Disruption of Orbitofrontal Cortex Laterality in Offspring from Multiplex Alcohol Dependence Families

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Background: Increased susceptibility for developing alcohol dependence (AD) might be related to structural differences in brain circuits that influence the salience of rewards and/or modify the efficiency of information processing. The role of the orbitofrontal cortex (OFC) in regulating emotional processing is increasingly being recognized along with its association with impulsive behavior.

Methods: Magnetic resonance imaging was used to measure the OFC in 107 high- and low-risk offspring (mean age 17.6 $\pm$ 4.69 years) from either multiplex AD families or control families. Region of interest measures including segmented values were obtained by reliable raters using BRAINS2 software. Statistical analyses were adjusted for intracranial volume, age, socioeconomic status (SES), IQ, and handedness. The Multidimensional Personality Questionnaire (MPQ) was administered to determine scale scores for Control. Genotyping was performed for the serotonin transporter (5-HTT) gene and the brain-derived neurotrophic factor (BDNF) gene.

Results: High-risk offspring from multiplex for AD families showed decreased right/left OFC volumes in comparison with control subjects. Smaller volume in the right hemisphere was significantly associated with variation in the 5-HTT and BDNF genes. White matter (WM) ratios showed a positive correlation with MPQ Control scale scores, indicating that reduced OFC WM is related to greater impulsivity.

Conclusions: Offspring from multiplex families for AD manifest genetic susceptibility by exhibiting disruption in the laterality of the OFC volume that is related to greater impulsivity (lower Control scale scores). This disruption in OFC laterality is related to variation in genes associated with neuronal growth.

Key Words: Alcohol dependence, high-risk offspring, MRI, OFC

Significant changes in brain structure and refinement of brain organization during adolescence and young adulthood lead to changes in cognitive, social, and emotional behavior (1,2). White matter volume increases well into adulthood, whereas grey matter volume tends to increase in childhood and adolescence, followed by a decrease (3–7), with female subjects reaching their peak 1–2 years earlier than male subjects (8). Cortical development follows a pattern that subserves the needs of the organism, with primary motor, sensory, and visual areas maturing earlier than those supporting more complex cognitive functions, such as the association areas (9).

These morphological changes are accompanied by changes in cognitive abilities, including development of mature decision-making strategies that begin to emerge in young adulthood. During adolescence risk-taking behavior appears to be normative (10). This may be due to the late development of the frontal cortex (2) and maturation of neocortical regions that modulate prefrontal systems (11). In comparison with adults, children and adolescents show greater orbitofrontal cortex (OFC) functional magnetic resonance imaging (MRI) activation and slower discriminative learning when performing a delayed two-choice response task cued to whether their response results in a small, medium, or large reward, suggesting that the protracted maturational changes in the OFC might be responsible (12).

Adolescent risk-related behavior and novelty-seeking often coincide with the onset of alcohol, cigarette, and drug use (13–15). Having an earlier age of onset to begin drinking during adolescence is an important predictor of adult alcohol dependence (AD) with those younger than 14 years having a rate of 40%, whereas for those age 20 and older just 10% (16,17). Also, for those starting before age 14, increased rates of stress-reactive drinking is seen (18).

The mechanism underlying the relationship between disinhibition and increased risk for developing alcohol and other substance use disorders (SUDs) continues to remain unclear. Reduced P300 amplitude appears to be one indicator of disinhibited behavior (14,15) and an important mediator of the relationship between age of onset to begin drinking and familial loading for AD (13,19). Developmental trajectories of P300 show marked change during childhood and adolescence (20,21). Brain morphological underpinnings of disinhibited behavior, particularly in those with greater familial loading for alcohol or other substance dependence, might provide clues regarding possible interventions.

The OFC region appears to be a neural substrate for a variety of impulsive behaviors, including SUDs (22). There is evidence that neurodevelopmental changes in decision making and social/emotional functioning are accompanied by changes in brain morphology that differ by hemisphere. Greater involvement of the right than left ventrolateral prefrontal cortex (VLPFC) is seen in tasks involving response selection and inhibition (23), with suboptimal response inhibition in children and adolescents related to insufficient recruitment of the right VLPFC (24). Functional asymmetry in social conduct, decision-making, and emotional processing has been found for the ventromedial prefrontal cortex (VMPFC), an area that includes the medial and lateral regions of the OFC, in rare patients with unilateral VMPFC lesions (25,26). Left VMPFC patients perform well on the Iowa Gambling Test (IGT), whereas right VMPFC patients perform this

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Received March 18, 2008; revised August 29, 2008; accepted September 3, 2008.

