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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 gray 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 gray 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 gray 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 circuitual determinants of vulnerability to drug addiction.

Keywords

alcohol; VBM; fMRI; gray matter; addiction; metabolism

1.1 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 of morphological, metabolic and functional changes associated with alcohol abuse in humans. Morpho-anatomical studies have revealed reduced gray 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 gray 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 gray 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.

Two recent neuroimaging 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 SF, 2003), and reductions in cortical and thalamic gray 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 psycho-stimulant 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.

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 to 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).

1.2 Materials and Methods

1.2.1. 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 hours), 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 animal was exposed to alcohol during gestation, or at any point during development.

1.2. 2 Magnetic resonance Imaging

Animal preparation and MRI acquisition parameters have been recently described in great detail (Ferrari et al., 2012; Gozzi et al., 2011; Gozzi et al., 2012). Briefly, rats were anaesthetized with 3% halothane, tracheotomised and artificially ventilated with a mechanical respirator. After surgery halothane level was set to 0.8%. Arterial blood gases ($p_a\text{CO}_2$ and $p_a\text{O}_2$) were measured prior to and after bCBV measurement and ventilation parameters were adjusted to keep gas levels within physiological range (Ferrari et al., 2012).

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 (Figure S1). DTI, anatomical and bCBV timeseries were acquired on a Bruker Biospec 4.7 Tesla scanner. DTI images were acquired using a single- gradient-echo EPI sequence (TR=3000 ms; $\text{TE}_{\text{eff}} = 35$ ms, FOV 40mm, 128×128 matrix, 20×1 mm slices). Six 3D data sets with diffusion weighting ($\delta = 20$ ms, $\delta = 4$ ms, b value $1,000 \text{ s/mm}^2$) in six uniformly distributed directions and one data set without diffusion weighting were obtained. T₂-weighted anatomical volumes were acquired using a RARE sequence ($\text{TE}_{\text{eff}} = 72$ ms, RARE factor 8, FOV 40mm, 256×256 matrix, 20×1 mm slices) followed by a time series acquisition (TR = 2700 ms, $\text{TE}_{\text{eff}} = 111$ 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) were injected to make the MRI signal changes sensitive to bCBV (Mandeville et al., 1998; Schwarz et al., 2003).

1.2.3 Data analysis

1.2.3.1 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 ($\text{bCBV}(t)$) 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.

1.2.3.2 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, 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 gray 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, voxelwise 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 5,000 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 $p=0.01$.

1.2.3.3 Tract-based spatial statistics of white matter fractional anisotropy—Inter-group voxelwise statistical analysis of the DTI data was carried out using TBSS (Tract-Based Spatial Statistics, (Smith et al., 2006), part of FSL (Smith et al., 2004). First, fractional anisotropy (FA) images were created by fitting a tensor model to the raw diffusion data. A common FA space including all the control subjects of the study was created by

linearly-aligning all subjects' FA data to a representative subject. A linearly-registered FA reference-template was created by affine registration (Jenkinson et al., 2002) of each FA volume to a reference FA chosen from the study. The template was then used as reference for two rounds of linear and non-linear registrations of individual FA images using FLIRT (affine) and FNIRT (non-linear) algorithms, respectively. The resulting images were averaged to create a reference FA template, to which all native control (wistar) FA images were then non-linearly re-registered to create the final FA study-template. The template was thinned at a threshold level of 0.3 to create a mean FA skeleton which represents the centres of all tracts common to the groups (Figure S2). Each subject's aligned FA data was then projected onto this skeleton and the resulting data fed into voxelwise cross-subject statistic as using a nonparametric permutation test (Nichols and Holmes, 2002). The null distribution for the data in the TBSS statistics was built over 5,000 permutations. Data were thresholded at a Z level of 3.1 and a corrected cluster-based thresholding of 0.01.

1.3 Results

1.3.1 msP rats display reduced resting brain function in limbic areas

In order to investigate the presence of inborn alterations on resting brain function in msP rats, we measured bCBV and mapped the regions exhibiting statistically significant differences with respect to control subjects. msP rats highlighted significantly reduced bCBV in several brain areas compared to controls (Figures 1 and 2). Prominent effects were observed in the striatum, cingulate cortex, thalamus, ventral hippocampus, hypothalamus, and corpus callosum. Foci of reduced bCBV were present in key components of the extended amygdala (Koob, 2003), such the central nucleus of the amygdala, bed nucleus of the stria terminalis and shell of the nucleus accumbens. Additional areas of reduced bCBV were observed in the orbito-frontal cortex, median raphe, superior colliculi and locus coeruleus. A trend for a lower total CBV in msP rats vs. control was found, although the effect did not reach statistical significance (Figure S3; $p=0.06$, student's t test).

