doi:10.1111/j.1369-1600.2006.00043.x

Gray matter volume abnormalities and externalizing symptoms in subjects at high risk for alcohol dependence

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ABSTRACT

Reduced right amygdala volumes have been reported in young, alcohol-naïve subjects at high risk (HR) for alcohol dependence. The differences in brain morphometry have been associated with an excess of externalizing behaviors in these subjects. This may reflect a neurobiological vulnerability to alcohol dependence. Existing Magnetic Resonance Imaging (MRI) studies on these subjects have examined only a few, pre-selected brain regions using the manual regions of interest (ROI) approach. MRI of HR subjects ($n = 20$) and age, sex, and handedness-matched low-risk (LR) subjects ($n = 21$) were analyzed using optimized voxel-based morphometry and ROI approach. The externalizing symptoms of these subjects and their fathers were measured using the Semi-Structured Assessment for the Genetics of Alcoholism. HR subjects had significantly smaller volumes of superior frontal, cingulate and parahippocampal gyri, amygdala, thalamus and cerebellum. These gray matter volumes correlated negatively with externalizing symptoms scores. Subjects at HR for alcoholism have reduced volumes of critical areas of brain gray matter, which are associated with increased externalizing symptoms. These represent key endophenotypes of alcoholism.

Keywords Alcoholism, brain volume, externalizing symptoms, region of interest, voxel-based morphometry, vulnerability.

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INTRODUCTION

Alcohol dependence is a common, etiologically complex disorder with genetic underpinnings. The disorder aggregates in families and the morbid risk to relatives of alcoholics is significantly higher than the risk to individuals in the general population. Evidence from twin and adoption studies has highlighted the significance of genetic influences and the heritability of alcoholism has been estimated at 40–60% (Enoch & Goldman 1999). The highest risk for developing alcoholism exists for individuals who start using alcohol as adolescents (Pitkanen, Lyyra & Pulkkinen 2005), have a high family loading for alcohol problems, and display a cluster of behavioral traits described as disinhibited, under-controlled, or impulsive, which are usually evident in childhood and persist into adulthood (Haber, Jacob & Heath 2005).

Subjects at high risk (HR) for alcoholism have been found to differ from low-risk individuals (LR), on a range of neurobiological markers, which include measures of brain electrical activity hypothesized to reflect an inherited state of central nervous system (CNS) hyperexcitability, such as increased beta power in the resting electroencephalogram and reduced amplitude of the P300 component of the event-related potential (Porjesz *et al.* 2005) as also increased body sway (Hill 2004). These measures of susceptibility appear more pronounced for early onset alcoholism rather than for the late onset form (Benegal *et al.* 1995). Preliminary evidence also shows that HR subjects demonstrate a delay in reaching age-appropriate P300 amplitude (Hill *et al.* 1999), and fail to show age-related improvements in sway at the same rate as control children (Hill *et al.* 2000). This has been interpreted as evidence that HR subjects may have subtle neurodevelopmental delay/deficit in certain brain areas that change rapidly during adolescence.

Hill *et al.* (2001) using magnetic resonance imaging (MRI) to study brain morphology in alcohol-naïve HR adolescent and young adult offspring from multiplex alcoholism families reported that HR subjects showed

reduced right amygdala volume compared with control subjects. They concluded that the volume of the right amygdala is smaller in individuals who carry an increased susceptibility for developing alcohol dependence, and appears to be present before drinking begins. De Bellis *et al.* (2000, 2005) reported smaller prefrontal lobe and hippocampus in alcohol-dependent adolescents, in comparison with age- and gender-matched controls. While they interpreted these differences as being the result of excessive use of alcohol, they acknowledged that these structural differences might have been present before the onset of drinking.

These MRI studies have utilized the measurement of manually delineated, anatomically defined regions of interest (ROI) within the brain. The ROI method has the strength of anatomical validity. However, the time-consuming nature of manual ROI drawings, limited to a priori defined regions and the rigorous training needed to ensure rater reliability, do not easily allow for comparison of many brain regions or large subject groups (Kubicki *et al.* 2002). Recently, investigators have employed voxel-based morphometry (VBM; Ashburner & Friston 2000), a fully automated whole-brain measurement technique, to examine structural magnetic resonance images of the brain. By surveying the whole brain, VBM provides a non-biased measure of highly localized regions that may not be investigated in hypothesis-based studies that employ more labor-intensive ROI measurement techniques. The VBM methodology has been updated and optimized (Good *et al.* 2001) to reduce errors due to systematic differences in head shape, variations in segmentation, inconsistent brain stripping, and errors introduced by spatial normalization. A recent study comparing these two methods has suggested that both the ROI and VBM analyses be used in tandem as they provide different types of information (Giuliani *et al.* 2005).

The current study was planned to investigate the differences in externalizing behaviors and brain gray matter volume between subjects at HR for developing alcoholism (child, adolescent and young adult offspring from multigenerational alcohol dependent families) and age-, gender-matched LR controls, without history of alcohol dependence in first or second degree relatives. We used both the optimized VBM and the ROI-based approach for MRI volumetric analyses. In order to explore the possibility of differences, between HR and LR subjects, in the developmental trajectories of certain brain areas that are known to be changing rapidly during adolescence, subjects and controls were selected to cover the developmental life-span between 10 and 24 years of age. We hypothesized that HR subjects would have: (1) gray matter volume deficits (with the prefrontal cortices, hippocampus and amygdala being the ROI); and (2) greater externalizing behaviors than LR controls.

