# Right Amygdala Volume in Adolescent and Young Adult Offspring from Families at High Risk for Developing Alcoholism

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**Background:** *Neurobiological factors have been implicated in the increased susceptibility for developing alcohol dependence that offspring from alcoholic families exhibit. The P300 component of the event-related potential shows developmental changes during childhood and adolescence that appear to be related to risk status. The underlying structural changes that accompany these neurophysiological changes are not well understood.*

**Methods:** *Magnetic resonance imaging was used to measure cerebral, amygdala, and hippocampal volumes in 17 high-risk adolescent and young adult offspring from multiplex alcoholism families and 17 age-, gender-, and IQ-matched control subjects without a family history for alcoholism or other substance dependence. Twenty-two of the subjects are part of a longitudinal prospective study and have been followed an average of 7.3 years, making it possible to relate P300 developmental trajectories to structural volumes.*

**Results:** *High-risk adolescents and young adults showed reduced right amygdala volume in comparison with control subjects. Right amygdala volume was significantly correlated with visual P300 amplitude.*

**Conclusions:** *Offspring from families having a high density of alcoholism differ in both neurophysiological and neuroanatomical characteristics that could not be explained by personal drinking history or particular childhood and adolescent psychopathology. Because the amygdala tends to increase in volume during childhood and adolescence, smaller volumes in high-risk children may indicate a developmental delay that parallels delays seen in visual P300 amplitude.* Biol Psychiatry 2001;49: 894–905 © *2001 Society of Biological Psychiatry*

**Key Words:** alcoholism, amygdala, P300, high-risk offspring

## Introduction

An extensive literature exists suggesting that children of alcoholics exhibit deficits in neuropsychological test performance (Drejer et al 1985; Knop et al 1985; Sher et al 1991). Verbal performance has been reported to be diminished in children of alcoholics (Knop et al 1985), as has nonverbal problem-solving skills including block design (Sher et al 1991) and Halstead category errors (Drejer et al 1985). Additionally, children of alcoholics have been reported to have poorer school achievement than children of nonalcoholics (Knop et al 1985; Marcus 1986; Sher et al 1991), although some studies have found no differences (Reich et al 1993; Vitaro et al 1996) and others have found only limited achievement deficits for high-risk children who were well matched for socioeconomic status (SES) and IQ to control subjects (Hill et al 1999a). In that study, Wide Range Achievement Test scores were significantly lower for math, but spelling and reading scores were not significantly different.

Although results for studies of school achievement have given mixed results, differences in P300 amplitude between offspring with high- and low-risk for alcoholism have frequently been observed (Berman et al 1993; Hill and Steinhauer 1993a; Hill et al 1990a, 1995a). Multiple studies have demonstrated that the amplitude of the P300 component of the event-related potential (ERP) is smaller in high-risk child and adolescent offspring of alcoholics than in control subjects (for review, see Polich et al 1994). The meta-analysis performed by Polich et al (1994) found that although P300 amplitude was reduced overall in high-risk compared with low-risk offspring, the differences were more pronounced in younger subjects and in those receiving a difficult visual discrimination task. Moreover, longitudinal data collected in our laboratory has identified electrophysiological differences between high- and low-risk children suggestive of developmental delays in cognitive development (Hill et al 1999a). Specifically, high-risk children and adolescents appear to be delayed in reaching age-appropriate P300 amplitude (Hill et al

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Received October 5, 2000; revised December 28, 2000; accepted January 4, 2001.

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0006-3223/01/$20.00
PII S0006-3223(01)01088-5

1999b). Using a longitudinal design, the children and adolescents were evaluated yearly with oddball tasks (two auditory paradigms and one visual) designed to elicit event-related potentials. Growth curves obtained for these children and adolescents studied between the ages of 8 and 18 showed differing patterns by modality (visual P300 shows a steady decline whereas auditory P300 shows an increase), with a consistent trend for the high-risk children to be delayed in reaching age-appropriate P300 levels when compared with control subjects.

Other evidence for subtle developmental delays in neurological capacity has been provided by longitudinal assessment of postural sway in these high- and low-risk children and adolescents (Hill et al 2000a). Using a sensitive movement platform, children and adolescents were evaluated yearly to determine the amount of sway present when the participants were asked to stand as steady as possible while assuming standard postural stances. Age-related improvements in postural balance during childhood have been documented in our laboratory (Hill et al 2000a) and others (Shumway-Cook and Woollacott 1985; Usui et al 1995). Using cross-sectional analyses, several groups have found that high-risk children show greater body sway than do control children (Hegedus et al 1984; Hill and Steinhauer1993b; Lester and Carpenter 1985; Lipscomb et al 1979). Follow-up of the same children at annual intervals during childhood and adolescence confirms that overall, in comparison to control children, high-risk children exhibit greater body sway and moreover appear to be delayed in achieving age-appropriate postural control (Hill et al 2000a). Together, the neuropsychological, ERP, and postural results suggest that subtle differences in neurocognitive capacity may be characteristic of offspring from alcoholic families. To date, these domains have not been investigated further using either structural or functional imaging techniques to explore possible sources of identified developmental lags.