1.3.2 msP rats display reduced fronto-cortical gray matter volume

The presence of inborn alterations in gray matter volume of msP rats was investigated via a VBM-approach. The analysis showed focal and robust reductions in gray matter volume in fronto cortical areas, with a predominant involvement of cingulate and insular cortex (Figures 3 and 4). The effect extended rostrally across the posterior cingulate (retrosplenial cortex) and dorso-lateral thalamic nuclei. Reduced GM volume was also observed in the lateral portions of the ventral tegmental area and in the substantia nigra. Foci of *increased* gray matter were observed in the visual cortex and dorsal hippocampus (Figure S4). Correlation measurements between bCBV and GM volume were performed in the regions exhibiting the largest VBM alterations, namely, the insular, cingulate and retrosplenial cortex, and the dorsolateral thalamus (Figure S5). No significant correlation between the two parameters was observed in any of the regions examined. The presence of micro-structural white matter alterations was investigated by comparing the mean FA within a tract-based skeleton. The analysis did not highlight any statistical differences in FA between msP and control Wistar rats ($Z>3.1$).

1.4 Discussion

Human neuroimaging studies in alcoholism have demonstrated the presence of long-lasting alterations in brain structure and function (Fein et al., 2009; Fein et al., 2006; Sameti et al., 2011; Volkow et al., 1994). Since brain structure is to a large extent inherited (Wright et al., 2002), and alcoholism is a familial disease (Disney et al., 1999; Slutske et al., 1999), these findings raise the question of whether such abnormalities could precede alcohol exposure, and represent vulnerability factor in alcohol-dependence. The present study addresses this question from a preclinical perspective. We used a multi-parametric MRI protocol to investigate the presence of inborn brain abnormalities in msP rats, an established model of genetic predisposition to alcohol addiction (Ciccocioppo et al., 2006), and documented that alcohol-naïve msP rats have discrete morpho-functional alterations in fronto-cortical and limbic areas.

The GM abnormalities observed in msP rats reproduce very closely some of the most replicated findings in neuroimaging studies of alcoholic patients. Decreased gray matter volume in fronto-parietal areas, with a predominant involvement of the anterior cingulate and insular cortex, have been consistently reported by several authors both in active heavy drinking and abstinent (6 months to 21 years) alcoholics (Cardenas et al., 2005; Demirakca et al., 2011; Fein et al., 2009; Jernigan et al., 1991; Makris et al., 2008; Mechtcheriakov et al., 2007; Rando et al., 2011). The effect mapped in the present study extends rostrally to include the posterior cingulate (retrosplenial) cortex, and the dorsolateral thalamus, to identify a neuro-anatomically coherent network of areas that has been shown to be altered also in alcoholic patients (Makris et al., 2008; Mechtcheriakov et al., 2007; Rando et al., 2011). We also observed reduced volumes in areas of the mesencephalon, including the lateral portions of the ventral tegmental area. This finding is consistent with recent reports showing decreased volume of the brain reward system in alcoholism (Makris et al., 2008), although no alterations in frontal terminal of the mesolimbic dopamine system, or the amygdala were observed in msP rats. Analogously, the pattern of reduced bCBV observed in our study can be correlated with previous reports of reduced resting-state metabolism in frontal-parietal, orbitofrontal cortex and striatal areas in active and abstinent alcohol-abusers (Gilman et al., 1990; Volkow et al., 1992; Volkow et al., 1994; Volkow et al., 1997; Wang et al., 2000). Interestingly, we also observed hypo-metabolism in areas whose functional reactivity has been shown to be altered in task- or cue-based fMRI activation studies in alcoholism, such as the striatum and nucleus accumbens (Kareken et al., 2004; Vollstädt-Klein et al., 2010) and thalamo-cortical circuits (George et al., 2001). Collectively, these findings show that predisposition to alcohol dependence in msP rats is associated to brain abnormalities reminiscent of those observed in alcoholic-patients. As the animals imaged in this study were alcohol-naïve, our work suggests that some of the morpho-functional alterations documented in alcoholics may reflect a pre-existing endophenotype predisposing to alcohol addiction. Recent clinical data lends preliminary evidence to this hypothesis. Reduced GM volumes in fronto-cortical and thalamic areas, as well as in the cerebellum and amygdala have been recently reported in subjects at high risk for alcohol dependence (Benegal et al., 2007). Individuals at high-risk for alcohol dependence have also been shown to have altered sensitivity of the reward circuitry (Acheson et al., 2009; Andrews et al.,

2011; Kareken et al., 2010; Tapert SF, 2003). Importantly, fronto-striatal abnormalities, including reduced GM volume in the insular cortex, have been recently documented in siblings of psycho-stimulant drug abusers (Ersche et al., 2012). Together with the results of the present study, the limited clinical data available point towards a role of heritable alterations in brain metabolism and frontal gray matter as an endophenotype of vulnerability to alcohol dependence.