MATERIALS AND METHODS

Subjects

Twenty alcohol-naïve HR male subjects were ascertained from among offspring of male treatment seeking alcohol-dependent patients. 'High risk' was defined as: offspring of early onset alcohol-dependent individuals (having developed dependence before 25 years of age) with two or more dependent first-degree relatives. Using a convenient sampling method, subjects were chosen to cover the developmental life-span between 8 and 24 years of age, with not more than three subjects of the same age. Twenty-one LR male subjects were recruited from among offspring of volunteers and hospital employees, selected for the absence of Diagnostic and Statistical Manual of Mental Disorders—Fourth Edition (DSM-IV) Axis I psychopathology (American Psychiatric Association 1994). Written informed consent was obtained from the parents of minor subjects and controls and from all adults for undergoing assessments as indicated. The institutional review board for research ethics approved the study protocol.

Clinical assessment of parents

Both parents of the HR and LR subjects were assessed using the Semi-Structured Assessment For The Genetics Of Alcoholism—II; Adult version (SSAGA-II-Adult; Bucholz *et al.* 1994) to confirm the diagnosis of DSM-IV Alcohol dependence (American Psychiatric Association 1994) in the male parent and to rule out any other lifetime psychiatric disorder; to rule out the presence of a lifetime psychiatric diagnosis (including alcohol dependence syndrome) in the both parents of LR and the female parent of HR subjects. Tobacco use among the parents was not controlled for, as smoking in men is rather ubiquitous in the Indian population. Moreover, alcoholism and nicotine addiction are thought to be both comorbid and cross-transmitted (Swan, Carmelli & Cardon 1997).

The SSAGA-II items pertaining to attention-deficit/hyperactivity disorder (ADHD) and antisocial personality disorder were summated to devise an externalizing symptom score for the fathers of all subjects. Information on the presence of family history of alcohol dependence, or major psychiatric illness in first-degree relatives of the parents, was gathered using the Family Interview For Genetic Studies (FigS) (Maxwell 1992) from three or more adult informants in the family.

Clinical assessment of subjects

All subjects were assessed on the SSAGA-II, child, adolescent or adult versions as indicated to specifically assess externalizing symptoms (attention deficit, hyperactivity, conduct and oppositional defiant symptoms) and to rule out any other syndromal psychiatric diagnoses

(psychoses, anxiety disorder, affective disorder). The items pertaining to inattention, hyperactivity, impulsivity and conduct symptoms were added to calculate a total externalizing symptoms score. A detailed physical examination, which included a neurological evaluation, was conducted to clinically rule out Huntington's chorea/Wilson's disease, seizure disorders, mental retardation, cerebral palsy. None of the subjects had a lifetime history of significant head injury or contraindication to MRI procedures. The Annett Handedness Questionnaire (Annett 1967) was used to include only right-handed subjects.

MRI scanning protocol

MRI was done with 1.5 T scanner (Magnetom 'Vision', Siemens, Erhlangen, Germany). T1 weighted three-dimensional Magnetization Prepared Rapid Acquisition Gradient Echo sequence was performed (repetition time = 9.7 ms; echo time = 4 ms; nutation angle = 12 degrees; field of view = 250 mm; slice thickness = 1 mm; number of excitation = 1; matrix = 200×256) yielding 160 sagittal slices.

Image processing

The optimized VBM protocol was implemented through Statistical Parametric Mapping 2 (SPM2) (Wellcome Department of Imaging Neuroscience, London; http://www.fil.ion.ucl.ac.uk/spm). Pre-processing of structural data followed a number of defined stages (Good *et al.* 2001; Ananth *et al.* 2002). First, customized templates were created from the whole subject group, imaged with identical methods on the same scanner. The customized templates from the whole subject group rather than a subset was chosen in order to reduce any potential bias for spatial normalization. Each structural MRI image was normalized to the standard statistical parametric mapping T1 template; segmented into cerebrospinal fluid (CSF), gray matter and white matter compartments; then smoothed (8-mm full width at half maximum isotropic Gaussian kernel) and averaged to create gray and white matter templates in stereotactic space. This study-specific customized gray matter template was utilized for the subsequent post-processing steps.

An automated brain extraction procedure that incorporated a segmentation step was used to remove non-brain tissue in the original structural magnetic resonance images (Good *et al.* 2001). The extracted gray matter images were normalized to the customized gray matter template. Spatial normalization used a residual sum of squared differences as the matching criterion and included affine transformations and linear combination of smooth basis functions modeling global non-linear shape differences (Ashburner *et al.* 1997; Ashburner & Friston 2000). The normalization parameters were then reapplied to the original structural images to maximize optimal segmentation of fully normalized images, and these normalized images were re-sliced to a final voxel size of 1 mm^3 and segmented into gray/white matter and CSF/non-CSF partitions. After correcting for non-uniformity in image intensity, the statistical parametric mapping segmentation employs a mixture model cluster analysis to identify voxel intensities that match particular tissue types combined with a priori probabilistic knowledge of the spatial distribution of tissues. After a further automated brain extraction step, the partitioned images were modulated by the Jacobian determinants from spatial normalization to correct for volume changes introduced during the non-linear spatial transformations. The modulation step helped to analyze the regional differences in absolute volume of gray matter (Good *et al.* 2001). Finally, all normalized, segmented modulated images were smoothed with a 12-mm full width at half maximum isotropic Gaussian kernel (Ashburner & Friston 1999).