Although neuroimaging studies have become quite common in the alcoholism literature, the emphasis has been on either documenting brain pathologies in long-term adult alcoholics or more recently in uncovering neuropathologies thought to be the result of adolescent substance abuse (De Bellis et al 2000a). De Bellis and colleagues have reported that alcohol-dependent adolescents have significantly smaller hippocampal volume in comparison with age- and gender-matched control subjects. These differences have been interpreted as being the result of excessive use of alcohol, although the authors readily acknowledge that these structural differences may have been present before the initiation of drinking. Similarly, Brown et al (2000) have shown that alcohol-dependent adolescents show significantly poorer neuropsychological performance including lower WISC-R Vocabulary, Information, Similarities, and Coding scores and reduced reproduction retention rates in comparison with control subjects. Additionally, evidence of a recent episode of alcohol withdrawal correlated significantly with block design scores. These observed differences in alcohol-dependent youth could be the result of alcohol use or, alternatively, may be neurobiological markers of vulnerability that are present before alcohol initiation.

Based on the longitudinal data suggesting that high-risk children experience developmental delays in acquiring age-appropriate postural control (Hill et al 2000a) and age-appropriate P300 (Hill et al 1999b), we hypothesized that brain areas that are changing rapidly during adolescence might show volumetric differences between high- and low-risk children and adolescents. Both progressive and regressive processes are characteristic of adolescence (Cowan et al 1984; Giedd et al 1996a, 1996b, 1999; Jernigan et al 1991; Jernigan and Sowell 1997; Jernigan and Tallal 1990; Paus et al 1999; Pfefferbaum et al 1994; Thompson et al 2000). In contrast to the decreases in cortical gray matter that is seen up to the age of 30, there is evidence for continued growth during the same developmental period in subcortical limbic structures including the septal area, hippocampus, and amygdala that show an increase in volume (Jernigan and Sowell 1997). The present study targeted the amygdala and hippocampus for volumetric analysis using a region of interest technique. This decision was based on four considerations.

First, neural generators for P300 have been described in both cortical and subcortical regions (Kropotov and Etlinger 1991; Mecklinger et al 1998), including the hippocampus (Halgren et al 1980; Yingling and Hosobuchi 1984). Although some have argued that the hippocampal formation may influence P300 only indirectly (Molnar 1994; Polich 1998) through the interaction of hippocampal–temporal-parietal functioning with the frontal lobe, the hippocampus nevertheless is thought to play a role in P300 production. Because P300 generation undoubtedly involves subcortical areas, we reasoned that the developmental changes in P300 previously observed (Hill et al 1999b) might reflect regressive or progressive events in subcortical anatomy during childhood and adolescence. Second, the amygdala has been directly implicated in the reinforcing effects of alcohol; microinjection of GABA and opioid antagonists in the central nucleus of the amygdala are quite effective in decreasing the acute reinforcing effects of ethanol (Heyser et al 1995; Hyytia and Koob 1995). Third, adolescent substance abusers have been found to have smaller hippocampal volumes than control subjects without substance abuse (De Bellis et al 2000a), a finding that may reflect alcohol use during adolescence or, alternatively, greater familial loading of alcoholism in those individuals who become alcohol

dependent by adolescence. If the latter interpretation is correct, we speculated that familial or genetic differences in subcortical structures might be found when high- and low-risk for alcoholism offspring are compared. Fourth, the hippocampus and amygdala can be measured noninvasively and reliably in children using MRI (De Bellis et al 2000a).

In addition to our hypothesis that high-risk offspring would show volumetric differences in selected subcortical structures, we also hypothesized that a relationship would exist between the developmental pattern seen for P300 amplitude and the volume of the targeted subcortical structures. Although the general tendency is for high-risk children to show smaller P300 amplitude than control subjects (Berman et al 1993; Hill et al 1990a; Hill and Steinhauer 1993a), variation clearly exists. Also, there is considerable variation in outcome among high-risk offspring; not all high-risk offspring develop substance dependence. Some of this variation in substance abuse outcome appears to be related to P300 amplitude (Berman et al 1993; Hill et al 1995b). We speculated that the mediating variable responsible for variations in outcome and P300 production might be a subcortical structure showing rapid alteration during child and adolescent development and associated with P300 generation. Therefore, we hypothesized that offspring showing the most extreme P300 trajectories, consistently lower or higher P300 throughout the developmental period studied, might be most likely to exhibit structural differences in the subcortical regions of interest.

A large cohort of children and adolescents are being followed for whom annual assessments of P300 and other measures are obtained (see Hill et al 2000b). Data obtained during childhood and adolescence has allowed for determination of the growth curve trajectories of high- and low-risk children and adolescents. High-risk individuals appear to show a developmental delay in achieving age-appropriate P300 amplitude (Hill et al 1999b). Existence of this data set made it possible to calculate quartiles for P300 amplitude by age and gender. For the MRI study, high-risk individuals were recruited from among the larger follow-up cohort so that those consistently showing P300 amplitude in the lowest quartile (at least two of the repeated measures fell in this quartile) could be contrasted with those in the highest quartile. Among the high-risk participants in the MRI study, seven had shown consistently low P300 over the developmental period of observation (9.4–16.7 years on average), whereas nine of the individuals scanned had been observed to be in the top quartile of the larger longitudinal cohort. Selection from among these two extreme patterns made it possible to determine if a relationship between the extreme endophenotype "consistently lower P300 amplitude" and the "consistently higher P300 amplitude" groups and volume of the amygdala and hippocampus might be manifest.