0006-3223/09/$36.00
doi:10.1016/j.biopsych.2008.09.001

Table 1. Demographic Characteristics of HR and LR Adolescents and Young Adults

	HR (n = 63)		LR (n = 44)				
	Mean	SD	Mean	SD	F	df	p
Age	18.33	4.47	16.68	4.88	3.43	1, 103	NS
Intracranial Volume (cc) Males[a,b,c]	1491.94	105.72	1433.48	105.95	4.84	1, 54	.05
Intracranial Volume (cc) Females[a,b,d]	1344.01	101.95	1276.73	102.37	5.17	1, 47	.03
SES[e]	41.00	10.79	44.77	11.26	4.05	1, 103	NS
IQ	108.73	14.20	112.70	17.98	2.00	1, 103	NS
BMI Male[a,b,c]	23.89	4.44	22.77	4.45	.84	1, 54	NS
BMI Female[a,b,d]	22.83	5.67	26.62	5.69	5.30	1, 47	.03
Right-Handed, n	60		40		.80[f]	1	NS
Right-Handed (%)	(95.2)		(90.2)				
MPQ Control Scale Scores[a]	11.68	5.16	13.18	5.13	1.54	1, 73	NS
Alcohol or Drug Abuse/Dependence (N)[g]	18		4				

HR, high-risk; LR, low-risk; SES, socioeconomic status; BMI, body mass index; MPQ, Multidimensional Personality Questionnaire.
[a]Adjusted means are presented.
[b]Analysis of risk, gender, and risk × gender showed a significant difference by gender and risk. Accordingly, results are presented by gender.
[c]There were 35 HR and 22 LR male subjects.
[d]There were 28 HR and 22 LR female subjects.
[e]Hollingshead Four Factor Index (61).
[f]χ^2 value.
[g]Number of cases meeting criteria for alcohol or drug abuse or dependence before their scan. Diagnoses were made with the age-appropriate diagnostic instrument, Kiddie-Schedule for Affective Disorders and Schizophrenia for those under age 19 years and Composite International Diagnostic Interview for those 19 years or greater. Three cases were diagnosed before the age of 19 years and had a mean exposure period of .33 ± .57 years prior to the magnetic resonance imaging scan. The remaining 19 cases were diagnosed after age 19 years and had a mean exposure of 2.47 ± 2.32 years before the scan.

decision-making task as poorly as bilaterally damaged patients (26). Unilateral right VMPFC damage is associated with severe deficits in social/emotional and decision-making processes in male patients (25).

Because adolescents with the poorest impulse control may be at the greatest risk for harmful behaviors to themselves and others (27,28), it is important to identify the neurobiological concomitants of impulsive behavior. Offspring from families selected for multiple cases of AD in comparison with control children have greater disinhibition, including earlier onset to begin drinking and greater externalizing pathology (13,17,29). We hypothesized that lateralized volumetric differences in the OFC would be seen between offspring from multiplex AD families (high-risk) and those from control families (low-risk) and that these differences would be related to impulsive temperament as measured by Control scale scores from the Multidimensional Personality Questionnaire (MPQ) (30). Persons scoring at the higher end of the Control scale tend to take a careful and cautious approach to life, whereas those at the lower end of the scale tend to act without much thought and are impulsive. Alcohol-dependent individuals typically have lower scores on Control than those without AD (31,32).

Additionally, although susceptibility genes have been implicated in the etiology of AD and other genes have been identified as having a role in central nervous system growth, we were interested in testing genes that might have both functions. Consistent with its role as a nerve growth factor, variation in the VAL/MET alleles of the brain-derived neurotrophic factor (BDNF) is associated with smaller volume of the hippocampus in healthy control subjects (33). Moreover, there is evidence that the behavioral effects of alcohol are regulated by BDNF (34). The short variant of the serotonin transporter (5-HTT) gene also appears to act as a nerve growth factor and has been associated with volumetric differences in both amygdala and hippocampus (35). Additionally, the "short" allele of 5-HTT has been shown to be associated with greater impulsivity, including increased risk for suicide attempts among male alcohol-dependent subjects (36). Therefore, our third goal was to determine whether differences in OFC volume would be associated with genotypic variation in the 5-HTT and BDNF genes.

Methods and Materials

Participants

A total of 107 participants (57 male subjects and 50 female subjects) with an average age of 17.6 ± 4.69 years were studied (Table 1). All participants were part of a longitudinal cohort of offspring from multiplex for AD pedigrees initiated in 1990. The high-risk (HR) offspring (n = 63) were from multiplex AD families selected through the presence of a pair of adult alcohol-dependent brothers. As a result, each HR offspring had an average of four first- and second-degree relatives with AD. Low-risk control offspring (n = 44) were identified through their families, who were selected for absence of Axis I psychopathology and had no first- or second-degree relatives with alcohol or drug dependence. Mothers of all offspring were free of heavy use of alcohol or drugs during pregnancy.