ROI measurement

The sagittal images were reconstructed into coronal section images in the magnetic resonance work station. The coronal images were transferred to a personal computer and coded. ROI analyses were performed using ImageJ (Version 1.28) (NIH, http://rsb.info.nih.gov/ij/) that provides valid and reliable measurements of specific structures using a semi-segmentation approach. Volumes of cerebral hemispheres, prefrontal gray matter, hippocampi, amygdala and caudate nuclei were measured by manual tracing in coronal MRI slices. The boundaries of these structures were defined as described earlier in MRI studies on child and adolescents (Castellanos *et al.* 2001). An investigator who was trained by an expert consultant neuroradiologist made all ROI volume measurements (with an inter-rater reliability of intraclass correlation coefficient > 0.9).

The boundaries of the individual ROI are as follows: (1) amygdala: the anterior most boundary of the amygdala was taken as the most anterior slice in which the temporal stem was first visible. The most anterior slice showing the mamillary bodies was used as the amygdala–hippocampal boundary. (2) Hippocampus: the ambient cistern defined the medial border of the anterior hippocampus. The superior border of the hippocampus was bounded anteriorly by the temporal horn and posteriorly by the fornix. Measurements of the hippocampal formation included the corna ammonis, dentate gyrus and the subiculum. (3) Caudate nucleus: the caudate nucleus was measured bilaterally on all coronal slices in which it appeared. The head, body and tail portions were included up to the point where the tail curved ventrally to border the lateral aspect of the atrium of the lateral ventricles. The anterior coursing of the tail was excluded. (4)

No.	*Brain region*	*HR subjects*	*LR subjects*	*F*[a]	*P*
1	Left amygdala	2.0 ± 0.4	2.3 ± 0.4	5.2	0.029
2	Right amygdala	2.4 ± 0.4	2.9 ± 0.6	8.3	0.007
3	Left hippocampus	3.6 ± 0.4	4.2 ± 0.6	11.8	0.001
4	Right hippocampus	3.6 ± 0.4	4.3 ± 0.6	18.0	< 0.001
5	Left caudate	5.9 ± 0.6	6.1 ± 0.8	0.05	0.8
6	Right caudate	5.9 ± 0.6	6.1 ± 0.7	0.25	0.6
7	Left prefrontal cortex	51.4 ± 7.6	51.4 ± 7.4	1.0	0.32
8	Right prefrontal cortex	53.7 ± 8.3	55.3 ± 9.9	2.0	0.053

[a]Analysis of covariance using cerebral volume as covariate. Cerebral volume (mean ± SD): HR subjects = 1452.6 ± 132.6 ml; LR subjects = 1513.6 ± 119.0 ml.

Table 1 Region of interest analyses: mean ± SD of regional brain volumes (ml) of high-risk (HR) subjects ($n = 21$) in comparison with low-risk (LR) subjects ($n = 21$).

Prefrontal cortex: the anterior boundary of the prefrontal cortex was defined as the most anterior coronal section containing gray matter (frontal pole). The most posterior coronal slice showing the genu of the corpus callosum was used to mark the posterior limit of the prefrontal cortex.

Statistical analyses

Socio-demographic, clinical and ROI data

The socio-demographic, clinical and ROI data were analyzed using the Statistical Package of Social Sciences –10. The data was tested for normality using Shapiro and Wilks W statistic before conducting the parametric analyses. The data were found to be of normal distribution ($P > 0.05$). The socio-demographic and clinical data were compared using the Independent Samples *t*-test. The ROI data were analyzed using the Analysis of Covariance with the cerebral volume as covariate.

VBM analyses

Group comparisons for regional gray matter volume differences were performed using 'single conditions and covariates' analysis within the framework of general linear model in SPM2 with gray matter volume as the confounding covariate and age as nuisance covariate. Statistical parametric maps were constructed to test for morphological differences between HR and LR subjects controls. Regional gray matter volume differences between the HR and LR subjects were assessed. Significance corrections for multiple comparisons over whole brain were done using false discovery rate (FDR) correction ($P < 0.05$) (Genovese *et al.* 2002). FDR is a new approach to the multiple comparisons problem. Instead of controlling the chance of any false positives (as in Bonferroni or random field methods), FDR controls the expected proportion of false positives among suprathreshold voxels. A FDR threshold is determined from the observed *P*-value distribution and hence is adaptive to the observations in a specific dataset. The coordinates of significant voxels were converted from Montreal Neurological Institute space to Talairach and Tournoux coordinates (Talairach & Tournoux 1988) using a nonlinear transform approach (Owen 2002).

