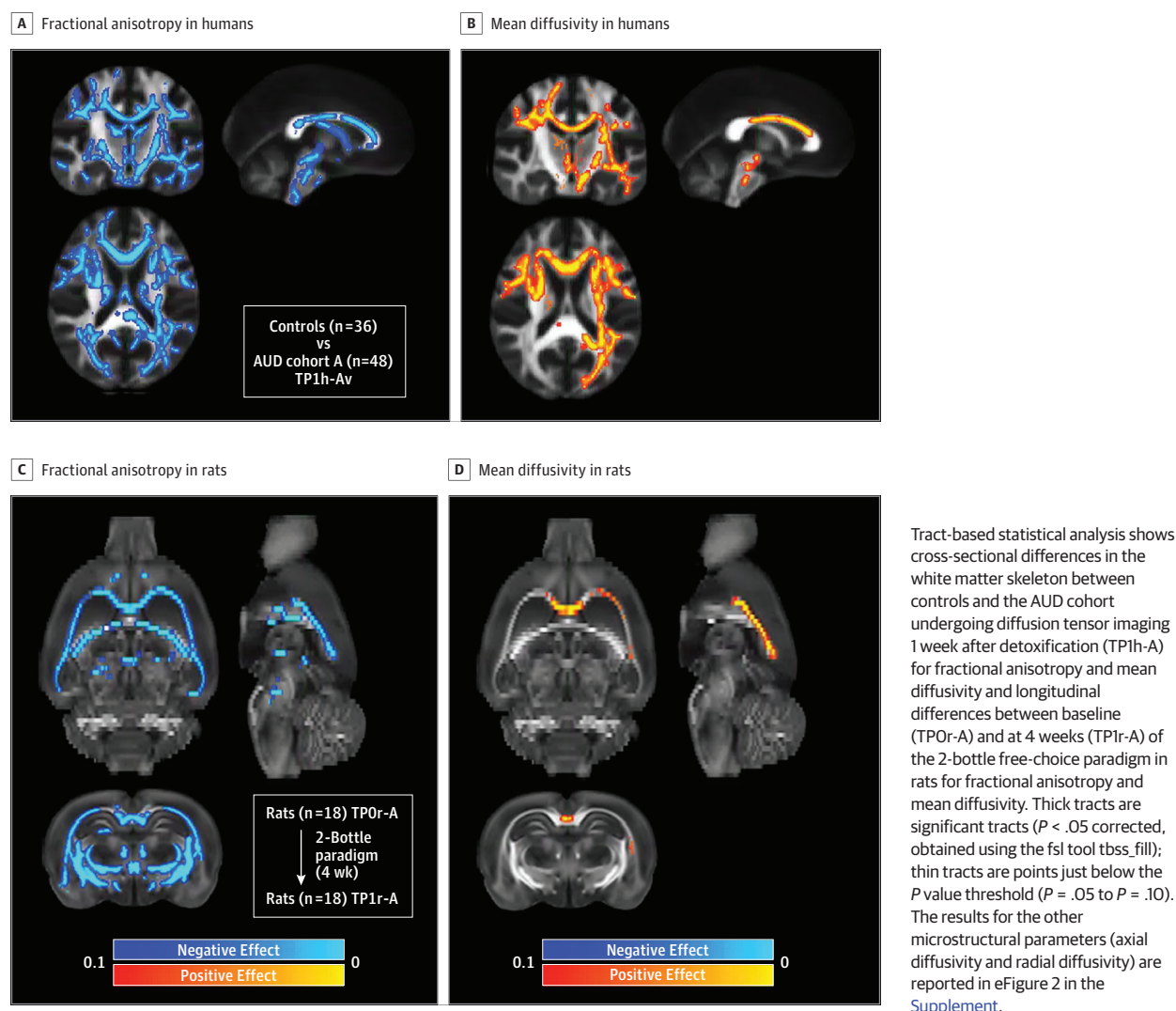
Statistical Analysis

Human data were analyzed from June 14, 2017, to May 31, 2018; rat data were analyzed from October 11, 2017, to May 28, 2018. Whole-brain statistical analysis of group differences and correlation with clinical variables were achieved using tract-based spatial statistics (TBSS),²³ combined with an advanced normalization approach.²⁴ For the cross-sectional analysis, a general linear model was used within a voxelwise, permutation-based, nonparametric statistical framework²³ to test for significant differences controlling for age and multiple comparisons across clusters using threshold-free cluster enhancement. For the longitudinal design, the statistic was applied on the difference between maps acquired at different points. To check for lateralization of the group differences, the hemisphere by group interaction was tested using the TBSS_sym routine in FSL,²³ as described in the eMethods in the Supplement. To test correlation with severity of AUD, the analysis was repeated by including measures of severity as an independent regressor in the general linear model for all the data at TP2h (cohorts A and B).

