Auditory Event-Related Potentials in Children at High Risk for Alcoholism*

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ABSTRACT. To determine if P300 and other event-related potentials (ERP) could serve as markers for risk of developing alcoholism, two groups of children (8-18 years old) were tested. The high-risk (HR) group consisted of 51 children with an average of 4.1 first- and second-degree relatives who were alcoholic. The low-risk group (LR) consisted of 42 children who had no first- or second-degree relatives who were alcoholic or met criteria for DSM-III Axis I psychopathology. Auditory stimuli varying in conditional probability were presented during a silent counting task, and during a choice reaction task. P300 amplitude was smaller in high-risk than low-risk children. When grouped according to gender and developmental status (8-12 and 13-18 year olds), P300 showed the greatest reduction for the older high-risk males compared to low-risk males. In addition, a previous finding was replicated: the prolonged centro-frontal negativity (232-352 msec), which decreased with age in low-risk children, showed significantly less reduction for high-risk children. Risk status was not related to either amplitudes of the N100 and P200 components of the ERP, or to latencies of any components. Decreases in P300 amplitude and delayed reduction of anterior negativity appear related to developmental processes in high-risk children. (J. Stud. Alcohol 54: 408-421, 1993)

THE P300 COMPONENT of the event-related potential (ERP) has received considerable attention as a possible neurophysiological risk marker for the development of alcoholism. P300 is a scalp-positive wave occurring approximately 300 msec after an informative event. The event-related potential is of interest for two reasons. First, long-latency components of ERPs, including P300, are associated with particular sensory and cognitive aspects of information processing (Donchin, 1979; Sutton et al., 1967). Second, the ERP waveform appears to be under genetic control (Bock, 1976; Polich and Burns, 1987; Rogers and Deary, 1991; Surwillo, 1980).

The promise of utilizing P300 as a marker for alcoholism risk has been clouded by controversy surrounding the fact that some research groups have presented strong evidence for P300 being a risk marker (Begleiter et al., 1984; Hill and Steinhauer, 1993; Whipple et al., 1988). Other groups have found moderate support (Elmasian et al., 1982; Hill et al., 1988; O’Connor et al., 1987). Still others have found no difference between high- and low-risk groups (Baribeau et al., 1987; Neville and Schmidt, 1985; Polich and Bloom, 1987, 1988; Polich et al., 1988a,b).

Understanding the critical differences in methodologies leading to these divergent results is of considerable interest. The age of the high-risk subjects employed (prepubescent and postpubescent minors, and young adults), as well as the proportion of males and females utilized, has varied across studies. Differences in task difficulty and the modality of stimulus presentation are additional factors that have not been systematically varied using the same subject pool. Some studies have employed ethanol administration to assess alterations in cognitive performance among high- and low-risk subjects, where the majority have not utilized any. Finally, criteria for “high-risk” status has varied considerably across studies.

While some of the variation in ERP results may be due to differences in task demands (modality, task difficulty with respect to age), the psychiatric background of the individual assessed is of equal importance. Few studies have systematically assessed the offspring (in some cases minor children) for psychopathology to determine if the high-risk for alcoholism group of offspring, often referred to as the family history positive group (FHP), has elevated rates. If higher rates of psychopathology were present (e.g., conduct disorder) among the FHP group, then any decrements in P300 might be due to this factor rather than to an alcoholism diathesis factor.

We have reserved the use of the term “high risk” to denote cases where the familial constellation is of sufficient density to produce lifetime risk of 50% or greater for young males. For an individual to have a predicted recurrence risk of this magnitude, multiple relatives must be alcoholic (Aston and Hill, 1990). Most studies using the family history positive and family history negative (FHN)
dichotomy to determine presence or absence of alcoholism among first- or second-degree relatives of these offspring utilize the family history report of a single informant to dichotomize the families. Therefore, “FHP” offspring are not necessarily at high-risk for developing alcoholism inasmuch as their single relative may be a sporadic (non-genetic) case. Administration of face-to-face structured psychiatric interviews for all first- and second-degree relatives of these offspring, or where this is impossible, family history of multiple informants of the offspring studied, is the ideal situation for classification, a standard that has not yet been adopted except in our own laboratory (Hill et al., 1990; Hill and Steinhauer, 1993).