## Methods and Materials

### *Subjects*

A sample of 34 adolescent and young adult male participants was studied. The high-risk group and a subset of the control subjects ($n = 22$) were members of the larger longitudinal cohort of offspring from high-density for alcoholism pedigrees initiated in 1990. The high-risk offspring were from multiplex alcoholism families selected through the presence of a pair of adult alcoholic brothers. As a result, each high-risk offspring had an average of four first- and second-degree relatives who were alcoholic. Low-risk offspring from the larger longitudinal cohort were identified through their families who were selected for absence of Axis I psychopathology. Low-risk historical control subjects ($n = 12$) were similarly selected for absence of Axis I psychopathology.

Participants who were members of the longitudinal cohort had been followed an average of 7.3 years (± 2.1 SD) and had received yearly evaluations that included a clinical interview to determine diagnostic status, administration of visual and auditory paradigms designed to elicit ERPs and to reliably assess P300 amplitude, and administration of a short battery of neuropsychological tests. Participants who were members of the longitudinal cohort were evaluated for neuropsychological performance on the occasion of their initial follow-up visit to our laboratory. This included administration of subtests from the Wechsler Intelligence Scale for Children (WISC-R) and administration of the Peabody Picture Vocabulary Test (PPVT-R) for determination of IQ. The PPVT-R was used as an indicator of overall intelligence because it does not require reading skill for completion. The WISC-R subtests given at the baseline evaluation included vocabulary and block design.

Because of the known variability in volume of brain structures during development (Giedd et al 1996a, 1996b; Pfefferbaum et al 1994), a yoked control design was used in which a control subject was selected on a case-by-case basis to match each high-risk case using gender and age (within 6 months) as matching characteristics. Where an appropriate gender and age-matched control subject was not available from the longitudinal follow-up, an historical control was included from the files of the coauthors (MDDB and MSK). Control subjects were selected with the intent of equalizing groups for IQ, SES, and handedness (all but one subject was right-handed). Handedness was determined using the subject's stated hand preference and confirmed by noting the number of handedness items scored for each hand using the Revised Physical and Neurologic Examination for Subtle Signs (PANESS) inventory (Denckla 1985) where 13 items are used to define right handedness. Socioeconomic status was determined using the Hollingshead Four Factor Index (Hollingshead 1975), which uses both education and occupation to determine the appropriate SES. As may be seen in Table 1, the high- and low-risk groups were well matched on age, SES, IQ, height, and weight. Only a single subject was left handed.

Table 1. Demographic Characteristics of High- and Low-Risk Male Adolescent and Young Adults

| | High risk | | Low risk | | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | *F* | *p* |
| Age | 17.6 | 2.9 | 17.3 | 2.2 | 0.13 | NS |
| SES | 40.7 | 10.6 | 43.4 | 14.6 | 0.40 | NS |
| IQ | 110.1 | 16.2 | 112.8 | 12.3 | 0.30 | NS |
| Height | 71.0 | 3.5 | 69.9 | 3.1 | 0.92 | NS |
| Weight | 166.7 | 38.2 | 186.4 | 36.0 | 2.38 | NS |

*df* = 1,32. SES, socioeconomic status.

## *Clinical Evaluation*

All children and adolescents were administered the Schedule for Affective Disorders and Schizophrenia for School-Aged Children (K-SADS; Chambers et al 1985) interview by trained clinical interviewers (M.A. in psychology) at each annual evaluation. K-SADS interviewers had diagnostic reliability of 90% or greater with interviewers trained by the authors of the instrument. The parent who accompanied the child to the testing session (usually the mother) also participated in the K-SADS, answering the same questions asked of the child in a separate interview. Also, the mother was queried extensively regarding alcohol, drug, and cigarette use during pregnancy as previously described (Hill et al 2000a; Steinhauer and Hill 1993).

A 3rd- or 4th-year resident who was specializing in child psychiatry in an integrated general and child psychiatry program conducted an unstructured interview independently with both the child and the parent. Both the interviewer and the psychiatrist were blind to the risk status of the subject's family. The presence of the following selected disorders was determined through a "best-estimate" consensus diagnosis between the interviewer and the psychiatrist: depression (major depression and dysthymia), phobia, anxiety disorders (panic disorder, separation anxiety, overanxious disorder), attention-deficit/hyperactivity disorder (ADHD), conduct disorder, substance abuse/dependence disorders, and oppositional disorder. Any discrepancies were resolved in the presence of a third clinician. Diagnoses of the 34 individuals who participated in the magnetic resonance imaging (MRI) study may be seen in Table 2.

Table 2. Percentage of Cases with a Lifetime Diagnosis by K-SADS Interviews ($N = 34$)

| Diagnosis | High risk | Low risk |
|---|---|---|
| Alcohol or drug dependence | 18.2 | 0 |
| ADHD | 27.3 | 0 |
| Anxiety disorders | 36.4 | 0 |
| Depression | 9.1 | 0 |
| Tic disorder | 4.5 | 0 |
| Oppositional/conduct disorder | 22.7 | 0 |
| No diagnosis | 36.4 | 100 |

Note that the high-risk children are all part of a longitudinal follow-up and have received annual diagnostic procedures. Some of the low-risk control subjects ($n = 5$) were similarly evaluated at yearly intervals. The remainder were evaluated a single time before being scanned and are historical control subjects from the files of the coauthors (MSK and MDDB). Anxiety disorders included simple phobia, overanxious disorder, separation anxiety, and social anxiety. ADHD, attention-deficit/hyperactivity disorder; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Aged Children.