We used 10 000 permutations, and a corrected voxelwise 2-sided $P < .05$ was considered statistically significant. Cluster location was achieved using a white matter DTI-driven parcellization for humans²⁵ and the Paxino-Watson atlas for rats.²⁶ To compare the effect size across different designs, we defined voxel wise, in voxels with significant differences across conditions, the percentage of change in DTI parameters as $\Delta P = (P_2 - P_1)/P_1$, where P indicates fractional anisotropy, mean diffusivity, axial diffusivity, or radial diffusivity; 1, initial or healthy condition; and 2, final or pathologic condition.

Tract-specific analysis was performed according to the tractometry approach.²⁷ Significant differences between healthy controls and patients with AUD among the human participants were calculated using a multivariate analysis of variance (MANOVA) on all microstructural parameters, with age as covariate. In rats, significant differences between different DTI points were tested using a repeated-measures MANOVA. Both MANOVAs were followed by post hoc t tests, corrected for multiple comparisons using the false discovery rate.²⁸ The effect size was calculated as the difference between the parameter in the alcohol group and in the healthy control group, divided by the pooled SD (Cohen d statistic). Survival analysis was performed as described in the eMethods in the Supplement.

Figure 1. Differences Between Alcohol Use Disorder (AUD) Cohorts and Controls in the White Matter Skeleton



Signal Simulation

Because all water pools present in cerebral tissue contribute to the measured diffusion tensor, we simulated the effect of combining restricted, highly anisotropic water pools (representing water in axons) with an isotropic compartment of increasing volume fraction (which can represent glia and/or fluid accumulation). Details are reported in the eMethods in the Supplement.

Results

Difference Between Alcohol and Controls in the Whole WM Skeleton

The analysis included 91 men with AUD (mean [SD] age, 46.1 [9.6] years) and 27 male rats in the AUD groups and 36 men (mean [SD] age, 41.7 [9.3] years) and 9 male rats in the control groups. Our first aim was to compare the maps measured in patients with AUD at TP1h-A with those measured in controls. Patients with AUD had widespread microstructural abnormali-

ties, namely reduced fractional anisotropy and axial diffusivity and increased mean and radial diffusivity, compared with controls (Figure 1A and B and eFigure 2A and B in the Supplement) at $P < .05$ level, corrected for multiple comparisons. The mean ΔP over the significant voxels was -7% for fractional anisotropy, 6% for mean diffusivity, -9% for axial diffusivity, and 11% for radial diffusivity. The differences followed a complex pattern, which depends on modality and region, with a preferential involvement of frontal and superior white matter, and were most widespread in fractional anisotropy. According to the lateralization analysis results reported in eFigure 3 in the Supplement, voxels with significant differences were preferentially located in the right hemisphere for fractional anisotropy, mean diffusivity, and radial diffusivity, whereas they were more prevalent in the left hemisphere for axial diffusivity.

Likewise, in the longitudinal rodent experiment, animals were exposed to alcohol for 1 month. During this period rats escalated their alcohol consumption from 2 to 3 g/kg per day in the first 5 days to 5 to 6 g/kg per day from the tenth day onward. Such daily consumption levels led to pharmacologically

relevant blood alcohol levels as high as 1 g/L in msP rats.²² This level of alcohol consumption caused widespread microstructural abnormalities, namely reduced fractional anisotropy and axial diffusivity (Figure 1C and eFigure 2C in the [Supplement](#)) and increased mean diffusivity and radial diffusivity (Figure 1D and eFigure 2D in the [Supplement](#)) at TP1r-A compared with TPO-A. The mean ΔP across the significant voxels was -6% for fractional anisotropy, 4% for mean diffusivity, -3% for axial diffusivity, and 5% for radial diffusivity. The nature of these changes was comparable to the human finding; however, unlike in humans, the observed changes were not lateralized.

