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Reduced limbic metabolism and fronto-cortical volume in rats vulnerable to alcohol addiction

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ABSTRACT

Alcohol abuse is associated with long-term reductions in fronto-cortical volume and limbic metabolism. However, an unanswered question in alcohol research is whether these alterations are the sole consequence of chronic alcohol use, or contain heritable contributions reflecting biological propensity toward ethanol addiction. Animal models of genetic predisposition to alcohol dependence can be used to investigate the role of inborn brain abnormalities in the aetiology of alcoholism. Here we used magnetic resonance imaging (MRI) in the Marchigian–Sardinian (msP) alcohol-preferring rats to assess the presence of inherited structural or functional brain alterations. Alcohol-naïve msP (N = 22) and control rats (N = 26) were subjected to basal cerebral blood volume (bCBV) mapping followed by voxel-based morphometry (VBM) of grey matter and tract-based spatial statistics mapping of white matter fractional anisotropy. msP rats exhibited significantly reduced bCBV, an established marker of resting brain function, in focal cortico-limbic and thalamic areas, together with reduced grey matter volume in the thalamus, ventral tegmental area, insular and cingulate cortex. No statistically significant differences in fractional anisotropy were observed between groups. These findings highlight the presence of inborn grey matter and metabolic abnormalities in alcohol-naïve msP rats, the localization and sign of which are remarkably similar to those mapped in abstinent alcoholics and subjects at high risk for alcohol dependence. Collectively, these results point for a significant role of heritable neurofunctional brain alterations in biological propensity toward ethanol addiction, and support the translational use of advanced imaging methods to describe the circuital determinants of vulnerability to drug addiction.

Introduction

Alcoholism is a chronic relapsing disorder with substantial heritability (Disney et al., 1999; Slutske et al., 1999). Brain imaging techniques have been extensively used to investigate morphological, metabolic and functional changes associated with alcohol abuse in humans. Morpho-anatomical studies have revealed reduced grey matter (GM) volume in alcoholic patients, with the frontal lobes showing the most pronounced abnormalities (Buhler and Mann, 2011; Demirakca et al., 2011; Fein et al., 2002; Rando et al., 2011). Reduced grey matter volumes have also been reported in limbic areas and in the cerebellum of alcoholic patients (Fein et al., 2006; Makris et al., 2008). Such alterations have been recently demonstrated to be predictive of relapse risk, suggesting a significant role for grey matter shrinkage in clinical outcomes in alcoholism (Rando et al., 2011). White matter abnormalities as well as numerous functional and neuro-metabolic deficits (reviewed by Buhler and Mann, 2011) have also been reported in heavy consumers of alcohol (Gazdzinski et al., 2010; Mechtcheriakov et al., 2007; Pfefferbaum et al., 1995). However, an unanswered question in alcohol research is whether these alterations are the sole consequence of chronic alcohol use, or also represent an innate factor contributing to biological propensity toward ethanol addiction.

Recent neuorimaging studies have begun to address this question. Individuals at high-risk for alcohol dependence have been shown to have altered sensitivity of the reward circuitry (Acheson et al., 2009; Andrews et al., 2011; Kareken et al., 2010; Tapert et al., 2003), and reductions in cortical and thalamic grey matter volumes (Benegal et al., 2007), two features commonly observed in abstinent alcoholic patients. Importantly, the presence of shared fronto-striatal abnormalities has also recently reported in drug-naïve siblings of psychostimulant drug abusers (Ersche et al., 2012). These preliminary findings highlight a putative role for inborn morpho-functional brain abnormalities in the aetiology of drug-dependence. However, the specific substrates underlying biological propensity to alcohol addiction remain to be elucidated.

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Animal models of genetic predisposition to alcohol dependence can help investigate the role of heritable brain abnormalities to the aetiology of alcoholism. The Marchigian–Sardinian alcohol-preferring (msP) rat is an established selection-based model for the investigation of the neurobiology of alcoholism (Ciccocioppo et al., 2006) closely mimicking several fundamental aspects of human disease such as the occurrence of binge-like ethanol drinking (Ciccocioppo et al., 2006), psychological withdrawal symptoms (Ciccocioppo et al., 1999), escalating alcohol intake upon abstinence and high-vulnerability to stress-mediated relapse (Hansson et al., 2006). Importantly, the model also reproduces important co-morbid states pervasively associated with alcoholism, such as increased sensitivity to stress, anxious phenotype and depressive-like symptoms (Ciccocioppo et al., 2006; Hansson et al., 2006). We reasoned that mapping morpho-functional parameters in alcohol-naïve msP rats vs. control animals could allow us to pin-point heritable brain alterations underlying vulnerability to alcoholism, which can be used to inform and guide clinical research in alcoholic patients. To this purpose, we used in vivo volumetric Magnetic Resonance Imaging (MRI) to investigate the presence of regional differences in grey matter volume using an automated voxel-based morphometry approach (VBM, Ashburner and Friston, 2000). We also acquired diffusion tensor imaging (DTI) data and used Tract-Based Spatial Statistical (TBSS) mapping of the fractional anisotropy (FA) to investigate the presence of inter-group white matter microstructure alterations (Smith et al., 2006). Finally, we assessed resting-state brain function in msP and control rats by mapping basal cerebral blood volume (bCBV), an established indicator of brain metabolism (Gaisler-Salomon et al., 2009; Gonzalez et al., 1995; Gozzi et al., 2011; Small et al., 2004).