Clinical Evaluation

An ongoing longitudinal study that follows youngsters from childhood through young-adulthood provided annual psychiatric diagnoses, including SUDs, prior to the time the MRI assessment was performed (57 were performed before the age of 19 years and 50 at 19 years or later). Children/adolescents under the age of 19 years were assessed yearly with the Kiddie-Schedule for Affective Disorders and Schizophrenia (37) with separate interviews of parent and offspring to determine the presence or absence of Axis I DSM-III diagnoses (DSM-III was the current methodology at the initiation of the study). Young adults (age 19 years or older) were assessed with the Composite International Diagnostic Interview diagnostic instrument (38) to obtain DSM-IV Axis I diagnoses.

Table 2 shows the clinical diagnoses for those scanned before the age of 19 years (n = 57). High-risk offspring had a greater

Table 2. Diagnoses for Participants Scanned During Childhood or Adolescence ($N = 57$)

	K-SADS Evaluation[a]			
	HR[b] $n = 29$		LR[c] $n = 28$	
Diagnosis	n	% with Diagnosis (n)	n	% with Diagnosis (n)
Alcohol or Drug Abuse	2	6.9	1	3.6
Alcohol or Drug Dependence	1	3.4	1	3.6
Either Abuse or Dependence	2	6.9	1	3.6
Anxiety Disorders	4	13.8	4	14.3
Depression	6	20.7	1	3.6
ADHD	7	24.1	0	.0
Oppositional/Conduct Disorder	5	17.2	2	7.1

K-SADs, Kiddie-Schedule for Affective Disorders and Schizophrenia; HR, high-risk; LR, low-risk; ADHD, attention-deficit/hyperactivity disorder.

[a]Participants scanned before their 19th birthday were evaluated with the K-SADs.

[b]The HR group included 10 female subjects and 19 male subjects.

[c]The LR group included 14 female subjects and 14 male subjects.

incidence of depression, attention-deficit/hyperactivity disorder (ADHD), and oppositional/conduct disorders. For those receiving the first MRI during young-adulthood ($n = 50$), a greater number of HR participants met criteria for SUD, anxiety disorders, depression, ADHD, and oppositional/conduct disorders during either childhood or young adulthood (Table 3).

Ethical Considerations

All participants were provided written informed consent. All were screened to insure absence of ferromagnetic metal in or on their body. Female subjects were screened for pregnancy using Icon 25 hCG (Beckman Coulter, Fullerton, California) pregnancy kits.

Structural Acquisition Methods

All subjects were scanned on a 1.5 Tesla GE (General Electric, Milwaukee, Wisconsin) scanner. T1 weighted axial images with slice thickness of 1.5 mm were obtained with a three-dimensional spoiled gradient recalled echo in the steady state (3D SPGR) (echo time [TE] = 5, repetition time [TR] = 24, flip angle = 45 degrees, acquisition matrix = 192 × 256, number of excitations [NEX] = 1, FOV = 24 cm). Slices were resliced in the coronal plane through the anterior commissures for quantitative limbic morphology. Additionally, axial proton density and T2 weighted images were obtained covering the whole brain at a slice thickness of 5 mm, slice gap = 0 mm ([double echo spin echo, TE = 17 ms and 102 ms; TR = 3000 ms], acquisition matrix = 256 × 192, NEX = 1, field of view [FOV] = 24 cm). Obtaining the dual echo study enabled us to adequately address segmentation. All scans were reviewed by a neuroradiologist where suspected structural abnormalities might be present.

Region of Interest Analysis

Regions of interest were drawn using BRAINS2 (39), a software that provides valid and reliable volume measurements of specific structures, and automated segmentation of grey, white, and cerebrospinal fluid (CSF) volumes. Segmentation of tissue into grey and white matter is done to optimize the kappa (κ) value obtained with successive iterations by the raters. The BRAINS2 software allows selection of tissue plugs for separation of tissue classes using discriminant function analysis. Once a best fitting function is found using training classes, it is applied to the entire image to verify the discriminant function classification. The predicted classification is then compared with a priori labeled grey matter, white matter, and CSF plugs, and a κ statistic is applied. In our laboratory, data are included only when κ values exceed .95.

Two raters (SW and HC) who were blind to risk group membership with inter-rater reliability > .95 traced the volumes of the OFC and intracranial volume (ICV) after first aligning the T1, T2, and proton density (PD) images. Region of interest (ROI) manual tracing was performed in the coronal plane.

The boundaries and landmarks for the OFC—right, left, and total—followed the guidelines established by Lacerda *et al.* (40) (see Figure 1). The OFC ratio was determined with the formula (Right – Left)/(Right + Left).