## *Event-Related Potential Assessments*

Each child and adolescent performed an auditory (Choice Reaction Time) task and a visual ERP task with electrodes placed at frontal, vertex, parietal, and occipital locations (Fz, Cz, Pz, Oz, P3, P4) at each follow-up assessment. Auditory ERPs were elicited with "high" (1500 Hz) and "low" pitched (800 Hz) tones, presented every 3 sec (70 dBA intensity; 40-msec duration, 2-msec rise/fall time) in a modified oddball paradigm as previously described (Hill et al 1990a; Steinhauer and Hill 1993). The visual task consisted of presenting brief (33-msec) target or nontarget stimuli. The target condition consisted of a stick-figure "head" with a nose and only one ear. The subject responded to the position of the ear with a button press as previously described (Hill and Steinhauer 1993a). P300 amplitude was measured at the midline parietal site.

## *Acquisition of MRI Scans*

The MRI scans were performed at the University of Pittsburgh Medical Center Magnetic Resonance Research Center by using a Signa 1.5-T system (GE Medical Systems, Milwaukee). A sagittal scout series verified subject position, cooperation, and image quality. A three-dimensional, spoiled gradient recalled acquisition in the steady state pulse sequence was used to obtain 124 contiguous images with slice thickness of 1.5 mm in the coronal plane (TE = 5 msec, TR = 25 msec, flip angle = 40°, acquisition matrix = 256 × 192, number of excitations = 1, field of view = 24 cm). Coronal sections were obtained perpendicular to the anterior commissure–posterior commissure line to provide a more reproducible guide for image orientation. Axial proton density and $T_2$-weighted images were obtained to enable exclusion of structural abnormalities on the MRI scan. All subjects tolerated the procedure well. No sedation was used.

## *Region of Interest Analysis*

The imaging data were transferred from the MRI unit to a computer workstation in the Developmental Traumatology Neuroimaging Laboratory and analyzed using the IMAGE software (version 1.52) developed at the National Institute of Health (Rasband 1996), which provides valid and reliable volume measurements of specific structures by using a semiautomated segmentation approach. All anatomical measurements were made by a trained and reliable rater (JH) who was blind to subject information. This individual was supervised by one of the authors (MDDB), who reviewed all tracings and final measure-

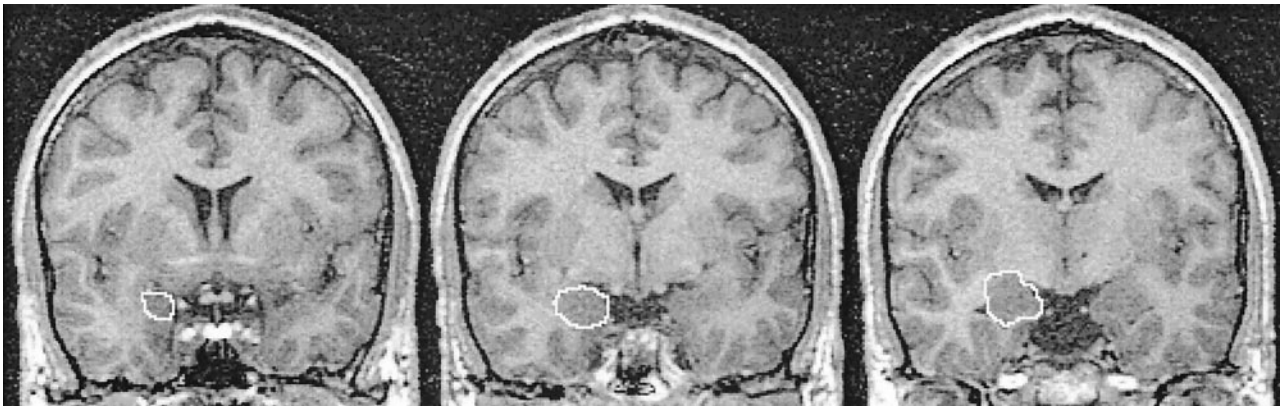

Figure 1. This figure illustrates the manual tracing technique used in quantifying the right amygdala. Starting at the left, the first full visualization of the amygdala is outlined. The middle image is posterior to the first visualization and is included to show the approximate midpoint of the amygdala. The image at the right illustrates the end point of the amygdala, defined as the slice with the most anterior visualization of the mamillary bodies (landmark method).

ments. Interrater reliabilities for this laboratory and detailed descriptions of the region of interest methodology have been described previously (De Bellis et al 1999) and are only briefly presented here.

Intracranial volumes were calculated by summing areas of successive coronal slices, including gray and white matter and cerebrospinal fluid (CSF) volumes, and multiplying by slice thickness. Cerebral volumes were measured in the same manner after exclusion of cerebellum and brain stem.

Total cerebral gray and white matter volumes were calculated by using a semiautomated segmentation algorithm. This computerized segmentation technique uses mathematically derived cutoffs for gray matter–white matter CSF partitions with histograms of signal intensities.

Figure 1 presents the method used to manually trace the amygdala and hippocampus in the coronal plane. The areas were summed for each slice and multiplied by slice thickness, using 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. This landmark method is commonly used in psychiatric studies of children and adolescents using MRI (Castellanos et al 1996; De Bellis et al 1999; 2000a; 2000b; Giedd et al 1996b). 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. Our measurement of the hippocampal formation included the cornu ammonis, dentate gyrus, and subiculum.