To control for the age difference in the longitudinal rat study between TPOr-A and TP1r-A and discard potential age effects, we repeated the DTI acquisition in the same strain after 1 month of normal aging. Importantly, we found that, for this period, fractional anisotropy was not affected and mean diffusivity was only marginally changed, with only 2 regions of interest (the second somatosensory cortex and the piriform cortex) showing significant differences (eFigure 4 in the [Supplement](#)). Furthermore, the aging effect on mean diffusivity was a slight reduction, opposite to the clear enhancing effect of alcohol.

Difference Between AUD and Control Groups in the Corpus Callosum and Fornix

Next, we focused on the corpus callosum and the fornix, 2 fiber tracts that are preferentially affected by alcohol according to literature,²⁹ using streamline-specific statistics to compare tract-specific DTI parameters between TP1h-A and controls in humans and TP1r-A and TPOr-A in rats. Alcohol exposure was associated with microstructural changes in the corpus callosum and fornix (Figure 2 and eFigure 5A-F in the [Supplement](#)). Specifically, in the corpus callosum, we observed a statistically significant reduction of fractional anisotropy (Cohen $d = -0.84$ [$P < .01$]) and an increase in mean diffusivity (Cohen $d = 1.05$ [$P < .001$]), axial diffusivity (Cohen $d = 0.73$ [$P < .05$]), and radial diffusivity (Cohen $d = 0.95$ [$P < .001$]), whereas in the fornix we observed a statistically significant reduction of fractional anisotropy (Cohen $d = -0.92$ [$P < .001$]). Alcohol intake was also associated with a statistically significant decrease in the tract volume compared with controls for the corpus callosum (Cohen $d = -0.75$ [$P < .01$]) and fornix (Cohen $d = -0.74$ [$P < .01$]).

Likewise, 1 month of excessive drinking in rats led to microstructural changes in the corpus callosum and fornix tracts (Figure 2 and eFigure 5G-L in the [Supplement](#)). In the corpus callosum we observed a statistically significant reduction of fractional anisotropy (Cohen $d = -1.17$ [$P < .001$]) and an increase in mean diffusivity (Cohen $d = 0.88$ [$P < .01$]) and radial diffusivity (Cohen $d = 1.21$ [$P < .001$]), whereas in the fornix we observed a statistically significant reduction of fractional anisotropy (Cohen $d = -1.24$ [$P < .001$]). Alcohol exposure also was associated with a decrease in the tract, which was not significant (all ANOVA results are reported in eTables 1 and 2 in the [Supplement](#)).

Development of the Microstructural Changes During Early Abstinence

To study the dynamics of microstructural changes, we compared DTI parameters measured at different points during ab-

stinence. In humans, we found a pattern of progressive decrease of fractional anisotropy (Figure 3A-B) and an increase of mean diffusivity (eFigure 6A-D in the [Supplement](#)) and radial diffusivity (eFigure 6B-E in the [Supplement](#)) in most white matter tracts. Axial diffusivity decreased and increased (depending on the region) during the initial 2 to 3 weeks of abstinence, but increased in most white matter between 2 to 3 weeks and 4 to 6 weeks. The mean ΔP over the significant voxels for TP2h-A vs TP1h-A was -6% for fractional anisotropy, 4% for mean diffusivity, 1% for axial diffusivity, and 8% for radial diffusivity; the ΔP over the significant voxels for TP3h-B vs TP2h-B was -7% for fractional anisotropy, 4% for mean diffusivity, 1% for axial diffusivity, and 9% for radial diffusivity. Notably, none of the microstructural parameters measured after early abstinence normalized toward levels measured in healthy controls. On the contrary, the difference compared with healthy controls increased during early abstinence for all parameters, except axial diffusivity in some regions.