Intracranial volume was measured, including cerebral hemispheres, brainstem, and the CSF surrounding these structures (41). Intracranial volumes were calculated by summing areas of successive coronal slices, including grey and white matter and CSF volumes, and multiplying by slice thickness.

Personality Assessment

All subjects were administered the MPQ (30). The MPQ provides 11 personality scales and 3 higher order scales. One of the primary scales measures Control. Those scoring low on this scale tend to be impulsive.

Genotyping

Blood was drawn from 87 of 107 individuals for whom structural MRI scans were assessed. The DNA containing the 5-HTT polymorphism was amplified by polymerase chain reaction (PCR) with a modification of a method previously described (42). The PCR was completed in 384-well plates in a 7.5-μL total reaction volume, containing 20 ng of human genomic DNA; 1X GeneAmp PCR Gold Buffer (Applied Biosystems, Foster City, California); 1.5 mmol/L magnesium chloride; .25 mmol/L deoxynucleoside triphosphates (dNTPs) (with equal concentration of each dNTP but substituting 7-deaza–deoxyguanosine triphosphate [dGTP] for one-half of the total dGTP); .3 units of AmpliTaq Gold (Perkin Elmer, Branchburg, New Jersey) taq polymerase;

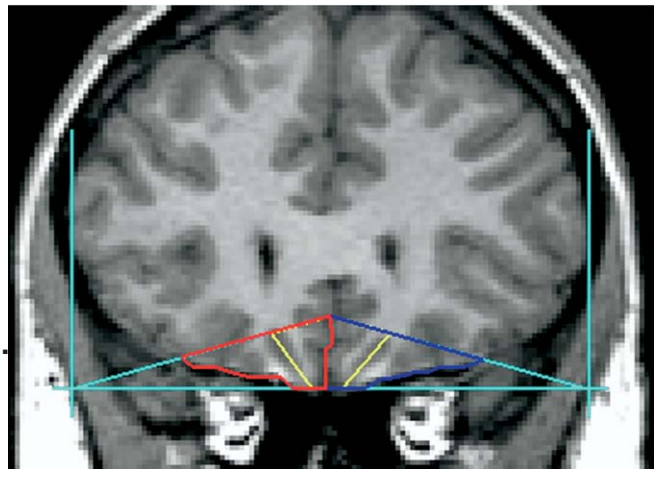

Figure 1. The boundaries and landmarks for the orbitofrontal cortex (OFC)—right, left, and total—followed the guidelines established by Lacerda *et al.* (40). Outlines for the left OFC are seen in blue, with the right OFC in red. The yellow line depicts the lateral and medial portions of the OFC in each hemisphere

Table 3. Diagnoses for Participants Scanned During Young Adulthood

	Evaluation by KSADS or CIDI[a]			
	HR[b] $n = 34$		LR[c] $n = 16$	
Diagnosis	*n*	% with Diagnosis (*n*)	*n*	% with Diagnosis (*n*)
Alcohol or Drug Abuse	12	35.3	2	12.5
Alcohol or Drug Dependence	11	32.4	3	18.8
Either Abuse or Dependence	16	47.1	3	18.8
Anxiety Disorders	11	32.4	4	25.0
Depression	9	26.5	2	12.5
ADHD	4	11.8	0	.0
Oppositional/Conduct Disorder	9	26.5	0	.0

CIDI, Composite International Diagnostic Interview; other abbreviations as in Table 1.

[a]Psychiatric evaluations were completed in both developmental periods for 46 of the 50 cases. The percent positive represents a positive diagnosis at either time point.

[b]The HR group included 18 female subjects and 16 male subjects.

[c]The LR group included 8 female subjects and 8 male subjects.

1.25 pmols of the fluorescently labeled forward primer 5′-GGCGTTGCCGCTCTGAATGCC-3′, and 1.25 pmols of the unlabeled reverse primer 5′-CAGGGGAGATCCTGGGAGAGGT-3′. Thermal cycling included 35 cycles at an annealing temperature of 61°C. Subsequent electrophoresis was performed on the ABI Prism 377 DNA Sequencer (Applied Biosystems). The wild type was detected by the presence of a 268-base pair (bp) "long" allele, whereas the variant is a 224-bp "short" allele, caused by a 44-bp deletion.

