Supplementary Online Content


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This supplementary material has been provided by the authors to give readers additional information about their work.
1. Inclusion criteria and Assessments

Alcohol dependent patients of cohort A had to meet the following inclusion criteria: i) diagnosis of alcohol use disorder according to the Diagnostic Statistical Manual of Mental Disorders (DSM-V), ii) age between 18 and 65 years, iii) completed detoxification (i.e. treatment of withdrawal symptoms with short-acting benzodiazepines or clomethiazole had to have been completed for at least 3 days; i.e. > 5x elimination half-life). In addition to criteria i) and ii), alcohol dependent patients of cohort B had to meet the following inclusion criteria: iii) abstinence from any substance for two to five weeks, and iv) at least six drinks per day (84g) in the previous 90 days. Exclusion criteria were similar for both cohorts: i) comorbid axis-I disorders (other than nicotine dependence) in the last year, ii) treatment with psychotropic or anticonvulsive medications in the last three months, iii) severe neurological or physiological disease (i.e. liver cirrhosis), iv) positive drug screening, v) ineligibility for MRI scanning (e.g. metal implants), v) history of severe head trauma, or vi) changes to vasoactive or antihypertensive medication during the last seven days. Healthy control participants were only included if they i) were aged between 18 and 65 years, ii) did not meet the clinical diagnosis of an alcohol dependence or any other axis-I disorder, iii) had an average alcohol consumption below one drink per day (14g) and iv) did not meet any of the exclusion criteria (see above).

All participants completed a series of questionnaires, including the Beck Depression Inventory (BDI, Beck et al, 1961), the Fagerström Test for Nicotine Dependence (FTND, Fagerstrom and Schneider, 1989), the Alcohol Dependence Scale (ADS, Kivlahan et al, 1989), the Alcohol Use Disorders Identification Test (AUDIT, Saunders et al, 1993), the
Obsessive Compulsive Drinking Scale (OCDS, Mann, 2000), the Clinical Institute Withdrawal Assessment scale (CIWA-Ar, Sullivan et al, 1989) and the Systematic Assessment for Treatment Emergent Events (SAFTEE, Johnson et al, 2005). Symptoms of acute withdrawal were assessed using the Clinical Institute Withdrawal Assessment scale [CIWA-A] (Stuppaec et al., 1994), and treated if needed, i.e. CIWA-R > 10, using short acting benzodiazepines. The initial detoxification treatment lasted for about 3 to 5 days, and patients could first enter the study after > 5x elimination half-life times had elapsed. Then, patients participated then in a standardized multiprofessional CBT-based relapse prevention program, i.e. an extended withdrawal treatment; for details, see [e1]. Drinking and relapse data were collected for three months following the experiment at baseline and at weeks 2, 4, 8 and 12 weeks using the Form 90 (F90) [e2].

2. Animal study details

We used the msP rat line, created by selective breeding for high alcohol preference and voluntary alcohol consumption [e3]. Rats were obtained from the breeding facility at the School of Pharmacy University of Camerino, Camerino, Italy. A total of 27 msP rats (370–480 g, 8 weeks of age) were used in these experiments. Additionally, 9 age-matched rats were used to control the effect of 1 month of ageing on the DTI parameters. The rats were individually housed for 30 days with access to two drinking bottles, one containing water and the other 10% (v/v) EtOH in water. Fluid consumption was registered every 2–3 days. While the animals start drinking moderate doses of alcohol (in the first 4-5 days, 2-3 g/kg/day), consumption rapidly escalates and after 10 days of drinking it reaches values of 5-6 g/kg/day, and it is maintained at this level until the end of the experiment. This pattern is consistent with earlier reports [e3]. We also observed escalating alcohol preference compared to water, which starts around 50% (same preference for alcohol and
water) and after about 10 days reaches a plateau of 80%. Individual alcohol preference over the exposed period are reported in eFigure 9.

3. MRI acquisitions and processing

Human scanning was performed with a 3 T whole-body tomograph (MAGNETOM Trio with TIM technology; Siemens, Erlangen, Germany). DTI data were acquired using an Echo Planar Imaging spin-echo diffusion sequence with the following parameters: repetition time (TR) 14 ms, echo time (TE) 84 ms, 41 gradient orientations uniformly distributed plus one non-diffusion weighted images, b-value 1000 s/mm², matrix size = 128 x 128 x 64, isotropic resolution of 2 mm³.