In rats, we found a widespread significant decrease in fractional anisotropy (Figure 3C) and in axial diffusivity (eFigure 6H in the [Supplement](#)) at TP2r-B compared with TP1r-B. At TP2r-A compared with TP1r-A, we found an even more widespread decreased fractional anisotropy (Figure 3D) and axial diffusivity (eFigure 6I in the [Supplement](#)) and increased radial diffusivity (eFigure 6J in the [Supplement](#)). The mean ΔP over the significant voxels for TP2r-B vs TP1r-B was -4% for fractional anisotropy and -3% for axial diffusivity; and for TP3r-A vs TP1r-A, -0.2% for fractional anisotropy, -1% for axial diffusivity, and 0.2% for radial diffusivity. The pattern of further progression of microstructural changes during abstinence was compatible with the human results.

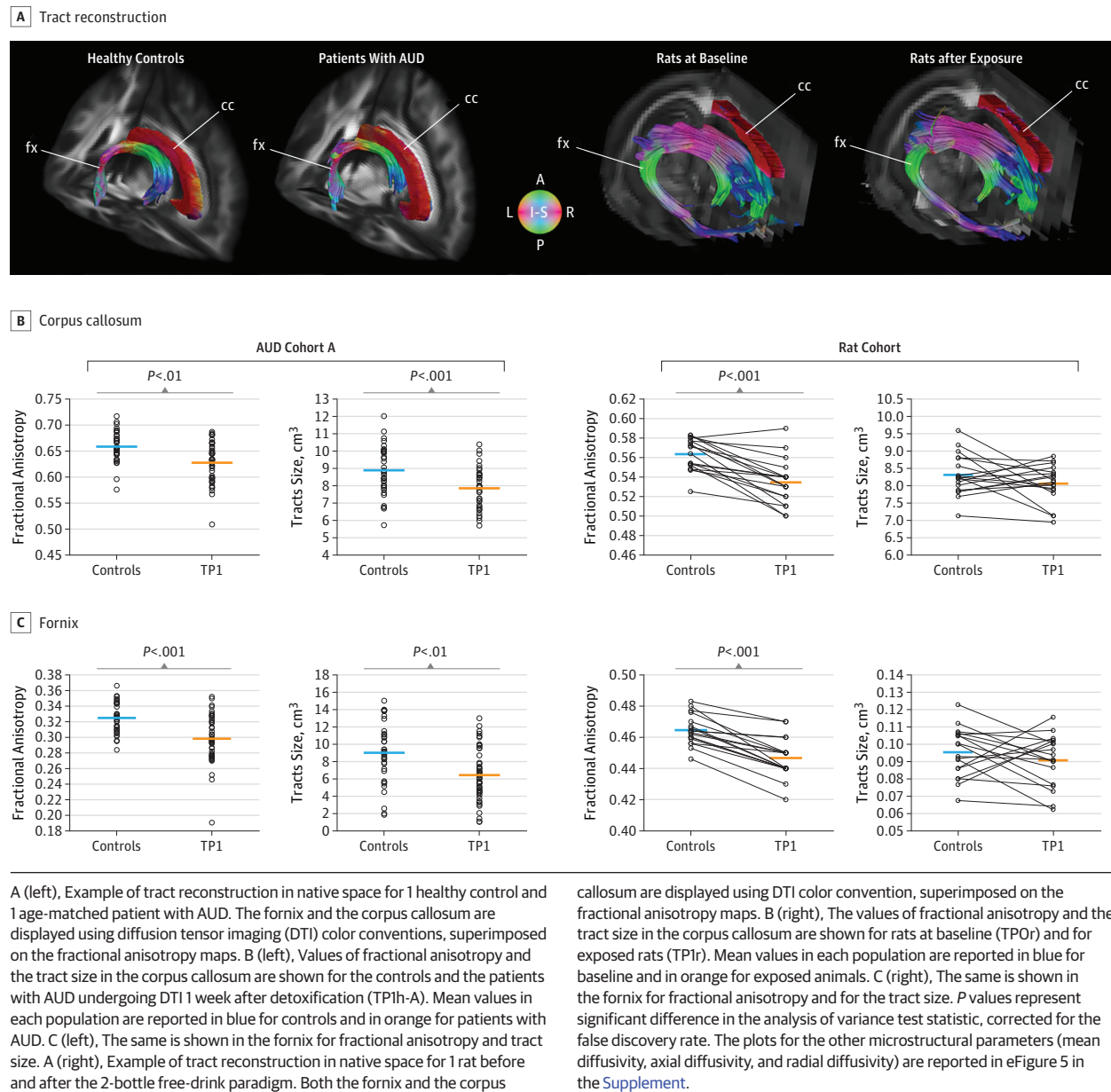
Correlation With Clinical and Behavioral Measures in Humans