Statistical parametric maps were examined for correlation between specific gray matter volumes and externalizing symptom scores. These specific regions of gray matter volume deficits were identified from the group comparison analysis (Table 1). This analysis examined for correlates in specific a priori regions rather than an exploratory whole brain analysis. As the analysis was examining voxels contained in the selected gray matter regions (that were chosen based on the results of regional gray matter volume deficits obtained after group comparison analysis as described above), multiple comparison error correction was also restricted to voxels in the same regions similar to the previous VBM studies (Job *et al.* 2002; Salgado-Pineda *et al.* 2003; Jayakumar *et al.* 2005). This was performed using the small volume correction (SVC) function in SPM [significance corrected for multiple comparisons using FDR ($P < 0.05$) over the voxels included in the analysis rather than the whole brain]. The voxels included in the analysis were defined by a mask created for the specific gray matter region using the Wake Forest University Pickatlas (Maldjian *et al.* 2003). The SVC analyses were done for each brain region separately as done in previous studies (Salgado-Pineda *et al.* 2003; Jayakumar *et al.* 2005).

RESULTS

Socio-demographic variables

The age of the HR (15.4 ± 4.6 years) and LR (15.7 ± 4.5 years) subjects ranged from 9 to 23 years. The fathers of the HR group all satisfied DSM-IV criteria for Alcohol Dependence and had developed dependence by 19.95 (2.54) years of age and had on average three other affected first degree relatives with alcohol dependence (minimum 3, maximum 8). They drank 17.3 (8)

standard drinks (12 g ethanol) daily or almost daily. Neither the mothers of the HR group nor both parents of the LR group satisfied criteria for a lifetime diagnosis of alcohol dependence.

All subjects in the HR and LR group were alcohol-naïve and there was no difference in tobacco use among the two groups [four subjects in the HR group and three subjects in the LR group reported any tobacco use ($\chi^2 = 0.2$, d.f. = 1, $P = 1$)].

Externalizing symptoms scores

The total externalizing symptoms score was significantly higher in the HR (18.6 ± 6.4) than the LR (1.8 ± 1.4) group ($t = 11.7$; d.f. = 39; $P < 0.001$). The paternal externalizing scores of the HR subjects (13.6 ± 6.9) was significantly higher than that of the LR (3.0 ± 1.7) subjects ($t = 6.9$; d.f. = 39; $P < 0.001$]. There was a significant positive correlation ($P = 0.57$; $P < 0.0001$) between paternal and child externalizing scores for the entire sample.

Comparison of brain volumes between HR and LR subjects

VBM analysis

Brain regions of significant gray matter volume deficits (after correcting for the confounding effect of global gray matter volume reduction and also for multiple comparisons over whole brain) in HR subjects are shown in Table 2 and Fig. 1.

ROI-based analysis

The ROI-based analysis revealed significant reductions of volume in bilateral amygdala and hippocampi in HR subjects in comparison with LR subjects. The difference in the right prefrontal lobe narrowly escaped being statistically significant. There were no significant group differences in total cerebral volume and caudate nuclei (Table 1).

Correlation between externalizing symptom scores and brain volumes

VBM analysis

Total externalizing symptom scores had a significant negatively correlation with gray matter volumes of left superior frontal gyrus, bilateral parahippocampal gyri, bilateral amygdala, right thalamus and right cerebellum (Table 3).

ROI analysis

Linear regression statistics (stepwise method), using cerebral, right and left amygdala, and right and left hippocampal volume as independent variables to negatively predict the externalizing symptom scores in the total sample of HR and LR offspring. The model incorporating right amygdala and the right hippocampus accounted for 43% ($P < 0.001$) and 38% ($P < 0.001$) of the variance in total ADHD score and the total externalizing symptom scores, respectively. The paternal externalizing symptom scores had a significant negative correlation with their offspring's right ($r = -0.504$, $P < 0.001$) and left hippocampal volumes ($r = -0.340$, $P = 0.024$).

Correlation of amygdala/hippocampus volume with age

Linear growth curve models showed a better fit for the volumes of the cerebrum, prefrontal lobes, amygdala and hippocampi, calculated for all subjects (HR and LR). The volumes of the cerebrum and the prefrontal lobes showed a downward trend with increasing age, whilst the subcortical structures such as the amygdala, hippocampi and caudate nuclei displayed an increasing trend in volume with increasing age. The differences in

Table 2 Optimized voxel-based morphometry: regional gray matter volume deficits in high-risk subjects in comparison with low-risk subjects.

No.	*Gray matter region*	*NVox*	*X*	*Y*	*Z*	*T*	*P**
1	Left superior frontal gyrus (BA 6)	47 040	−03	12	58	6.58	< 0.001
2	Left parahippocampal gyrus (BA 19)	15 449	−17	−55	−04	4.70	< 0.001
3	Left amygdala	12 362	−20	−01	−18	3.85	< 0.001
4	Right parahippocampal gyrus (BA 28)	3 102	18	−12	−20	3.78	< 0.001
5	Left cingulate gyrus (BA 24)	864	−01	−03	31	3.77	< 0.001
6	Right amygdala	3 102	18	−07	−14	3.75	< 0.001
7	Right cingulate gyrus (BA 23/24)	864	04	−11	29	3.53	< 0.001
8	Right thalamus	454	10	−30	08	3.51	< 0.001
9	Left thalamus	2 659	−02	−06	08	3.34	< 0.001
10	Right cerebellum	552	17	−43	−07	3.07	< 0.001
11	Right superior frontal gyrus (BA 6)	127	18	14	56	3.00	< 0.001

**P*-value corrected for multiple comparison with false discovery rate < 0.05. BA = Brodmann's Area; NVox = number of voxels in each cluster (cluster size); X, Y, Z = Talairach and Tournoux Coordinates.