The BDNF genotyping was completed with the single nucleotide polymorphism rs 6265 analyzed on the Biotage PSQ 96MA Pyrosequencer (Biotage AB, Uppsala, Sweden). An amplimer containing the polymorphism was generated by PCR in 96-well plates in a 50-μL total reaction volume, containing 10 ng of human genomic DNA; 1X GeneAmp PCR Gold Buffer (Applied Biosystems); 2.5 mmol/L magnesium chloride; 200 μmol/L dNTPs; 1 unit of AmpliTaq Gold (Perkin Elmer) taq polymerase; and 1 pmol of each of the unmodified forward primer 5′-GGACTCTGGAGAGCGTGAAT-3′ and the biotinylated reverse primer 5′-CCTCATCCAACAGCTCTTCTATC-3′. Thermal cycling included 45 cycles at an annealing temperature of 60°C. The Biotage workstation was used to isolate the biotinylated single strand from the double strand PCR products. The isolated product was then sequenced using the complementary sequencing primer 5′-GGCTGACACTTTCGAAC-3′. The minor allele was detected by the presence of an A nucleotide at the polymorphic site, whereas the major allele was detected by the presence of a G nucleotide at this site.

Statistical Analysis

Because our central hypothesis was that lateralized effects would be seen, an initial analysis was planned using right/left ratios of OFC volume analyzing risk, gender, and risk × gender effects**.** A General Linear Model (GLM) was implemented in SPSS (Version 15; SPSS, Chicago, Illinois) with age, any prior SUD, and handedness as covariates on the ICV adjusted volumes. With support from this analysis showing differences by risk and gender, three separate univariate analyses were planned for right OFC volume (total, grey, and white) to determine whether differences would be seen by risk group, gender, and their interaction. Because the OFC reaches full development in late adolescence to young adulthood, exploratory regression analyses were planned to determine if age-related changes in OFC by risk group and gender (grey and white) would be seen. Because the OFC is thought to be involved in impulsive disorders, additional exploratory analyses (partial correlations controlling for age) were planned using MPQ control scale scores to detect possible associations between these scores and total, white, or grey matter volume. An additional goal was to determine whether OFC volume would be related to the BDNF or 5-HTT genes or their interaction. Confirmatory analyses were planned to address the possible impact of individual psychiatric disorders on the obtained results.

Results

Preliminary analyses were performed for age, socioeconomic status, IQ, body mass index, hand preference, and ICV by risk group, gender, and risk × gender (Table 1). Psychiatric diagnoses by risk group were also analyzed (Tables 2 and 3). The HR offspring from multiplex families were more likely to have a lifetime childhood/adolescent or young adult disorder than control subjects, consistent with data from the larger sample (29).

Results of our primary analysis revealed a significant difference by risk group for the right/left ratios adjusting for ICV, age, hand preference, and previous SUD diagnosis [$F(1,100) = 9.95$, $p = .002$] (Figure 2). The OFC ratios were larger for low-risk than HR offspring (adjusted means = $2.92 \times 10^{-5} \pm 3.33 \times 10^{-5}$ cm^3 versus $.82 \times 10^{-5} \pm 3.32 \times 10^{-5}$ cm^3). Three univariate analyses were performed for right volume (total, grey, and white), adjusting for left volume, ICV, age, handedness, and SUD. For total volume, risk was significant [$F(1,98) = 10.44$, $p = .002$] as was risk × gender [$F(1,98) = 5.05$, $p = .02$]. For grey, risk was significant [$F(1,97) = 9.36$, $p = .003$]. For white, risk was significant [$F(1,97) = 4.96$, $p = .028$], as was risk × gender [$F(1,97) = 5.32$, $p = .023$].

Because personal exposure to alcohol and drugs or the presence of psychiatric disorders might explain the familial risk group differences seen, analyses were performed removing cases with anxiety or depression ($n = 38$) or SUD ($n = 22$). Risk group differences remain significant for total right volume adjusting for left, age, and handedness [$F(1,62) = 4.62$, $p = .04$] and for risk × gender [$F(1,62) = 4.37$, $p = .04$] when cases with anxiety or

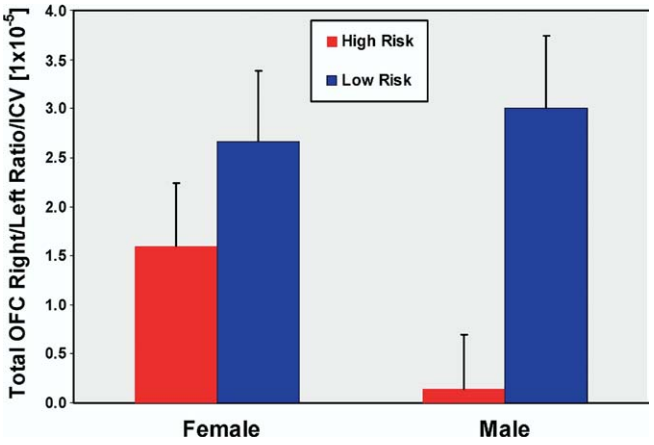

Figure 2. Orbitofrontal cortex (OFC) ratios were determined for each participant with the formula Right – Left/Right + Left. Volumes were adjusted for intracranial volume (ICV) before statistical analyses were performed. Depicted here are the adjusted means (adjustment for age) and SDs for the male and female high- and low-risk participants.