In our next analysis, we investigated whether the microstructural alterations found in humans were associated with clinical and behavioral measures. Correlation analysis between the microstructural parameters and alcohol use history in the combined AUD TP2h-A plus TP2h-B cohorts unveiled a significant negative association between fractional anisotropy and axial diffusivity and the mean ethanol daily intake before treatment (Figure 4A and eFigure 7 in the [Supplement](#)). Plots of age-adjusted fractional anisotropy vs ethanol intake in the corpus callosum and fornix are reported in Figure 4B-C. No significant correlations were found with the other measures of addiction reported in the assessment. Survival analysis did not reveal association of any DTI parameters with relapse risk within 3 months after discharge from the hospital.

Interpretation of the Imaging Results: Simulations of Diffusion in Multiple Water Pools

To better interpret the observed DTI changes, we simulated the presence of an isotropic water pool of increasing volume fraction, compatible with a glial reaction and/or extracellular fluid accumulation, and determined the effect on the DTI indices. We report simulations for 2 different geometries of the restricted pool: single orientation (eFigure 8I in the [Supplement](#)), a model for highly coherent tracts like the corpus cal-

Figure 2. Tract-Specific Differences Between Alcohol Use Disorder (AUD) Cohorts and Controls



A (left), Example of tract reconstruction in native space for 1 healthy control and 1 age-matched patient with AUD. The fornix and the corpus callosum are displayed using diffusion tensor imaging (DTI) color conventions, superimposed on the fractional anisotropy maps. B (left), Values of fractional anisotropy and the tract size in the corpus callosum are shown for the controls and the patients with AUD undergoing DTI 1 week after detoxification (TP1h-A). Mean values in each population are reported in blue for controls and in orange for patients with AUD. C (left), The same is shown in the fornix for fractional anisotropy and tract size. A (right), Example of tract reconstruction in native space for 1 rat before and after the 2-bottle free-drink paradigm. Both the fornix and the corpus

callosum are displayed using DTI color convention, superimposed on the fractional anisotropy maps. B (right), The values of fractional anisotropy and the tract size in the corpus callosum are shown for rats at baseline (TP0r) and for exposed rats (TP1r). Mean values in each population are reported in blue for baseline and in orange for exposed animals. C (right), The same is shown in the fornix for fractional anisotropy and for the tract size. P values represent significant difference in the analysis of variance test statistic, corrected for the false discovery rate. The plots for the other microstructural parameters (mean diffusivity, axial diffusivity, and radial diffusivity) are reported in eFigure 5 in the [Supplement](#).