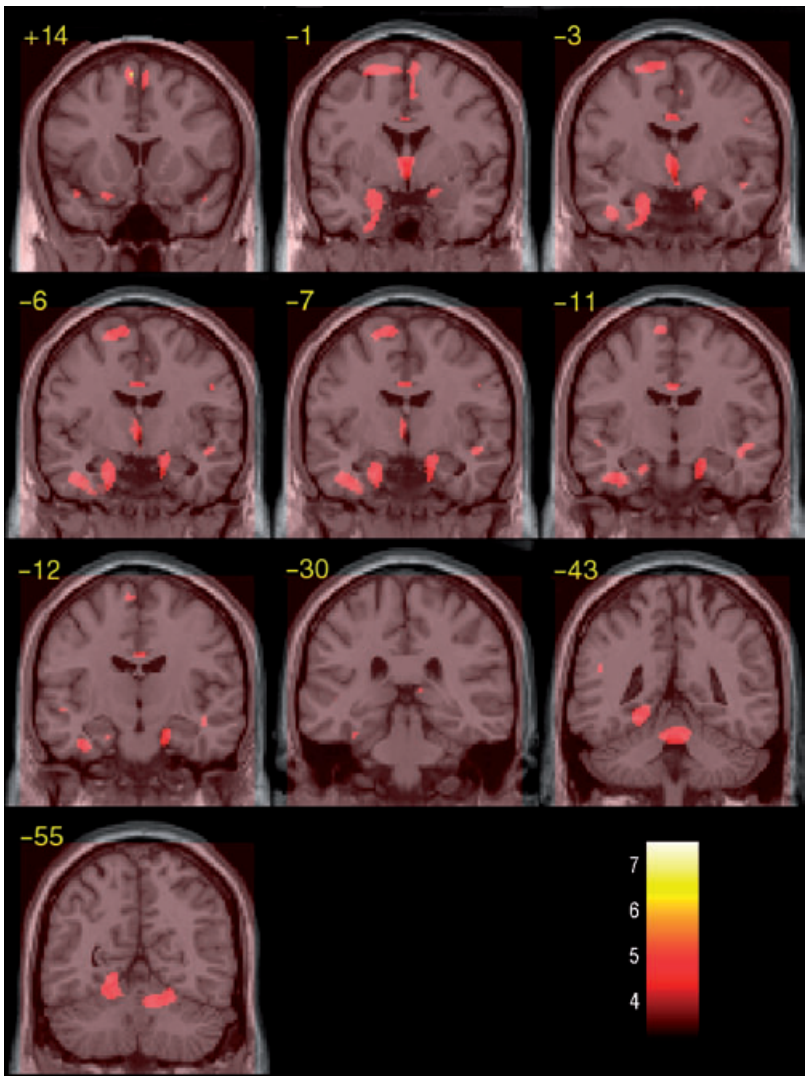

Figure 1 Differences in brain volume between low-risk and high-risk subjects. Coronal section of images illustrating the gray matter volume deficits (red) in high-risk subjects (each number indicates the corresponding Talairach and Tournoux 'y' coordinate of the section; the color bar represents the T scores)

Table 3 Optimized voxel-based morphometry analyses: regional gray matter volumes with significant negative correlation with total externalizing symptom scores.

No.	*Gray matter region*	*NVox*	*X*	*Y*	*Z*	*T*	CC_{Max}	*P**
1	Left parahippocampal gyrus (BA 19)	307	−18	−55	−04	4.01	0.22	< 0.001
2	Right cerebellum	1843	14	−57	−17	3.89	0.22	< 0.001
3	Left superior frontal gyrus (BA 6)	101	−02	15	62	3.39	0.21	< 0.001
4	Right parahippocampal gyrus (BA 19)	340	22	−57	−06	3.35	0.21	< 0.001
5	Right thalamus	121	18	−32	11	3.34	0.21	< 0.001
6	Right amygdala	17	26	01	−15	2.99	0.21	< 0.001
7	Left amygdala	3	−24	01	−15	2.77	0.20	< 0.001

**P*-value corrected for multiple comparison with false discovery rate < 0.05. BA = Brodmann's Area; CC_{Max} = maximum correlation coefficient for each cluster; NVox = number of voxels in each cluster (cluster size); X, Y, Z = Talairach and Tournoux Coordinates.

volumes of the above structures appeared to be maximal at earlier ages. Although not statistically significant, there appeared to be a visible trend toward narrowing of the difference in the developmental trajectory between the subjects of the HR and LR groups with increasing maturity (Fig. 2).