The detection of inherited brain alterations in subjects that have never been exposed to alcohol may seem at odds with reports of GM and WM volume recovery upon discontinuation of alcohol abuse (Cardenas et al., 2007; Demirakca et al., 2011; Ende et al., 2005; Gazdzinski et al., 2010; Pfefferbaum et al., 1995; Rando et al., 2011; Volkow et al., 1994). However, chronic ethanol intoxication is known to trigger the release of pro-inflammatory species and oxidative stress leading to neurodegenerative processes that can be partially reversed by brain cell genesis (Crews and Nixon, 2009). It is therefore plausible that the GM and WM recovery observed in alcoholics reflect the onset of regenerative process in response to the acute toxic effect of ethanol. However, long-lasting, possibly irreversible, GM and functional alterations have been documented in abstinent alcoholic patients even after years of alcohol-detoxification (6 months to 21 years) (Cardenas et al., 2007; Fein et al., 2009; Fein et al., 2006; Makris et al., 2008; Sameti et al., 2011). Our study represents an attempt to identify and differentiate the neurodegenerative effect produced by ethanol intoxication from pre-existing inheritable endophenotypes of vulnerability to alcohol-dependence. It is conceivable that both these components are present in clinical populations and contribute to the morpho-anatomical and neuropsychological impairments exhibited by alcoholic patients. The present results, in agreement with recent clinical reports (Andrews et al., 2011; Benegal et al., 2007; Ersche et al., 2012) suggests that inherited endophenotypes may contribute to determining the brain abnormalities observed in alcoholics. Within this framework, it should be noted that no major WM alterations were found in msP rats, whilst reversible WM damage is often observed in alcohol-dependent patients (Gazdzinski et al., 2010; Harris et al., 2008; Pfefferbaum et al., 1995). Although the msP rat is unlikely to closely reproduce all the clinical facets of alcoholism, it is tempting to speculate that the WM abnormalities observed in patients do not represent a major etiological factor for ethanol addiction, but may simply reflect alcohol-induced neurodegeneration. Further clinical research is warranted to clarify this point.

Epidemiological studies and analysis of patient samples have established the pervasiveness of co-occurring psychiatric diagnoses in alcoholism, with a particular prevalence of mood, anxiety and externalising disorders (Brady and Sinha, 2005; Grant et al., 2004; Kessler et al., 1997). Such a prevalent co-occurrence of psychiatric states seems to be well-modelled by the msP rat, as this strain exhibits pronounced sensitivity to stress, anxious phenotype and depressive-like symptoms (Ciccocioppo et al., 2006; Hansson et al., 2006). Consequently, the morpho-functional abnormalities described in the present study may contain significant contributions from co-morbid states, particularly those underlying maladaptive responses to stress. In this respect, it is interesting to note that reduced GM volume in the rostro-dorsal cingulate reminiscent of those seen in msP rats have been recently documented patients suffering from depression and anxiety disorders (Spampinato et al., 2009; van Tol et al., 2010). The development of refined rodent model mimicking alcohol vulnerability without

the presence of associated co-morbid traits may help segregate morpho-functional characters typical of alcoholism, from those underlying co-morbid psychiatric states.

Several of the regions affected by the GM and bCBV changes in msP have been shown to be implicated in drug addiction (Koob and Volkow, 2009). Neurobiological models of addiction suggest that substance taking may be related to reward deficiency (Blum et al., 2000), impaired response inhibition and salience attribution (Goldstein and Volkow, 2011). The presence of reduced GM volumes in the VTA and reduced basal activity in the extended amygdala represent a plausible substrate for the increased stress reactivity exhibited by msP rats and their innate propensity to develop drug addiction. The extended amygdala and its interactions with certain limbic structures, many of which appeared to be dysregulated impaired in this work (e.g. cingulate, hippocampus, hypothalamus, septal area and thalamus) appears to promote or inhibit motivated drives and is therefore critical in drug addiction and reinstatement of drug self-administration (Koob and Le Moal, 2001). Moreover, the presence of reduced metabolism and GM in cingulate areas is also of great interest because impaired function of these areas represents a clinical endophenotype for impulsive and disinhibitory behaviour (Goldstein and Volkow, 2011). Collectively, the morpho-functional alterations identified in the present work serve as plausible neurobiological substrate for the behavioural expression of vulnerability to alcohol abuse exhibited by msP rats, and further corroborate the construct- validity of this model to study the aetiology of alcoholism.