## Results

All of the categorical demographic variables were tested to determine if differences between groups were present. As may be seen in Table 1, no differences were seen for these variables. Also, none of the mothers of these offspring reported heavy use of alcohol or drugs during pregnancy. Although the subjects were well-matched for IQ and SES, they did differ with respect to neuropsychological performance at entry into the study. For the 22 children who were part of the longitudinal follow-up, we found risk differences in block design and vocabulary scores ($t = 3.75$, $df = 20$, $p = .001$ and $t = 2.16$, $df = 20$, $p = .04$, respectively). Similar findings of poorer block design and vocabulary performance in high-risk offspring have been obtained using the full longitudinal sample (Hill, unpublished data). Additionally, the high-risk children who were scanned were more likely to have a lifetime childhood or adolescent disorder than control subjects, consistent with our previously published data from the larger sample (Hill et al 1999a).

Data for all regions of interest were statistically analyzed using an ANOVA for a two-group comparison after controlling for individual differences in intracranial volume. Alcohol consumption in the past month was used as a covariate to control for possible effects of drinking on tissue volumes for the brain structures of interest. Analyses were conducted separately for right and left amygdala, total amygdala, right and left hippocampus and total hippocampus and intracranial volume (see Tables 3 and 4). Data were checked for normality (Shapiro and Wilks $W$ statistic) before conducting the parametric analyses. The distribution of volumes for the structures of interest met normality requirements. No significant differences were seen for the hippocampus (total, left or right), total amygdala, left amygdala, or for total intracranial volume (see Table 3). Based on analysis of all 34 cases, significant differences were seen for the right amygdala ($F = 6.72$, $df = 1,32$, $p = .014$). When this analysis was repeated using alcohol use in the past month as a covariate (information available for 22 individuals), the results remained significant ($F = 6.54$, $df = 1,19$, $p = .02$). There appears to be no evidence that the findings obtained were due to alcohol consumption differences between the high-

Table 3. Global Brain Volumes of High- and Low-Risk Control Children and Adolescents (Mean ± Standard Deviation)

| | High risk ($N = 17$) | | Low risk ($N = 17$) | | Statistic | |
|---|---|---|---|---|---|---|
| Structure ($cm^3$) | Mean | SD | Mean | SD | $t$ ($df = 32$) | $p$ |
| Intracranial volume | 1569.66 | ±96.05 | 1528.92 | ±134.87 | 1.10 | .32 |
| Cerebral volume | 1356.61 | ±89.00 | 1321.34 | ±118.06 | 0.98 | .33 |
| Cerebral gray matter | 806.42 | ±70.41 | 781.86 | ±57.55 | 1.11 | .27 |
| Cerebral white matter | 517.56 | ±59.06 | 502.51 | ±72.64 | 0.66 | .51 |

and low-risk groups. The correlation between drinking in the past month and right amygdala volume was nonsignificant ($r = -.17$, $df = 20$, $p = .45$).

Figure 2 presents data points resulting from volumetric calculation of the right amygdala with each data point adjusted for the subject's intracranial volume. Because the results were significant for the right but not the left amygdala in the two group comparisons, further analyses were performed ($2 \times 2$ ANOVA) to determine if an interaction between risk group and laterality would be found. In this analysis, each individuals right and left amygdala volumes were analyzed along with risk status. A significant risk by laterality finding ($F = 5.37$, $df = 1,32$, $p = .027$) was revealed, indicating that persons with familial loading for alcohol dependence differ from control subjects by having reduced amygdala volume that is specific to the right side.

These results suggest that the volume of the right amygdala is smaller in individuals who carry an increased susceptibility for developing alcohol dependence. Moreover, this morphological difference appears to be present before drinking or drug use begins and reflects a possible neurobiological vulnerability to substance dependence rather than a result of excessive drinking or drug use.

Analyses were also performed to determine the relationship between P300 amplitude during the child and adolescent developmental period and the size of the amygdala measured at an average age of approximately 17 years. Those children who were in the lowest quartile for visual P300 amplitude when assessed an average of seven times at yearly intervals were assigned to the Lower P300 group, whereas those who were in the upper quartile over the longitudinal observation were assigned to the Higher P300 group. An ANOVA was performed to compare the higher and lower P300 groups with respect to amygdala volume and controlling for a large number of covariates (SES, weight, height, IQ, and alcohol consumption in the past month). This analysis revealed that those in the Higher P300 group tended to have smaller right amygdala volumes than those in the Lower P300 group ($F = 8.09$, $df = 1,14$, $p = .013$). Comparison of each subgroup with control subjects revealed significant differences between the Higher P300 group and control subjects ($F = 13.14$, $df = 1,24$, $p = .0014$) but no differences for the Lower P300 high-risk group and control subjects ($F = 0.86$, $df = 1,22$, $p = .36$). (None of the covariates were significant.)