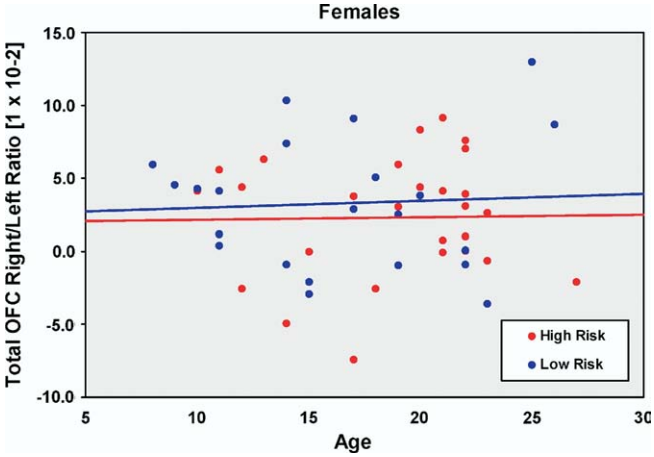

Figure 3. Regression lines for orbitofrontal cortex (OFC) ratios for female participants show a relatively flat progression from childhood to young adulthood.

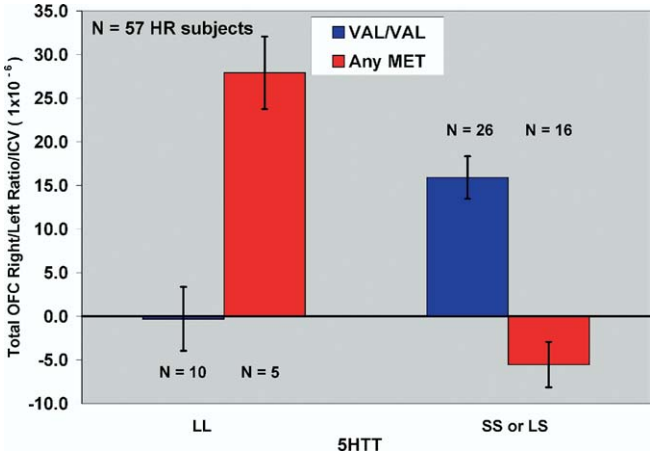

Figure 5. A statistically significant association between the presence of the S allele of the serotonin transporter (5-HTT) gene, the Met allele of the VAL/Met variation of the brain-derived neurotrophic factor gene and volume of the orbitofrontal cortex (OFC) in the right hemisphere (Right – Left/Right + Left) (intracranial volume [ICV]-corrected) was seen for the 57 high-risk (HR) participants genotyped. A significant association was also seen for all 87 participants.

depression are removed. Similarly, removal of SUD cases shows that risk remains significant [$F(1,78) = 8.98$, $p = .004$], as does risk × gender [$F(1,78) = 4.91$, $p = .03$].

We hypothesized that reduced volume of the right OFC in the HR group might reflect a developmental delay in reaching age-appropriate volume. To test this, right/left ratios were regressed on age and slopes were tested to determine if they differed from zero. For the female sample ($n = 50$), the slope did not differ from zero, remaining approximately the same with age (see Figure 3). In contrast, the relationship between age and the OFC ratios for the male sample ($n = 57$) showed a significant effect, with right/left ratios increasing with age for the HR male subjects only [$t(33) = 4.10$, $p = .001$; see Figure 4].

We hypothesized that genotypic variation in 5-HTT and the BDNF might explain the differing risk-group OFC ratios that reflect reduced right hemisphere volume. A significant interaction between the presence of the S allele of the 5-HTT gene and the Met allele of the VAL/Met variation of the BDNF gene was seen in association with volume of the OFC in the right hemisphere (Right – Left)/(Right + Left), corrected for ICV, for the 87 participants. For total volume, the BDNF × 5-HTT × risk interaction was significant [$F(1,77) = 5.55$, $p = .021$], although no main effects of either gene were seen. This interaction was also significant for white [$F(1,77) = 4.92$, $p = .03$] and for grey [$F(1,77) = 4.16$, $p = .045$] volumes. An analysis restricted to the 57 HR participants was also significant [$F(1,51) = 6.32$, $p = .015$] (Figure 5).