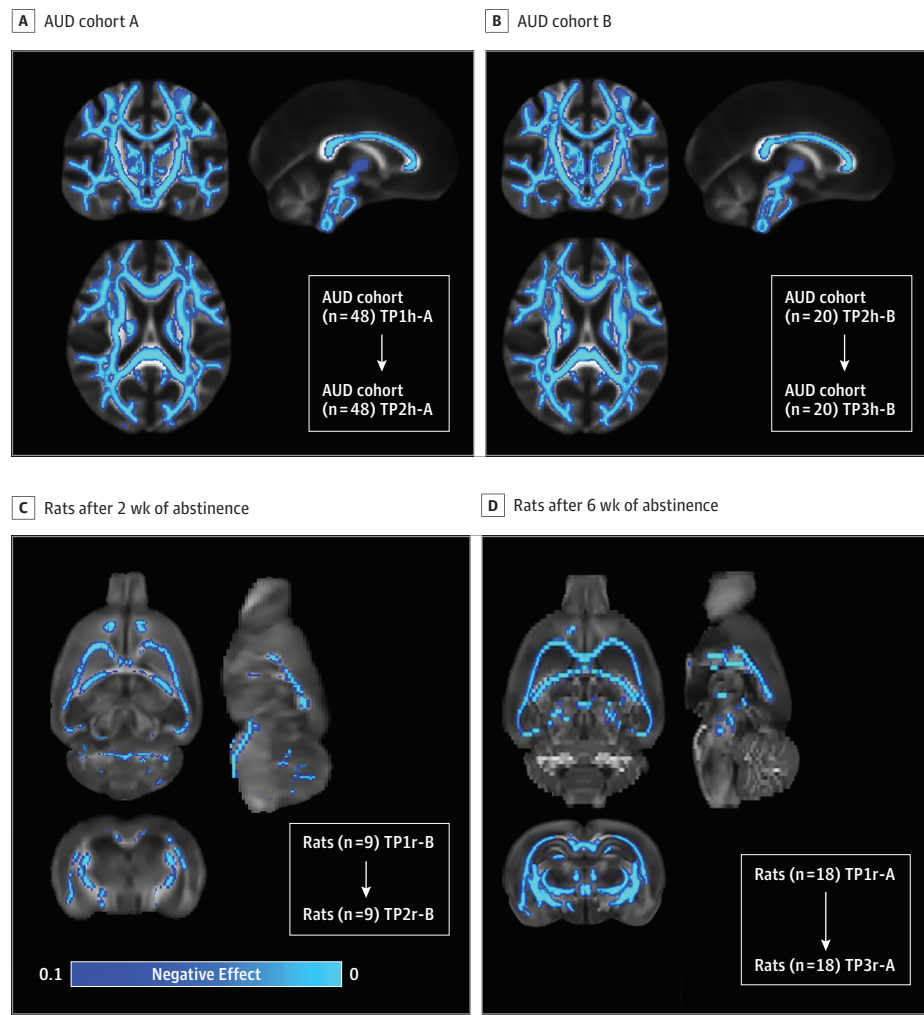
losium) vs 2 orthogonal populations (eFigure 8J in the [Supplement](#), a model for most white matter tracts). In the single restricted orientation (eFigure 8A in the [Supplement](#)), fractional anisotropy showed a nonmonotonic increase in volume fraction of the isotropic water pool, while it decreased monotonically when 2 restricted populations were present (eFigure 8E in the [Supplement](#)). Similarly, mean diffusivity and radial diffusivity had a nonmonotonic increase in volume fraction in single restricted orientation (eFigure 8B and D in the [Supplement](#)), but they increased monotonically with 2 orthogonal cylindrically restricted populations (eFigure 8F and H in the [Supplement](#)). Axial diffusivity always increased, but the rate depended on the volume of the restricted vs the hindered compartment (eFigure 8C and G in the [Supplement](#)). All

4 microstructural indices, following the same increase in the isotropic pool, can increase and decrease, depending on the fiber geometry (single or crossing fibers). This process suggests that a pattern of reduced fractional anisotropy, like that observed herein, can also be caused by a glial reaction and/or extracellular fluid accumulation.

Discussion

The most important findings from this translational neuroimaging study are the coherence in white matter microstructural changes observed after heavy alcohol exposure in a cohort of patients and experimental animals and the progression

Figure 3. Evolution of White Matter Alterations Into Early Abstinence



Tract-based statistical analysis shows longitudinal fractional anisotropy differences in the white matter skeleton between patients in alcohol use disorder (AUD) cohort A undergoing diffusion tensor imaging (DTI) at 2 weeks after detoxification (TP2h-A) vs 1 week after detoxification (TP1h-A) (A) and longitudinal DTI differences between patients in AUD cohort B undergoing DTI 4 to 6 weeks after admission (TP3h-B) vs 2 to 3 weeks after admission (TP2h-B) (B). The same analysis is shown in rats that underwent DTI after 4 weeks of alcohol access (TP1r-B) vs after 2 weeks of abstinence TP2r-B (C) and after 4 weeks of alcohol access (TP1r-A) vs after 6 weeks of abstinence (TP3r-A) (D). Thick tracts are significant tracts ($P < .05$, obtained using the fsl tool *tbss_fill*); thin tract are points just below P value threshold ($P = .05$ to $P = .10$). The results for the other microstructural parameters (mean diffusivity, axial diffusivity, and radial diffusivity) are reported in eFigure 6 in the Supplement.