To further examine this relationship, P300 amplitude for each modality was correlated with the right amygdala volume using the earliest age that it was obtained for the majority of subjects (approximately age 11) and for the most recent measure taken for the majority of participants (approximately age 17). Figure 3 presents the relationship between right amygdala volume and visual P300 amplitude obtained for the high-risk subjects studied. Correlations performed between the right amygdala volume and visual or auditory P300 measured at age 11 and at age 17 revealed a substantial significant relationship between visual P300 and right amygdala volume at age 11 and 17, $r = -.55$, $df = 15$, $p = .02$, and $-.50$, $df = 15$, $p = .04$, respectively. Correlations between auditory P300 at age 11 and 17 were not significant ($r = -.12$, $df = 15$, $p = .64$, and $-.20$, $df = 15$, $p = .45$ respectively). These results strongly suggest that individuals exhibiting a visual P300 pattern in which the visual P300 amplitude shows the least rate of decline, remaining in the higher quartile for age over the multiple assessments performed over the 8- to 18-year-old period studied, are those for whom the greatest visual P300 developmental delay may be present. It

Table 4. Regional Brain Volumes of High- and Low-Risk Control Children and Adolescents (Mean ± Standard Deviation)

| | High risk ($N = 17$) | | Low risk ($N = 17$) | | Statistic | |
|---|---|---|---|---|---|---|
| Structure | Mean | SD | Mean | SD | $t$ ($df = 32$) | $p$ |
| Right amygdala[a] | 2.96 | ±0.45 | 3.37 | ±0.60 | −2.63 | .01 |
| Left amygdala[a] | 2.62 | ±0.49 | 2.60 | ±0.47 | −0.28 | .78 |
| Right hippocampus[a] | 4.33 | ±0.77 | 4.24 | ±0.72 | −0.13 | .89 |
| Left hippocampus[a] | 4.14 | ±0.67 | 4.32 | ±0.61 | −1.56 | .13 |

[a]These values were adjusted for intracranial volume before statistical analyses were performed.

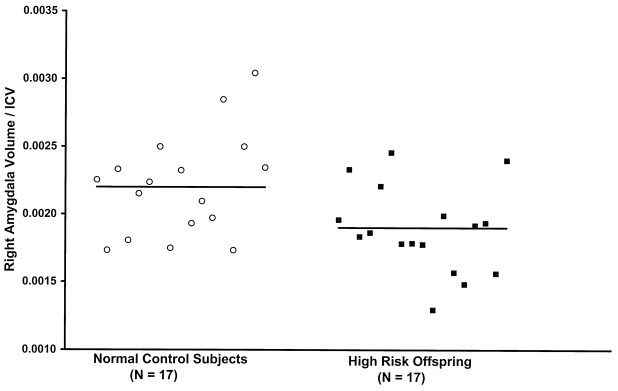


Figure 2. Right amygdala volume of individual subjects is plotted by group with low-risk control subjects on the left and the high-risk group on the right. Each volume was divided by intracranial volume before plotting. The horizontal line represents the mean of the group.

should be recalled that analysis of the larger cohort revealed a nonlinear trajectory in which the amplitude of the visual P300 is higher at age 8 than at age 18. The smaller right amygdala volume observed in the high-risk offspring suggests a maturational lag (Jernigan and Sowell 1997). Therefore, our results demonstrating that high-risk individuals who show the greatest delay in achieving age-appropriate visual P300 appear to have smaller amygdala volumes are consistent.

## Discussion

### *The Amygdala and Addiction*

Our results suggest that high-risk offspring differ from control subjects both neuroanatomically and neurophysiologically. One possibility is that genetic factors predispose the high-risk offspring to differ from control subjects. These differences cannot be explained by personal drinking histories or the mother's prenatal use of substances. Why these differences are specific to the right amygdala is unknown; however, there is evidence that lateralized neuroanatomical anomalies occur in association with psychiatric disorders, especially schizophrenia (Crow et al 1989; Jacobsen et al 1998). Demonstration that developmental changes proceed at varying rates from side to side for specific structures would ensure that findings seen only on the right or left side are not spurious or artifactual. For example, a faster rate of growth in the right versus left brain during childhood and adolescence might make it more likely that developmental lags would be seen in structures on the right. Differential development of the hemispheres has been suggested using autopsy material that compared infants and adults (Wada et al 1975) and more recently by MRI assessment in adolescents receiving follow-up scans (Jacobsen et al 1998). The right amygdala was found to be larger than the left for both healthy control subjects and childhood onset schizophrenics. Of particular relevance to the present findings, Jacobsen and colleagues (1998) found that over a 2-year follow-up period, the healthy control adolescents exhibited increases in right amygdala volume, whereas left amygdala volume decreased slightly, confirming that lateralized differential development occurs. The observed reduction in volume of the right amygdala seen in offspring from families with a high density for alcohol dependence suggests that these offspring may be delayed in reaching age-appropriate volumes. How and what specific genes might regulate this process during adolescence is currently unknown; however, documented differences in developmental trajectories of the temporal lobe have been suggested for childhood onset schizophrenia (Jacobsen et al 1998).

The present findings showing decreased amygdala volume in high-risk offspring is particularly interesting in view of speculations concerning the extended amygdala system that has been implicated in the etiology of drug and alcohol addiction (Koob 1999). Koob (1999) hypothesized that the acute reinforcing actions of drugs of abuse may be mediated by the striatopallidal and extended amygdala systems that includes the shell of nucleus accumbens, the central nucleus of the amygdala, and the sublenticular extended amygdala. In this view, chronic administration of drugs of abuse leads to increasing dysregulation of brain reward systems. Changes in neurochemical components of the extended amygdala, including decreases in dopamine and serotonin neurotransmission in the nucleus accumbens and increases in corticotropin releasing factor in the central nucleus of the amygdala, are thought to produce changes in the hedonic set point, leading to compulsive drug seeking and drug use (for review, see Koob 1999).