1.4.1 Methodological Considerations and Limitations of the Study

A few cautionary statements should be made about this work. Firstly, we point out that the main objective of this work was not aimed at establishing a direct relationship between brain abnormalities and drinking behaviour, but rather to assess what are the inherited brain alterations that may predispose to excessive alcohol intake. This is why we focused our analysis on naïve high alcohol drinking rats (msP) vs. heterogeneous Wistars (which is the background strain of msPs). In this respect our study represents an attempt to reproduce human studies where drug-naïve siblings of alcoholic patients have been shown to present brain abnormalities that are considered to be conducive to excessive alcohol intake (Benegal et al., 2007; Venkatasubramanian et al., 2007), or psychostimulant drug abuse (Ersche et al., 2012). The comparison of msP with outbred Wistar rats may have some limitations for determining the extent to which morpho-anatomical traits of msP rats are indicative of vulnerability to ethanol dependence, because an alcohol-avoiding line that is a direct counterpart to the msP line is not available. However the choice of an alcohol-avoiding control phenotype would also reflect segregation of features conferring alcohol avoidance rather than reproducing morpho-anatomical features representative of a general control population. In this regard, the good correspondence between our results and the clinical findings (discussed above) supports the choice of heterogeneous Wistars as control population, and the specificity of our imaging results in relation to the high propensity to alcohol addiction exhibited by msP subjects. The specificity of our findings is also indirectly corroborated by the results of recent neuroimaging studies where different inbred rat strains (not showing alcohol preference) were compared. For example, Prinssen and colleagues (Prinssen et al., 2012) reported increased perfusion in the cingulate cortex and hippocampus and decreased perfusion in the thalamus of F344 rats compared to control Sprague-Dawley

rats, a pattern of alterations that is substantially different from the one seen in the present study. Similarly, Sawiak and colleagues (Sawiak et al., 2012) used VBM to test morphometric differences in the brain of high- and low-impulsivity rats (e.g. animals characterized by different propensity to drug addiction (Belin et al., 2008)), and reported reduced GM volume in the nucleus accumbens but no alterations in any of the areas we found to exhibit reduced GM volume in msP rats (Jeff Dalley, personal communication; Sawiak et al., 2012). Collectively, these findings, together with the striking correspondence between our findings and analogous studies in humans, strongly corroborate the specificity of our findings in relation to the innate propensity to alcohol addiction shown by msP rats. In this article we used bCBV as an index of basal metabolism. The presence of a tight correlation between bCBV and neuronal metabolism has been previously demonstrated by measuring intra-subject correlations between bCBV and deoxy-glucose uptake in clinical cohorts of subjects (Gonzalez et al., 1995; LEENDERS et al., 1990). An indirect confirmation of this has been recently obtained in our lab, where we observed closed spatial correspondence between the distribution of bCBV in halothane-anaesthetized rats, and the expression profile of cytochrome oxidase subunit 1 mRNA, an established marker of neuronal metabolism (Meissner et al., 2007). As msP subjects do not present any histopathological alterations (Dr. P. Cristofori, unpublished results) it is reasonable to assume that a linear coupling between bCBV and basal metabolism would be conserved also in msP rats.

Because bCBV measurements were performed in anesthetized rats, contaminating contributions from the use of general anaesthesia cannot be ruled out. Experiment in freely moving animals, for example using the 2-deoxy-glucose technique may be used to overcome the limitations related to the use of anaesthesia, although methods in freely-moving animals do not permit to clearly differentiate resting metabolic states from activity evoked by behavioural responses. It should also be emphasized that the same imaging and anaesthetic protocol used here have been recently applied to map basal alterations produced by chronic cocaine self administration in rats, and produced a pattern of focal metabolic alterations very consistent with analogous clinical readouts in cocaine addicts (Gozzi et al., 2011) thus corroborating the translational validity of the approach. Additional examples of the use of the protocol for translational studies in other areas of psychiatric research have been reported by other groups (Gaisler-Salomon et al., 2009; Small et al., 2004). As msP rats present genetically-determined alteration in several genes involved in ethanol metabolism (reviewed by Ciccocioppo et al., 2006), group differences in the metabolic rate of the anaesthetic could play a role in the bCBV alterations observed. This hypothesis is however unlikely for a number of reasons. Firstly, we imaged alcohol naïve subject, which permits ruling out any direct interfering action of alcohol metabolism with the anaesthetic used. Secondly, animal studies show that halothane does not necessarily exert significant interactions with the central mediators of alcohol effects (Baker et al., 1980; Baker and Deitrich, 21995). Moreover, inter-strain differences in sensitivity to anaesthesia would be expected to produce generalized cortical alterations (Alkire et al., 1999; Heinke and Schwarzbauer, 2002) as well as differences in functional cardiovascular parameters such as blood pressure (Gelman et al., 1984; Steffey et al., 2003), two findings in contrast with the exquisitely focal bCBV effect observed in msP rats, and the observation of comparable