To test the possible contribution of right OFC volume to behavioral disinhibition, partial correlations were performed for right OFC volume (total, white, grey) and Control scale scores adjusting for left OFC volume and age. A highly significant relationship between Control scale scores and right OFC white matter [$r(74) = .36$, $p = .001$] was seen along with a significant relationship with grey matter [$r(74) = .28$, $p = .01$], although total right OFC volume was not significantly related to Control scale scores. Because white matter volume in the right hemisphere

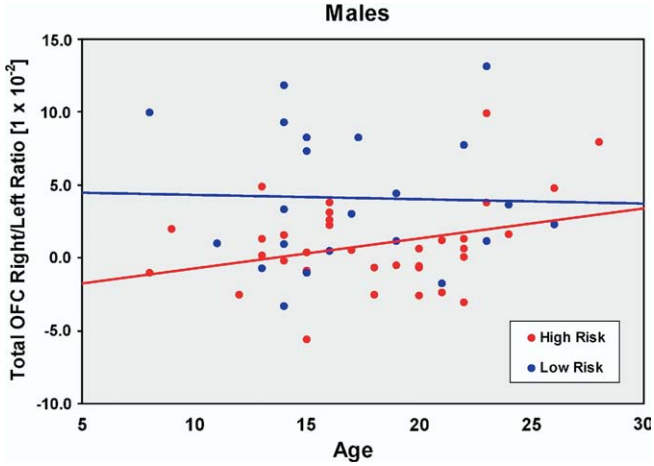

Figure 4. Regression lines for orbitofrontal cortex (OFC) ratios for male participants show a relatively flat progression from childhood to young adulthood for control subjects. In contrast, high-risk male subjects appears to show increased volume in the right hemisphere over the age range studied.

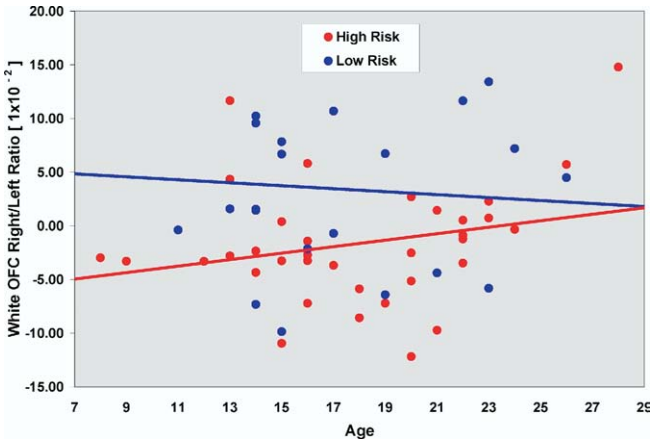

Figure 6. The growth in volume seen in high-risk male subjects (Figure 4) is largely due to increases in white matter volume. Orbitofrontal cortex (OFC) white matter volumes show a statistically significant correlation with Multidimensional Personality Questionnaire Control scale scores (reduced white matter being associated with greater impulsivity).

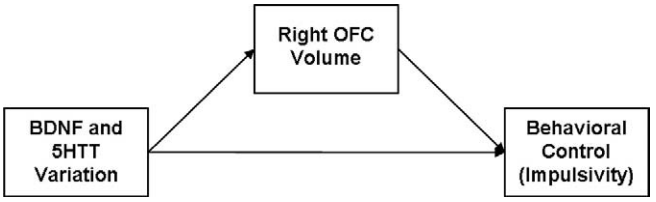

Figure 7. This empirically derived model suggests that developmental change in right orbitofrontal cortex (OFC) volume is influenced by the interaction of serotonin transporter (5-HTT) and brain-derived neurotrophic factor (BDNF) genes, which leads to developmental changes in impulsive behavior. With maturation, white matter volume increases in the OFC and leads to increasing behavioral control.

shows a greater age-related change in HR male subjects than in low-risk control subjects (Figure 6), it seems likely that delay in attaining white matter volumes for age in HR male subjects could result in greater disinhibited behavior for age. Collectively, the present results provide an empirical model (Figure 7) that would predict a relationship between genetic variation, right OFC volume, and Control scale scores.

A mixed model analysis was performed to test the main effect of the presence of any short allele of the 5-HTT polymorphism, any Met allele, and their interaction on Control scale scores, adjusting for age and gender. A significant relationship between the presence of the short 5-HTT allele and Control scores was seen [$F(1,69) = 7.15$, $p = .009$], as was the relationship between the BDNF allele and Control [$F(1,69) = 4.51$, $p = .037$], although the interaction of the two genes was not significant. However, including risk with the two genes showed a significant interaction between the BDNF gene and risk in prediction of Control scale scores [$F(1,65) = 8.79$, $p = .004$].

Discussion

Neuroimaging findings for AD adults (43) and adolescents (44) clearly suggest that alcohol has neuropathological effects on neuronal integrity. Reduced hippocampal volume (45) and smaller prefrontal cortex volume (44) along with neurocognitive changes (46) have been reported in adolescent-onset alcohol use disorders. What has not always been clear is whether the differences observed were antecedent to the development of AD. Reduced volumes of limbic structures have been observed in association with alcohol exposure and independently in association with familial/genetic susceptibility for AD. Adolescents with significant alcohol exposure show reduced hippocampal volume (45) that is not seen in HR male offspring with minimal alcohol exposure (47), although right amygdala volume is reduced.