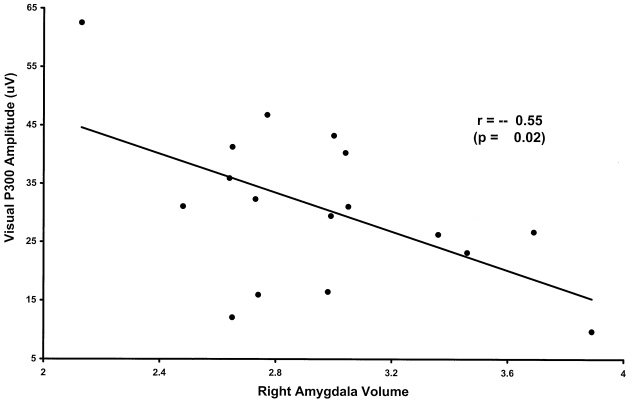


Figure 3. The amplitude of visual P300 determined when the subjects averaged 11 years of age is plotted with right amygdala volume. The correlation between right amygdala volume measured at a mean age of 17 and P300 amplitude measured at age 17 was also significant.

### *The Amygdala and Emotional Facial Expression*

Studies of animals (LeDoux 1956) and brain-damaged patients (Adolphs et al 1998; Bechara et al 1995) indicate a central role for the amygdala in emotional learning. Functional MRI studies of conditioned fear and extinction suggest that the amygdala contributes to both acquisition and extinction of associative emotional learning tasks in humans (LaBar et al 1998). Early lesion studies of both monkeys (Weiskrantz 1956) and rats (Blanchard and Blanchard 1972) also have implicated the amygdala in emotional behaviors. Electric stimulation of the amygdala results in fearful behavior (e.g., freezing), increased heart rate, increased blood pressure, and increases in plasma corticosteriods (for review, see Davis 1992). These responses appear to be eliminated by lesions of the amygdala (for review, see Davis 1997). Additionally, the amygdala has been long recognized for its influence on social behavior (Rosvold et al 1954; Kling and Brothers 1992). Studies of subjects with complete bilateral amygdalar damage who are asked to judge faces for their "approachability and trustworthiness" fail in doing so when cues are visual but have normal judgment when characteristics are presented to them verbally (Adolphs et al 1998). The amygdala appears to be an important neural component that aids in the retrieval of socially relevant knowledge gained through appraisal of facial appearance.

Recently, amygdalar function has been shown to be highly sophisticated with respect to an individual's ability to discriminate between stimuli on the basis of their acquired behavioral significance. This ability has been shown to be anatomically lateralized with the right amygdala having a greater role in conditioning occurring outside the individual's awareness (Morris et al 1998). These investigators analyzed positron emission tomographic data acquired by presenting two angry faces, one of which was paired (CS+) and the other unpaired (CS−) with an unconditioned stimulus (UCS), a 100-dB burst of white noise. Following conditioning, the CS+ and CS− faces were presented sequentially in either a masked or unmasked condition (masking consisted of presentation of a neutral face that was either a target or nontarget) and without the UCS. Subject awareness was determined by asking subjects to report what they saw. Masked faces were detected in 0% of cases, whereas the unmasked faces could be detected 100% of the time. A significant response in the region of the right amygdala (predominantly medial and inferior portion of the complex) was seen to the presentation of the masked CS+ faces with no response seen in the left amygdala to masked CS+ stimuli. In contrast, there was a left-sided activation by unmasked CS+ and CS− faces (subject was aware of the angry face). The right amygdala response occurred to the masked conditioned faces (previous pairing with the UCS) that were not consciously perceived. Characteristics of the stimulus (both were angry faces) were not responsible for the response but rather on the associative history of the stimulus. These new findings clearly indicate that lateralization of amygdalar response occurs as a function of the level of awareness of target stimuli. A similar specificity of response has been suggested by brain recordings in monkeys in which a similar lateralized region of the amygdala was reported to contain units selective for faces and visually aversive cues (Leonard et al 1985). The findings of Morris et al (1998) and Leonard et al (1985) are also consistent with previously published findings indicating a right-hemisphere advantage for processing emotional facial expressions (DeKosky et al 1980; Gazzaniga and Smylie 1983).

### *The Amygdala, Emotional Learning, and High-Risk Status*

The findings indicating reduced volume of the right amygdala in high-risk offspring are intriguing in view of current knowledge concerning the role of the amygdala in emotional learning and capacity for making accurate social appraisals. To the extent that the amygdala is necessary in the retrieval of information on the basis of prior social experience with certain classes of faces, those with overt damage (e.g., bilateral amygdalar damage) are clearly disadvantaged. Whether those with more covert alteration (reduced amygdalar volume) are disadvantaged is open to question. Might it be the case that individuals with such a deficit find it difficult to maintain friendships because of misunderstandings arising from misreading of visual social cues? Alcoholics report poorer social skills than nonalcoholics (Miller 1978). Some of these deficits may precede the onset of alcohol dependence. Adult alcoholics and their high-risk nonalcoholic adult relatives report higher levels of alienation than do control subjects (Hill 1993; Hill et al 1990b). Persons scoring high on this trait, as measured by the Multidimensional Personality Questionnaire (Tellegen et al 1985, 1988), report believing they are mistreated, that others wish them harm, and that they often feel betrayed and used by "friends."