blood pressure between the two groups of animals. Furthermore, intensity normalized bCBV maps exhibited a virtually unaltered pattern of distribution of the signal changes (Figure S6), thus ruling out unspecific global contribution attributable for example to variability in contrast agent dosing or anaesthesia levels.

A slight increase (~ 6 mmHg) in arterial $p\text{CO}_2$ was observed in the control group but not in msP subjects. This is unlikely to have played a contribution in the bCBV changes mapped because it has been demonstrated that vasodilatory effects of CO_2 produce global rCBV increases that are particularly prominent in cortical areas (Lu et al., 2009), whilst the bCBV effect observed in msP rats was more focal, and did not show major cortical involvement, with the exception of the cingulate cortex. Consistent with this, we did not observe significant correlation between bCBV (measured in the cingulate cortex) and individual $p\text{CO}_2$ levels when expressed as absolute values, or pre-post difference ($p>0.18$).

Finally, the lack of significant WM FA changes between groups could reflect poor sensitivity of the method consequent to the use of large anisotropic voxels, and a limited number of diffusion-encoding directions. Although similar imaging parameters have been successfully employed in rats to identify FA and WM changes induced by psychostimulant drugs (Narayana et al., 2009), additional studies using higher resolution and more isotropic voxels are warranted to provide a definite answer to this experimental question.

Conclusions

In conclusion, we have documented focal gray matter and metabolic abnormalities in alcohol-naïve msP rats, an established model of genetic predisposition to alcoholism, the localization and sign of which closely reproduce recent neuroimaging findings in 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 circuitual determinants of vulnerability to drug addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Orthogonal view (A: horizontal, B and C: coronal, D: sagittal) of the brain regions exhibiting reduced bCBV in msP rats (N=22) versus Wistar control subjects (N=26, $Z > 2.3$, cluster correction $p = .001$). [AccS, nucleus accumbens shell; CC, corpus callosum; Cg, cingulate cortex; CPu, caudate-putamen; dITh, dorso-lateral Thalamus; Hyp, Hypothalamus; Sc, superior collicoli; VHc; ventral hippocampus; vmTh, ventro-medial Thalamus]

Figure 2.

Mean bCBV ($\pm$ SEM) in representative 3D anatomical volumes (Gozzi et al., 2007) for mSP (N=22) versus Wistar control subjects (CTR; N=26) [Acc, nucleus accumbens; Amy, amygdala; CC, corpus callosum; Cg, cingulate cortex; CPu, caudate-putamen; ExtAmy, extended amygdala; Hipp, hippocampus; Hypoth, hypothalamus; Ins, insular cortex; OFC, orbito-frontal cortex; mPFC, medial prefrontal cortex; Rs, retrosplenial cortex; Sp, septum; SS, somatosensory cortex; Thal, thalamus; VHc, ventral hippocampus, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, one-way ANOVA, followed by Fisher LSD test]

Figure 3.

Map of the regions exhibiting reduce gray matter volume in msP (N=22) versus Wistar control subjects (N=26; $Z > 3.1$). [Cg, cingulate cortex; dlTh, dorso-lateral Thalmus; Ins, insular cortex; Rs, retrosplenial cortex; VTS, ventral tegmental area]

Figure 4.

Mean relative GM volume (±SEM) in representative 3D anatomical volumes (Gozzi et al., 2007) for msP (N=22) versus Wistar control subjects (CTR; N=26) [Acc, nucleus accumbens; Amy, amygdala; CC, corpus callosum; Cg, cingulate cortex; CPu, caudate-putamen; ExtAmy, extended amygdala; Hipp, hippocampus; Hypoth, hypothalamus; Ins, insular cortex; OFC, orbito-frontal cortex; mPFC, medial prefrontal cortex; Rs, retrosplenial cortex; Sp, septum; SS, somatosensory cortex; Thal, thalamus; VHc, ventral hippocampus, **p<0.01; one-way ANOVA, followed by Fisher LSD test]