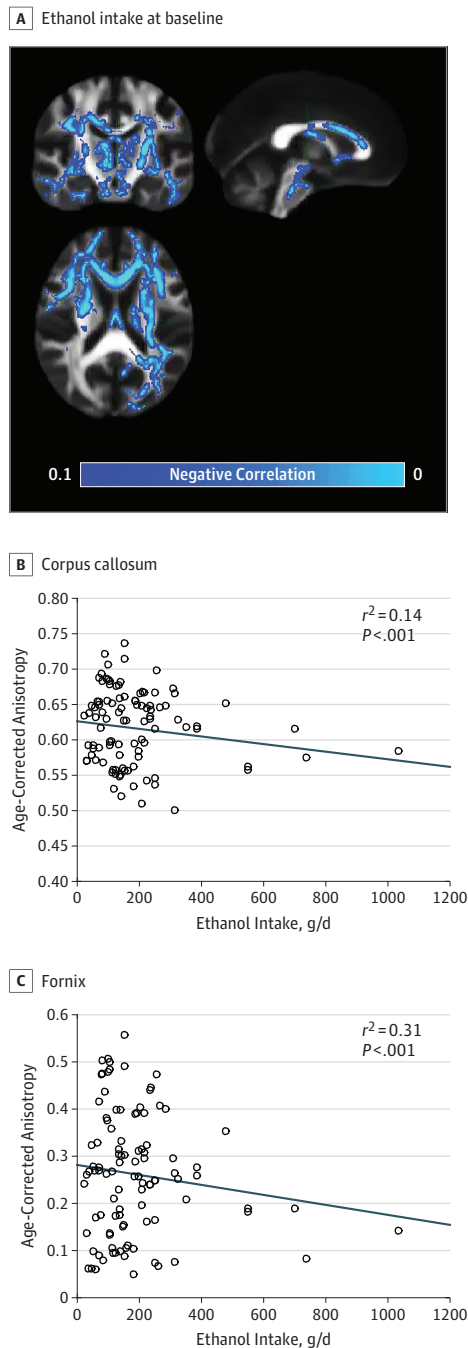
of such changes during early abstinence. Furthermore, by mathematical modeling of tissue diffusion properties, we challenge the current interpretation of DTI changes as reflecting myelin and/or axonal damage; we present evidence that this phenomenon may be associated with, for instance, a glial reaction.

In humans, we found diffuse microstructural differences in patients with AUD compared with controls, which affects preferentially the right hemisphere and the frontal area, in agreement with recent literature.^{9,30} We also found a negative correlation between fractional anisotropy and daily alcohol intake before admission, consistent with recent work.³¹ Using an established rat model of excessive voluntary alcohol consumption, we demonstrated that alcohol consumption during a relatively short period (1 month, but significantly longer if adjusted for the different life span of rats compared with humans) was associated with diffuse microstructural changes in DTI indices largely comparable to the findings in humans with AUD. Furthermore, using tractography to focus on the corpus callosum and the fornix, 2 tracts identified as particularly affected in AUD,^{8,10} we demonstrated that the microstructural

changes have similar effect size (0.7-1.0) in humans and rats. Minor differences between the 2 analyses can be attributed to the different sensitivity of region of interest-based vs whole brain approaches.²³ We also found an association with reduced tract volume in AUD, significant in humans only. This finding is consistent with magnetic resonance imaging volumetric studies that report shrinkage of white matter tracts of alcohol-dependent individuals.²⁹ The lack of significance in rats suggests its association with longer exposition times and higher alcohol levels in patients vs the 1-month consumption period in rats, but other differences between humans with AUD and the rat model (eg, medication) cannot be excluded.

The fact that the findings in humans mirror those in rats may establish a relationship between the observed changes and alcohol consumption, which is difficult to verify based on human results only, given the large heterogeneity of the abuse patterns, medication for relief of withdrawal symptoms, and comorbidities among patients with AUD. This result establishes the utility of diffusion imaging for monitoring the brain status as a possible noninvasive biomarker of AUD progression and, potentially, of treatment response.