Much of our social learning involves incidental memory and is often outside our conscious awareness. The recent findings demonstrating a unique role for the right amygdala in this process (Morris et al 1998) further suggest the importance of adequate functioning of the right amygdala. Although volume reduction does not necessarily imply functional impairment, the reduction in right amygdala volume seen among the high-risk offspring does suggest that further study of amygdala is warranted.

### *Amygdala Volume and Addiction Potential and Relapse*

If a functional alteration is associated with structural alteration of the right amygdala, reduced right amygdala volume may have implications for acquisition of visually acquired reinforcers associated with drug and alcohol use. Individuals with smaller right amygdala volume may develop conditioned responses to previously neutral visual stimuli more easily in association with drug or alcohol use or, alternatively, experience negative reinforcement in association with environments with visual cues associated with drug withdrawal more readily than individuals with larger amygdala volume.

Subjective reports of euphoria associated with saline injections have been noted among heroin abusers, especially when the injections take place in the abuser's usual injection environment (Mirin and Meyer 1979). Similarly, conditioned withdrawal has been reported for patients returning to environments associated with drug dependence (O'Brien 1975). Because the right amygdala appears to be activated more readily in association with stimuli for which the individual is unaware (Morris et al 1998), the potential number of neutral stimuli that can acquire reinforcer status may be much larger than those for which an individual has awareness and can control, to some degree, by limiting exposure. Relapse prevention programs that rely heavily on cognitive therapy utilize the individual's self-reported reinforcement history to target places, people, and situations that are known to be associated with relapse to train the individual in avoiding such stimuli or modifying the response to the cues (Marlatt 1990); however, neutral stimuli can become reinforcers without the individual's awareness. Acquisition of these reinforcers appear to be dependent on the functional integrity of the right amygdala. Our results demonstrating that offspring from families with a high-density for alcoholism have smaller amygdalar volume suggest that this structural alteration may have implications for the development of addiction, possibly through the acquisition of reinforcers, many of which the individual may not be aware, from among a large array of previously neutral visual stimuli.

### *P300 Generation and the Orbital and Medial Prefrontal Cortex*

The greater association between visual P300 and right amygdala volume than between auditory P300 and right amygdala volume merits comment with respect to brain regions that have been implicated as possible P300 generators. The amygdala has been suggested as a possible source for P300 generation based on clinical data acquired in patients undergoing probing for epileptic foci in which endogenous potentials to infrequently occurring visual stimuli occurred in both hippocampus and the amygdala (Halgren et al 1980). Patients with amygdala lesions appear to be more impaired in visual as opposed to verbal emotional learning (Adolphs et al 1998). Moreover, the amygdala has extensive connections with the orbital and medial prefrontal cortex (OMPFC; Price 1999). The OMPFC has been described as having both cortico-cortical networks and limbic connections and appears to have a probable role in eating behavior, guidance of behavior, and regulation of mood (Price 1999). The OMPFC, which connects extensively with limbic areas including the amygdala, would appear to overlap the hippocampal–temporal-parietal regions currently thought to be involved in P300 generation (for review, see Polich and Comerchero, in press).

### *Developmental Lags in High-Risk Offspring and Amygdala Volume*

Developmental issues presented by our larger longitudinal cohort study have been shown to have relevance to the issue of neuroanatomical abnormalities in high-risk offspring. Previously, it has been demonstrated that in comparison with control subjects, high-risk children have a slower rate of change in P300 amplitude with increasing age along with lower amplitude at the youngest age studied. For the visual modality, the developmental task appears to be to exhibit a steady decline in amplitude with age and eventually approaching control levels by late adolescence or young adulthood (Hill et al 1999b). Therefore, the significant relationship seen between the pattern of P300 development and volume of the right amygdala is particularly interesting. The amygdala shows a steady increase in volume during childhood and adolescence (Giedd et al 1996a, 1996b, 1999; Jernigan et al 1991; Jernigan and Tallal 1990; Jernigan and Sowell 1997; Pfefferbaum et al 1994). Therefore, individuals showing reduced amygdala volume might be considered as delayed. The significant differences in amygdala volume seen for the offspring who had a consistently delayed visual P300 (Higher P300 group) in contrast to those who were in the Lower P300 group suggest that neuroanatomical changes during childhood and adolescence are related to developmental changes in visual P300. The reduced right amygdala volume seen in high-risk offspring could result in a functional deficit in emotional and social learning that is largely mediated through the visual modality. This deficit would be expected to be differentially related to visual, in contrast to auditory, P300.

This research was supported by the National Institute of Alcohol Abuse and Alcoholism (Grant Nos. AA 05909 and AA 080820) and an award from the University of Pittsburgh Medical Center, Functional Imaging Research Program, for the cost of the scans. The principal investigator thanks the many families who have participated over the years and especially those who made time for this study. The principal investigator wishes to acknowledge the assistance of other members of the clinical team, particularly Diane Muka, and the many residents in child psychiatry who have helped in the evaluations over the years, especially David Averbach, M.D. We thank those involved in ERP data collection over the course of the study (Jeannette Locke-Wellman, Colleen Konicky, Shawn Gronlund, Kathleen Walsh) and Stuart Steinhauer, Ph.D., for assistance in ERP peak detection.

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