Materials and methods

Experimental subjects

msP rats (University of Camerino, N = 22) were compared with outbred Wistar rats (Charles River, Feld, Germany, N = 26), from which the msP line was derived (Ciccocioppo et al., 2006). Subjects (350–450 g at the time of the experiments) were housed on a reverse 12-hour light–dark cycle (lights off at 09:00 h), at 20–22 °C and 45–55% humidity, with restricted access to food pellets and unlimited access to tap water. All procedures followed the EU Directive for Care and Use of Laboratory Animals. No animals were exposed to alcohol during gestation, or at any point during development.

Magnetic resonance imaging

Animal preparation and MRI acquisition parameters have been recently described in great detail (Ferrari et al., 2012; Gozzi et al., 2011, 2012). Briefly, rats were anaesthetised with 3% halothane, tracheotomised and artificially ventilated with a mechanical respirator. After surgery halothane level was set to 0.8%. Arterial blood gases (p_aCO_2 and p_aO_2) were measured prior to and after bCBV measurement and ventilation parameters were adjusted to keep gas levels within physiological range (Ferrari et al., 2012) (Inline Supplementary Table S1).

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2012.12.015.

Body temperature was maintained within physiological range (37 ± 1 °C) by using a water heating system. Mean arterial blood pressure (MABP) was monitored continually through a transducer placed in the femoral artery (Fig. S1). DTI, anatomical and bCBV-weighted images were acquired on a Bruker Biospec 4.7 Tesla scanner. DTI images were acquired using a single-gradient-echo EPI sequence (TR = 3000 ms; TE_{eff} = 35 ms, FOV 40 mm, 128 × 128 matrix, 20 × 1 mm slices). Six 3D data sets with diffusion weighting (Δ = 20 ms, δ = 4 ms, b value 1000 s/mm^2) in six uniformly distributed directions and one data set without diffusion weighting were obtained. T_2-weighted anatomical volumes were acquired using a RARE sequence (TE_{eff} = 72 ms, RARE factor 8, FOV 40 mm, 256 × 256matrix, 20 × 1 mm slices) followed by a time series acquisition (TR = 2700 ms, TE_{eff} = 111 ms) with same spatial coverage but lower in-plane resolution (128 × 128) as recently described (Gozzi et al., 2011). Following five reference images, 1.5 ml/kg of the contrast agent Molday Ion (BioPal, Worcester, USA) was injected to make the MRI signal changes sensitive to bCBV (Mandeville et al., 1998; Schwarz et al., 2003).

Data analysis

Basal CBV (bCBV)

The procedure used to calculate bCBV has been recently described in greater detail (Gozzi et al., 2011). Briefly, bCBV time series were spatially normalized to a stereotaxic rat brain MRI template set (Schwarz et al., 2006) and signal intensity was converted into basal cerebral blood volume (bCBV(t)) on a pixel-wise basis (Gozzi et al., 2011; Mandeville et al., 1998). bCBV time-series were calculated over a 5 minute time-window starting 10 min after contrast agent injection. Mean bCBV volumes for individual subjects were created by averaging the time-series time-wise. The images were smoothed with an isotropic Gaussian kernel with a sigma of 0.7 mm. Voxel-wise group statistics was carried out using FSL using multi-level Bayesian inference and a Z threshold > 2.3 and a corrected cluster significance threshold of p = 0.01 (Smith et al., 2004). Volume of interest (VOI) mean bCBV values were extracted from the MRI template set as previously described (Gozzi et al., 2008). A detailed description of the location of the anatomical regions used for VOIS statistics can be found in Schwarz et al. (2006). Statistical analysis of VOI-based bCBV was performed using a one-way ANOVA test followed by Fisher's test for multiple comparisons.

Voxel-based morphometry (VBM)

Automated VBM analysis was performed using FSL (Smith et al., 2004). Briefly, bias-field corrected brain images were used to remove extra-cranial tissue using FSL's BET. The resulting images were then segmented into GM, WM, and cerebrospinal fluid (CSF) using FSL's FAST4 (Smith et al., 2004). Given the lack of a priori information on the probability distribution of different tissue classes in the rat brain, the images were segmented using an intensity based tissue classification kernel. The kernel was set to classify brain tissue into 6 compartments for a more refined classification of T2-weighted images of the rodent brain as recently described (Li et al., 2009) (3 GM classes, 1 CSF class, 1 WM class, 1 extra-brain CSF and residual tissue class). The 3 GM compartments captured independent portions of grey matter and were added to constitute the final GM class used for statistical analysis. An initial reference GM template was created by affine registration (Jenkinson et al., 2002) of each GM volume to a representative GM image. The linear template thus obtained was then used as reference for two additional rounds of linear and non-linear registrations of all subjects' individual GM volumes to the linear template using FSL's FLIRT and FNIRT algorithms, respectively (Schnabel et al., 2003). The resulting images were averaged to create a reference GM template, to which a group-balanced number (N = 22) of randomly chosen native grey matter images were then non-linearly re-registered to create the final GM study-template. The registered partial volume GM images were then modulated (to correct for local expansion or contraction) by dividing by the Jacobian of the warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with a sigma of 0.7 mm. Finally, voxel-wise GLM was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space (Nichols and Holmes, 2002). The null distribution for the data in the VBM statistics was built over 5000 permutations. All results were thresholded at Z level of 3.1. The results at this threshold closely reproduce the voxel distribution obtained using threshold-free cluster correction at a significance level of p = 0.01.