Animal imaging experiments were performed under anesthesia on a 7 T scanner (Bruker, BioSpect 70/30, Ettlingen, Germany). DTI data were acquired using an Echo Planar Imaging spin-echo diffusion sequence. For the first group of rats and for the control group, a diffusion protocol was employed with the following parameters: TR 8000 ms, TE 29 ms, 30 gradient orientations with b-value 1000 s/mm² plus three non-diffusion weighted images, matrix size = 128 x 128 x 16, in-plane resolution = 0.225 x 0.225 mm², slice thickness = 1 mm. For the second group of rats, we scanned a protocol with 30 uniform distributed gradient directions, b = 670 s/mm², with four non-diffusion weighted images, repetition time (TR) = 4000 ms, and echo time (TE) = 23 ms. Fourteen horizontal slices were planned for every subject (field of view [FOV] = 32X32 mm², matrix size = 128X128X14, in-plane resolution = 0.25 x 0.25 mm², slice thickness = 1 mm).

All data were preprocessed to correct for Eddy current and motion distortions using affine registration. DTI analysis was done with the software ExploreDTI v.4.8.4 [e4]. The tensor was fitted using a Robust Estimation of Tensors by Outlier Rejection approach [e5],
which uses iteratively reweighted least-squares regression to identify potential outliers (due to thermal noise and spatially and temporally varying artifacts such as residual subject motion and cardiac pulsation) and excludes them from the fit. On average, 0.7% of the volumes were excluded from the fit across all subjects.

To control for potential group differences in the movement inside the scanner in the human cohorts, for each subject we calculated the total motion index (TMI, as defined in [e6]), which takes into account for each subject the average translation, rotation, skew and scale factors across different volumes. An independent sample t-test was used to compare the TMI in AUD versus control, and no significant differences were found (p=0.21). A paired sample t-test was used to compare TMI across the two timepoints, for both datasets, and no significant differences were found either for cohort A or B (p=0.77, p=0.43, respectively).

From the preprocessed data, the following parameter maps were computed for each subject: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD). Whole brain tractography was calculated using a deterministic, tensor-based approach; tract termination criteria were FA<0.15 and angle>30 degrees.

4. Supplementary statistical analysis

Lateralization of group differences: We also tested for hemisphere by group interactions using the TBSS_sym function in FSL to directly compare adjacent white matter regions in each hemisphere. This procedure generates a symmetric skeleton by using the original (asymmetric) skeleton and dilating it by one voxel. The symmetric mean FA image is generated by flipping and averaging the mean FA map and then skeletonizing to generate the initial symmetric skeleton. This is masked by the dilated original skeleton to ensure
that only those areas that are already close to being symmetric in the original data are included. Finally, the skeleton is flipped and masked with the non-flipped version, creating a symmetrized skeleton, from which a thresholded skeleton mask and derived distance map were generated.

Survival analysis: In order to test for associations between fractional anisotropy in the body, genu, fornix and splenium of the corpus callosum, as well as in the overall white matter skeleton and relapse risk within the three months after the experiment, cox regression models using a stepwise forward selection were computed (stepwise inclusion p < 0.05). In accordance to our earlier studies, we used time to relapse to heavy drinking as outcome variable in our survival analyses [e7-9]. Other values, i.e. mean diffusivity, axial diffusivity and radial diffusivity in the areas specified above did not meet the requirements for cox regression analyses, e.g. proportional hazards. Hence, associations between these values and relapse risk were analyzed after dichotomizing the values using the median as separator.

5. DTI Simulations

Given that DTI measures are strongly dependent on the local fibre architecture (single or crossing) [e10], we simulated two different configurations: 1) one restricted, cylindrically symmetric water pool, one hindered water pool, and one isotropic water pool of increasing volume fraction; and 2) two restricted, cylindrically symmetric populations, orthogonal to each other, one hindered water pool and one isotropic water pool of increasing volume fraction. Signal simulations were generated in Matlab (R2012b, The Mathworks). The signal expression for the restricted population was modeled according to Assaf et al. [e11] and the hindered water pool was modeled according to [e12]. The simulated acquisition scheme was the same one used for the in vivo experiments. Other
simulation parameters were: diffusion coefficient of the isotropic pool $D_{iso}=1*10^{-9}$ mm$^2$/s, diffusion coefficient of the restricted pool orthogonal to the cylinder axis $D_{ortho}=1*10^{-9}$ mm$^2$/s, radius of the cylinder according to histological findings [e13], volume fraction of the restricted pool versus the hindered water pool = 0.2/0.4/0.6; volume fraction of the isotropic compartment in the range 0-0.5, increasing in 10 steps of 0.05. 10000 noisy repetitions were generated adding Rician noise at SNR=30. The fitting routine was written in Matlab and based on nonlinear least-squares estimation of the diffusion tensor. For each configuration, mean FA, MD, AD and RD and standard deviation over the noisy repetitions were estimated.
Supplementary Figures

eFigure 1. Study Design

![Study Design Scheme](image)