DISCUSSION

Differences in morphology of critical brain structures between HR and LR subjects

In this study, alcohol-naïve HR subjects (child, adolescent and young adult offspring of early onset male alcoholics

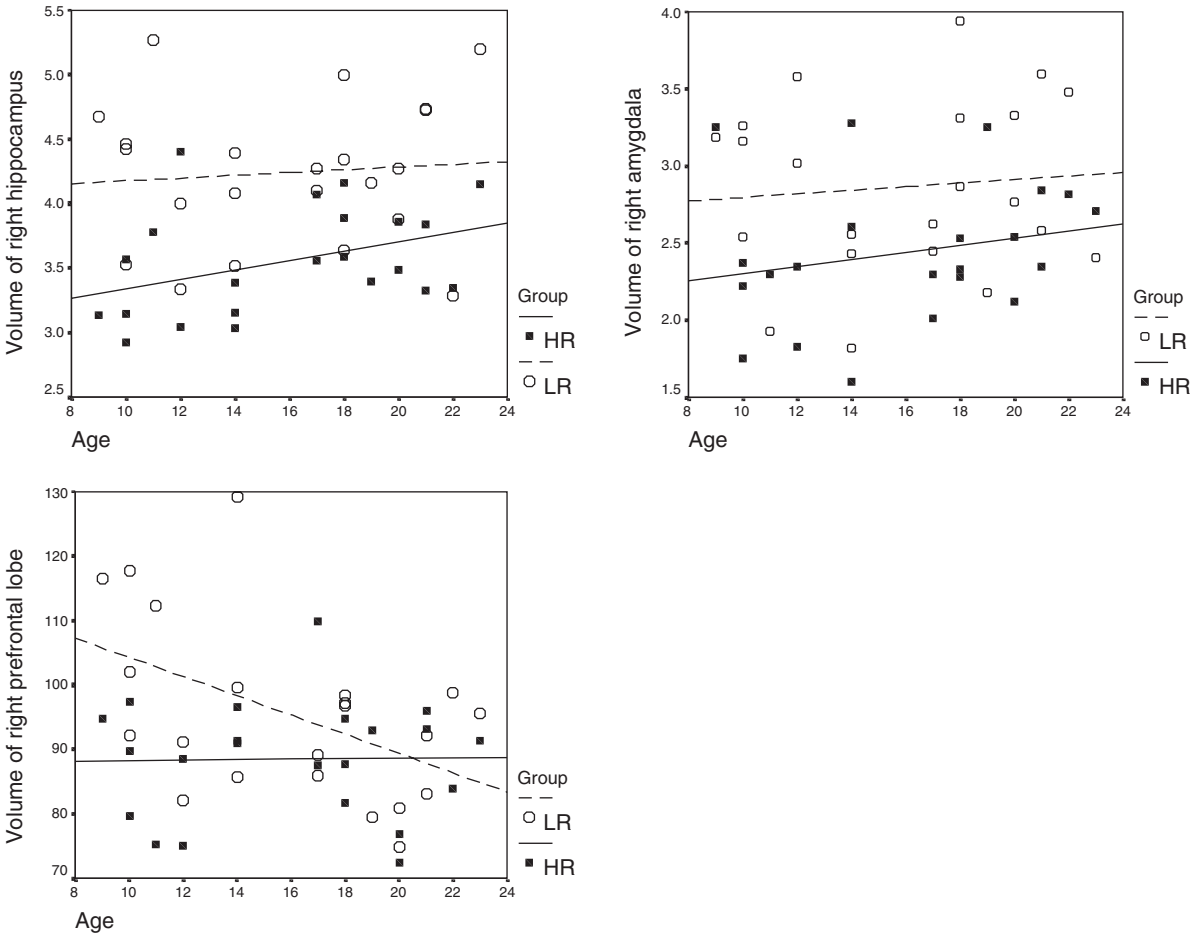

Figure 2 Trajectories of growth of brain areas across age. Curve-fitting, using a linear model, illustrates the differences in the trajectories of growth of the right amygdala, right hippocampus and the right prefrontal lobe. The demonstrated delay in reaching age-appropriate volumes in the high-risk (HR) subjects, in comparison with low-risk (LR) controls, is suggestive of a neurodevelopmental lag

with a high family loading for alcoholism) appeared to differ from LR subjects (offspring of non-alcohol-dependent fathers without family history for alcohol dependence) in having smaller volumes of bilateral amygdala (both ROI and VBM analyses), hippocampi (ROI analysis) and parahippocampal gyri (VBM analysis). In addition, VBM analysis detected additional reductions of gray matter volume in the HR group in bilateral superior frontal gyri (Brodmann Area 6), bilateral cingulate gyri (Brodmann Area 23/24), both thalami and the right cerebellar hemisphere. The prefrontal lobe was also smaller in the HR group (ROI analysis), though the difference narrowly evaded statistical significance.

The differences in morphology found in this study replicate earlier reports of smaller volume of the right amygdala in HR children (Hill *et al.* 2001), and suggest that additional regions of altered brain development may be involved in the vulnerability to early alcohol problems. There is some support for these additional regions being abnormal in substance dependence, from the findings of smaller volumes of the prefrontal cortex (De Bellis *et al.* 2005) and hippocampus (De Bellis *et al.* 2000) in adolescents and young adults with early onset alcoholism.