Figure 4. Correlation Between Diffusion Tensor Imaging Parameters and Ethanol Daily Intake



Tract-based statistical analysis of the correlation between fractional anisotropy measures in patients with alcohol use disorder (AUD) and ethanol intake at baseline, corrected for age (A). Thick tracts are significant tracts ($P < .05$, obtained using *tbss_fill*); thin tracts are points just below P value threshold ($P = .05$ to $P = .10$). Scatterplots between age-corrected fractional anisotropy (using the multiparametric regression $\alpha + \beta \times \text{age} + \gamma \times \text{ethanol}$) and ethanol intake are shown for the genu of the corpus callosum (B) and the fornix (C).

Although many previous studies have included individuals with abstinence ranging from days to years or included individuals during sustained remission (>1 year), the few works

focused on the early abstinence phase^{9,14,15} present conflicting results. Herein we found that at 2 and 6 weeks of abstinence, the microstructural changes progressed with further decrease of fractional anisotropy and increase of radial diffusivity in humans and rats. These results challenge the conventional idea that the microstructural alterations start to revert to control values immediately after discontinuing alcohol consumption and provide insights into the neuroadaptations occurring during abstinence.

Only 1 parameter, axial diffusivity, showed differential development during abstinence between humans and rats. However, its correlation with preadmission alcohol consumption in humans suggests its clinical relevance. Although axial diffusivity was initially proposed as a marker for axonal integrity, recent literature highlighted its pitfalls, especially in disease³²; our simulations showed that axial diffusivity was the only parameter with a nonmonotonic trend as a function of the restricted fraction, suggesting that more information is needed to understand the exact pathophysiological underpinning of its contrast.

Given the lack of specificity of DTI, interpreting the underlying neurobiological substrate that produces the observed change is challenging. Alcohol use induces loss of mainly small fibers, myelin irregularity, and segmental demyelination or remyelination,²⁰ accompanied by neuroinflammation.³³ Excessive intracellular and extracellular fluid accumulation was also proposed to explain DTI changes in AUD.⁸ Our simulation results challenge the idea that we might infer the specific microstructural alteration causing the observed changes, showing that a different balance among restricted, hindered, and isotropic water pools affects the observed DTI indices. We showed that an increase in the proportion of the isotropic pool, which can be a model for a glial reaction, was associated with an increase of fractional anisotropy in areas of single fibers, and a decrease of fractional anisotropy in areas of crossing fibers. The observed further decrease of fractional anisotropy in early abstinence may thus be explained by progressive myelin and axonal damage, but also by a glial or a cellular reaction, for instance, during an ongoing inflammatory process.

Limitations

Aging is a possible confounder in this longitudinal study because DTI indices also change with normal aging. However, in humans, the effect size of the change is too small to explain the observed changes, because the annual rate of change is smaller than or equal to 1%.^{34,35} For rats, we reported no change in fractional anisotropy and marginal changes in mean diffusivity after 1 month of aging, with mean diffusivity and changes going in the opposite direction compared with alcohol-induced changes. Another recent study reported similar trends in Sprague-Dawley rats.³⁶

Other limitations include differences in the age composition of the 3 human cohorts (healthy controls and AUD cohorts A and B), although these were controlled for in the statistical analysis. The alleviation of severe withdrawal symptoms is a medical requirement; although the direct pharmacological effects of benzodiazepines have been avoided by the study design, little is known about their long-term effect on

DTI parameters. To address this limitation, we have presented data from well-controlled animal models.

Only male participants were included in the study, because most patients with AUD admitted to our inpatient care are male. The prevalence of alcohol dependence in women, although lower than in men, is nevertheless significant. Thus, sex effects on the observed microstructural alterations need to be investigated more closely in the future.

On the methodological side, the limitations of the tensor model, especially in accounting for crossing fibers, are well known. Future studies with multishell diffusion data and more advanced diffusion models are needed to increase specificity and sensitivity.

Conclusions

This study reported diffuse white matter microstructural changes observed after heavy alcohol exposure in patients that mirrors changes obtained in experimental animals. We found that in humans and rats, a progression of DTI alterations into early abstinence (2-6 weeks), suggesting an underlying process that evolves soon after alcohol cessation. Owing to the inherent lack of specificity of diffusion MRI, further studies are needed to clarify the biological underpinnings of the observed signature. This study may lead the way for biomarker development with translational value and suitable for big data approaches.

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