The present results demonstrate reduced volume of the OFC in the right hemisphere in HR offspring selected for increased genetic risk (multiplex familial loading for AD). These results suggest that disruption in OFC laterality is antecedent to exposure to alcohol and drugs. This conclusion is supported by three considerations: 1) analyses demonstrating risk-group differences were performed with presence of SUDs as covariates, 2) supplemental analyses in which all 22 SUD cases were removed confirmed the significant risk-group differences, and 3) comorbid internalizing disorders do not explain these findings. Analyses performed with cases with depression or anxiety disorders removed show significant risk-group differences.

Drug/alcohol craving that leads to continued use in spite of adverse consequences suggests dysfunction of a neurologically based system designed for decision-making processes (48). The OFC has been a candidate region for addiction studies, because it is thought to be involved in inhibitory decision-making processes. Individuals with SUDs perform more poorly on the Iowa Gambling Task (26,49–51), a task that requires inhibitory decision making for successful performance.

Because the OFC has previously been reported to influence inhibitory decision-making processes (26), it was of interest to determine if volume of the OFC would be related to behavioral control (MPQ Control scale scores). With greater involvement of the right OFC in this process (26,52,53), it was of particular interest to determine whether an association between right OFC volume and Control scale scores would be seen. Our results suggest that the reduced volume of right OFC seen in HR offspring has implications for behavioral disinhibition and development of SUDs. Developmental changes in disinhibition appear to be most profound during adolescence and young adulthood. Structural MRIs acquired during childhood and adolescence (3–7) and even up to age 30 years (54) have consistently shown increases in total white matter volume. Diffusion tensor imaging of children and adolescents has shown increased white matter diffusion anistropy with age, particularly in prefrontal regions, suggesting that increased volume is due to increased myelination rather than loss of synapses (55). Age regression for OFC white matter volume shows a significant increase with age in our HR male subjects. Importantly, this dramatic increase appears to be due to the HR male subjects having less white matter at younger ages. The highly significant association between OFC white volume and MPQ Control scale scores is intriguing in suggesting that maturation of brain white matter pathways in the OFC might be important in regulation of emotional/cognitive processes involved in decision-making. A relationship between white matter microstructure and impulsivity in adolescence has previously been reported (56).

The present OFC findings and those seen for the amygdala (47), suggest that circuitry involving these structures might be altered in offspring from multiplex families through genetic mechanisms. There is substantial evidence that BDNF and 5-HTT contribute to central nervous system growth. The BDNF has an integral role in the normal development and plasticity of the cortex, with BDNF messenger RNA levels increasing by approximately one-third from infancy to young adulthood (57). Whereas BDNF and its receptor tyrosine kinase B (trkB) peak in the neonatal temporal cortex (57), the peak for prefrontal cortex occurs in young adulthood (58). This significant increase in BDNF at young adulthood corresponds to the point when the frontal cortex matures both structurally and functionally. Animal studies show lateralization of serotonin (5-HT) cortical innervation occurs in prefrontal and frontal regions (59), although the extent of lateralization of 5-HT innervation in humans is currently unknown. However, if greater 5-HT innervation is present in the right OFC, it might be influenced by BDNF expression, because this gene has previously been reported to influence 5-HT expression. Male HR participants show the greatest disparity in OFC volume in the right hemisphere but appear to "catch up" by young adulthood. This observation might be the result of genetic variation in genes influencing the growth of the OFC that, in turn, have implications for risk-taking behavior and development of SUDs.

It is important to note that total volume of the OFC did not differ between risk groups. Rather, volume of the right OFC, relative to the left, appear to characterize the offspring with increased genetic susceptibility to AD. Interestingly, all verte-

brates show lateral biases (preferences in use of a limb) due to brain specialization occurring at the population level (60) that might increase fitness. Most toads, chickens, and fish react faster when a predator approaches from the left, presumably as a result of genes that specify the direction of the asymmetry, genes that have been selected under "social" pressure (60). Both benefits and costs appear to be associated with lateralization. Reduced volume of the OFC in the right hemisphere might have conferred some selection advantage (those with greater tendency for risk-taking behavior are more likely to move on to new environments when the environment becomes adverse). However, risk-taking during adolescence can have lethal consequences. Identification of genetic variation associated with OFC reduction and increased impulsivity may provide important clues for medication development for those at highest risk.

This research was supported by Grants from the National Institute on Alcohol Abuse and Alcoholism AA05909, AA08082, and AA 015168.

The authors report no biomedical financial interests or potential conflicts of interest.

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