Vulnerability to alcoholism: frontal and limbic abnormalities

Neurobiological models of addiction suggest that substance taking may be related to two processes, namely reward deficiency (Blum *et al.* 2000) and the impaired response inhibition and salience attribution (Goldstein & Volkow 2002). The former process relates to abnormal activity in the limbic brain structures of the extended amygdala system, thereby resulting in exaggerated processing of the incentive values of substance-related stimuli (Blum *et al.* 2000). The other process relates to abnormal activity of the prefrontal cortex system necessary for inhibiting the substance-seeking action associated with immediate reward (Goldstein & Volkow 2002). An integrated model involving the extended amygdala (reward); the orbitofrontal cortex and the subcallosal cortex (motivation/drive); the amygdala and the hippocampus (memory and learning); the frontal and cingulate

cortices (control), and the thalamus, insula, and cerebellum has been implicated in the development of drug abuse and addiction (Volkow, Fowler & Wang 2003).

Similar areas have been implicated in a spectrum of externalizing disorders, such as ADHD (Seidman, Valera & Makris 2005) and antisocial personality (Blair & James 2003).

Externalizing symptoms and brain volume deficits

HR subjects had significantly higher scores of externalizing symptoms than the LR subjects. Importantly, there was a strong inverse correlation between the volume of many of these brain structures and expressed externalizing behaviors of the subjects (Table 3). The amygdala and the hippocampal volumes predicted around 40% of the variance in scores of externalizing symptoms. This inverse predictive relationship suggests that these brain areas might critically influence the appearance of a range of disruptive behaviors, thought to be the inherited general vulnerability in HR children.

Morphological deficits in areas like the prefrontal lobe, cingulate gyrus, amygdala, hippocampus, corpus callosum, thalamus and cerebellum have been repeatedly implicated in studies in children and adults with disruptive behavior disorders and ADHD (Seidman *et al*. 2005). An extensive literature has documented the association of an excess of externalizing/disruptive behaviors in HR children (McGue *et al*. 2001). Hill & Shen (2002) have suggested that the greater externalizing symptoms found in HR subjects in comparison with LR subjects implicate CNS hyperexcitability in the former. It is suggested that CNS hyperexcitability is heritable, and is critically involved in a genetic predisposition toward alcoholism and the development of dependence (Begleiter & Porjesz 1999). The proposition is that behavioral phenomena such as psychopathy, antisocial and impulsive traits, substance and alcohol abuse, should be viewed as variable expressions of a *generalized disinhibitory complex* (Gorenstein & Newman 1980). The strong predictive relationship between externalizing symptoms in alcoholic fathers and reduced brain volumes in their offspring, as well as the strong positive association between paternal externalizing symptoms and externalizing behaviors in their children—lends further weight to this argument.

An endophenotype for a generalized disinhibitory complex

The implicated brain regions appear to be part of the causal pathway in the development of this *generalized disinhibitory complex*. Dysregulation of the prefrontal cortex is implicated in impulsive decision making (Rogers *et al*. 1999) and ADHD children have significant gray matter deficits in right superior frontal gyrus and right posterior cingulate gyrus (Overmeyer *et al*. 2001). Functional MRI of alcohol-naïve youths with a strong family history of alcoholism have shown less frontal lobe brain activation (superior frontal, middle frontal, and right inferior frontal gyri) on response-inhibition tasks, relative to family history-negative youths. This is associated with disinhibition and impulsivity which may relate to resisting alcohol use opportunities and developing early alcohol problems (Schweinsburg *et al*. 2004).

The dorso-lateral-prefrontal cortex, with strong connections to the Dorsal Anterior Cingulate Cortex, plays a critical role in target detection, response selection, error detection, and reward-based decision making (Bush *et al*. 2002), functions that are thought to be impaired in ADHD. The anterior cingulate critically mediates in the assessment of the motivational content of internal and external stimuli, regulating approach and avoidance learning (Devinsky, Morrell & Vogt 1995) and in the inhibition of responses to previously rewarded affective and non-affective stimuli. Defects in this mechanism of behavioral control may contribute to the formation of a drug habit and the subsequent inability to break an established drug habit. (Bechara, Dolan & Hindes 2002). Cingulo-thalamic neuronal plasticity may be crucial for the acquisition of avoidance responses and the amygdala projections play an important role in the modulation of these plastic changes (Poremba & Gabriel 1997).

The extended amygdala and its interactions with certain limbic structures (cingulate gyrus and the parahippocampal gyrus, 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 & LeMoal 2001). The basolateral amygdala seems critical in acquiring associations between cues and the incentive properties of outcomes. The orbitofrontal cortex subsequently uses them to guide behavior: maintaining these representations in memory, updating them with new information, and expressing them in behavior (Pickens *et al*. 2003). This is critical for judgment and decision making, and in acquiring associations between cues and the incentive properties of outcomes.

The hippocampal formation and parahippocampal gyrus (collectively termed the hippocampal system), the superior parietal gyrus, the superior frontal gyrus, and the cerebellum are the regions most commonly found active during mismatch between the expectation and the delivery of painful stimulation. Learning of a cue–outcome association only takes place when there is a mismatch between outcome and the expectations based on perceived cues. Learning cues of impending pain allows future painful events to be anticipated and avoided (Ploghaus *et al*. 2000).

The midline cerebellar structure (vermis, fastigial nucleus) has been shown to modulate dopamine outflow

in reward-related limbic structures (Snider & Snider 1977). Neuroimaging data implicate a cerebellar–prefrontal circuit in the pathophysiology of ADHD (Middleton & Strick 2001). Side-by-side, several studies have found increased postural sway in HR children compared with LR subjects (Hegedus *et al.* 1984; Hill *et al.* 2000) and developmental markers of postural control and cerebellar functioning in infancy appear to predict alcohol dependence at 30 years of age (Manzardo *et al.* 2005).