The study design scheme for human (panel a) and rat experiments (panel b). The human participants were 127 men enrolled in three different groups: 1) a cohort of 36 healthy controls (HC); 2) 48 treatment seeking alcohol dependent patients (cohort a) scanned at two timepoints (TP): within 1 week after admission to the clinic, TP1h-A, and after 2-3 weeks, TP2h-A); and 3) 53 treatment seeking AUD patients (cohort b) scanned after 2-3 weeks of admission into the clinics (TP2h-B), 20 of which were scanned again after 4-6 weeks of admission into the clinics (TP3h-B). Group a and b share 10 patients. Two
groups of rats, cohort a and b, underwent the 2-bottle free-choice paradigm during four weeks. The first one was scanned three times: before alcohol access (TP0r-A), after four weeks of alcohol drinking (TP1r-A), and after six weeks of abstinence (TP3r-A). The second group was scanned twice: after four weeks of alcohol drinking (TP1r-B) and after two weeks of abstinence (TP2r-B).
Tract-based statistical analysis showing cross-sectional differences in the WM skeleton between controls and alcohol-dependent at TP1h-A for AD (a) and RD (b), and longitudinal differences between baseline (TP0r-A) and four weeks (TP1r-A) of two-bottle free-choice paradigm in rats for AD (c) and RD (d). Thick tracts are significant tracts ($P<.05$ corrected, obtained using the fsl tool tbss_fil), thin tract are points just below $P$ value threshold ($P=.05-.10$).
eFigure 3. Lateralization of Group Differences in the White Matter Skeleton

Tract-based statistical analysis showing lateralization of the group differences reported in Figure 1 and eFigure 2 for FA (a), MD (b), AD (c) and RD (d). Green voxels are voxels with significant ($P<.05$) group*hemisphere interactions according to the TBSS_sym routine.
eFigure 4. Effect Size of DTI Changes in Control Rats

Effect size $\Delta P$ measured after 1 month of normal ageing in msP rats, showing little or no change in DTI parameters over 1 month of ageing. Upper panel (a) shows $\Delta P$ for FA in 46 ROIs taken from the Paxinos atlas, while the lower panel (b) shows the same for MD. Asterisk indicates significant differences between the two timepoints in the paired t-test, corrected for multiple comparisons.
Left: values of MD (a), AD (b) and RD (c) in the corpus callosum are shown for the control subjects at the left and the AUD patients at TP1h-A at the right. Mean values in each population are reported in blue for controls and in orange for AUD patients. In the lower line, the same is shown in the fornix: MD (d), AD (e) and RD (f). Right: values of MD (g), AD (h) and RD (i) in the corpus callosum are shown for baseline rats at the left and for exposed rats at the right. Mean values in each population are reported in blue for baseline and in orange for exposed animals. In the lower line, the same is shown in the fornix: MD (j), AD (k) and RD (l). Asterisks represent significant difference in the ANOVA test statistic, corrected for the false discovery rate (*\(=P<.05\), **\(=P<.01\), ***\(=P<.001\)).
eFigure 6. Evolution of White Matter Damage Into Early Abstinence

Tract-based statistical analysis showing longitudinal DTI differences in the WM skeleton between alcohol-dependent at TP2h-A vs TP1h-A upon admission into the clinic for MD (a), AD (b) and RD (c), and longitudinal DTI differences between TP3h-B versus TP2h-B for MD (d), AD (e) and RD (f). The same analysis is shown in rats.
between TP1r-A and TP2r-A for MD (g), and AD (h), and between rats at TP1r-B and at TP3r-B for AD (i) and RD (j). Thick tracts are significant tracts (\( P<.05 \), obtained using the fsl tool tbss_fill), thin tract are points just below \( P \) value threshold (\( P=.05-.10 \))
eFigure 7. Correlation Between DTI Parameters and Ethanol Daily Intake

Tract-based statistical analysis of the correlation between DTI measures in AUD patients and ethanol intake at baseline for AD, corrected for age. Thick tracts are significant tracts ($P < .05$, obtained using tbss_fill), thin tracts are points just below $P$ value threshold ($P = .05$ to .10).
Signal simulations of FA, MD, AD and RD calculated for a single cylindrically symmetric restricted population (upper line, a-d) and for two orthogonal cylindrically symmetric restricted populations (middle line, e-h). Data are plotted for increasing volume fraction of the isotropic pool (x axis), as depicted in panel I for single fiber and J for crossing fibers. The curves are calculated for three different total volumes of the restricted compartment (0.2, 0.4 and 0.6, from light to dark green).
eFigure 9 Individual Alcohol Preferences for the 27 Rats Used in the Experiment

For each rat, the alcohol preference, defined as the ratio between the alcohol consumption and the total fluid consumption (alcohol + water) is reported as a function of the days elapsed since beginning of alcohol exposure.
Supplementary Tables

eTable 1. Results of the Multivariate ANOVA Analysis for Human Tractography Data

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eTable 2. Results of the Multivariate ANOVA Analysis for Rat Tractography Data

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