Immaturity or hypofunction of some of the brain structures implicated in the current study may thus lead to compromised impulse control, including high-novelty seeking or sensation seeking, increased response to reward, a tendency to pay more attention to immediate rewards and neglect long-term outcomes when making decisions, reduced response to punishment, and a disregard for social norms. These are attributes commonly associated with the vulnerability to alcoholism and a spectrum of externalizing disorders (Cloninger, Sigvardsson & Bohman 1988).

Genetic factors appear to account for most of the individual differences in whole brain (90%), gray (82%) and white (88%) matter volume (Baaré *et al.* 2001). Several researchers have reported a high heritability of gray matter volume in several cortical regions (Posthuma *et al.* 2000), the cerebellum and subcortical structures (White, Andreasen & Nopoulos 2002). Inherited gene variations influencing brain development and function (e.g. catechol-o-methyl transferase affecting variation in prefrontal-cortex function) are found in human populations (Winterer & Goldman 2003).

Further, the brain morphometric differences and the externalizing behaviors it appears to mediate appear to be found in affected individuals as well as in non-affected family members at a higher rate than in the general population. Together, these observations suggest that these brain abnormalities might constitute an endophenotype (Gottesman & Gould 2003) for the general disinhibitory complex which is thought to underlie the vulnerability to alcoholism.

Developmental lag

Our results also appear to suggest that the deficits in volume may not be static. The differences in volume between HR and LR subjects are maximal in younger subjects and tend to decrease among the older subjects. This can be interpreted as a difference in developmental trajectories of brain growth between the two groups. This finding lends substance to earlier observations (Hill & Shen 2002) that brain development in HR offspring of alcoholics might be subject to a process of maturational lag, affecting regions known to be actively developing during adolescence, and critical to the neurocircuitry of motivation and reward.

Adolescent boys with a history of conduct disorder and potentially at risk for substance dependence fail to exhibit normal maturational increases in P300 amplitude found in boys without such histories (Bauer & Hesselbrock 2003). Topographic analyses of current source densities suggest that the source of the maturational deficit involves P300 generators within the frontal brain (superior and middle frontal gyri). Nonetheless, our finding must be tempered by the caveat that the cross-sectional design of this study is by no means ideal for tracking developmental patterns, which ideally requires repetitive measurements across the developmental life-span.

Methodological issues

To our knowledge, this is the first study to examine the gray matter volume abnormalities in alcohol-naïve HR subjects using both ROI and VBM methods. As both the ROI and VBM methods have their own advantages and limitations, it has been suggested that both these techniques should be used in tandem in brain morphometric analysis (Giuliani *et al.* 2005). The VBM methodology has been updated and optimized recently (Good *et al.* 2001) to reduce errors due to systematic differences in head shape, variations in segmentation, inconsistent brain stripping, and errors introduced by spatial normalization. We have used the optimized method to improvise upon the VBM analyses with possible reduction of potential errors. Also, in contrast to the classical VBM, which analyzes gray matter concentration differences (Ashburner & Friston 2000), the optimized VBM examines the gray matter volume differences (because of incorporation of the 'modulation' step) (Good *et al.* 2001). Employing both the techniques (especially, the optimized VBM) add to the methodological rigor of our study.

Both ROI and VBM analyses revealed gray matter volume deficits in bilateral amygdala. Also, they concurred on absence of caudate nucleus volume abnormalities. However, there were some discrepancies between ROI and VBM findings. ROI analyses revealed hippocampal deficits but VBM did not. Instead, the contiguous parahippocampal gyri showed differences. Functionally, the hippocampus and the parahippocampal gyri are thought to be part of the hippocampal formation and overlapping. The important reason for the discrepancy between ROI and VBM results might be because of the methodological differences between voxel-averaged, landmark-based ROI analyses and the single, voxel-by-voxel whole brain VBM measurements (Giuliani *et al.* 2005). Nonetheless, in our study, concurrent analyses using both the methods have helped in: (1) establishing the brain abnormalities in amygdala and hippocampi; and (2) identifying additional brain regions that might potentially constitute the abnormal neural network that might underlie the risk toward alcohol dependence.

In conclusion, the findings demonstrate that HR offspring differ from the LR control subjects both neuroanatomically and in manifest behavioral pathology. The gray matter volume deficits involving frontal and cingulate gyri, amygdala, hippocampus, parahippocampus, thalamus and cerebellum offer a neuroanatomical basis for the hypothesized brain circuitry abnormalities, which might be contributing to the reward deficiency as well as impaired response inhibition and salience attribution. It is significant that these deficits appear to predict the expression of externalizing spectrum behaviors, which are thought to represent the inherited vulnerability to alcohol dependence in HR individuals. There is also a significant predictive relationship between paternal externalizing behaviors and deficits in brain morphology in their children. Moreover, paternal externalizing symptoms scores correlated positively with that of their children's scores. Together, these suggest that heritable differences in brain morphology and associated externalizing CNS hyperexcitability might be critically involved in the predisposition toward the development of alcohol dependence.

Acknowledgement

The paper is based on a reanalysis of the data set collected by Dr. George Antony for his MD dissertation.

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