Most of the early ERP studies of high-risk populations were conducted using auditory paradigms. Elmasian et al. (1982) compared young adult university students with (FHP) and without (FHN) a family history of alcoholism, finding reduced amplitude of the P300 component of the event-related potential (ERP) among the FHP subjects. Early attempts to replicate the Elmasian finding, using the same undergraduate population of young adults and employing an auditory paradigm but without placebo manipulation, were unsuccessful (Neville and Schmidt, 1985). Polich and Bloom (1987, 1988), contrasting two family history groups of male undergraduates and utilizing an auditory paradigm, failed to find significant differences between family history groups either with respect to P300 latency or amplitude. Polich et al. (1988), reporting on results obtained using a larger sample of both male and female college students, found no significant differences between groups. Schuckit et al. (1988) also failed to find family history differences in P300 latency except when ethanol was administered. (FHP subjects returned to baseline more quickly than did FHN subjects.)

Steinhauer et al. (1987) and Hill et al. (1988), using an auditory paradigm, also failed to find differences in P300 amplitude in high- and low-risk young men, though differences in latency were noted. When the same paradigm was used with minor children, mixed results were obtained (Hill et al., 1990). In this paradigm the target probability is .25 overall. When the probability of a nontarget following a nontarget is calculated, the conditional probability is .67. Similarly, because a target never follows a target (subjects are so instructed), the presence of a target stimulus leads to virtual certainty that the next stimulus will be a nontarget (1.00). While high-risk children displayed decreased amplitude when the conditional probability was greater (.67 and 1.00), they did not show a decrease in the low-probability (.33) condition. (In this paradigm the .33 condition represents those situations in which the infrequent stimulus is preceded by a nontarget stimulus.)

One other study has utilized auditory ERPs recorded in young children (7-12 years old) who were the offspring of alcoholic and control fathers (Begleiter et al., 1987). Rare and frequent tones were presented with varying interstimulus intervals. Significantly decreased amplitudes for both the parietal P200 and P300 components were found among FHP compared to FHN children (though no differences due to interstimulus interval were found between groups).

The results for P300 using visual tasks have also been explored with generally more consistent results. Three types of tasks have been employed. One involved a head orientation task with two levels of discrimination difficulty (Begleiter et al., 1984; O’Connor et al., 1987). A second involved matching forms that varied on three dimensions (color, shape and number) using a modification of the Continuous Performance Test, which required subjects to count the number of times two consecutive stimuli matched on all three dimensions (Whipple et al., 1988). The third visual task that has been used with high-risk individuals employed a discrimination task in which subjects were asked to discern two intensities of light and silently count the number of times the dim light appeared (Polich et al., 1988b).

Findings in adult FHP subjects have been mixed. For a line orientation task, Porjesz and Begleiter (1990) observed reduced P300 amplitudes in adult children of alcoholics in response to an infrequent visual target. Polich et al. (1988b) were unable to document visual P300 differences between adult FHP males and controls when required to detect an infrequently presented dim light. Parsons et al. (1990), using auditory and visual stimuli, observed some differences in early negative components for adult FHP subjects, but found no differences in P300 between FHP and FHN nonalcoholic subjects.

To date, five studies have employed prepubescent boys (Begleiter et al., 1984, 1987; Hill et al., 1990; Hill and Steinhauer, 1993; Whipple et al., 1988), while the majority of other studies have utilized young adult college students. Only one study has contrasted both pre- and postpubescent boys and girls (Hill and Steinhauer, 1993). This strategy has the advantage of removing the unwanted effects of either acute or chronic alcohol use upon brain functioning, both of which appear to be associated with changes in ERP characteristics, inasmuch as chronic alcoholics have repeatedly been shown to exhibit ERP changes relative to nonalcoholics (Parsons et al., 1990; Patterson et al., 1987). The present investigation was conducted in order that these extraneous factors might be removed by employing minor children who had not yet reached young adulthood.

Additionally, because age and gender distributions of the various samples utilized in previous studies may have contributed to the inconsistencies of the reported findings, a larger sample size was employed making it possible to statistically control for these critical variables. Finally, because important age and gender differences had emerged using a visual paradigm (Hill and Steinhauer, 1993), it was of interest to study the same age and gender groups using an auditory paradigm. Thus, this study provides the
Recruitment of families

ERPs measured by the peak of the N250 component, over the same sample of children. The opportunity for comparing results across modalities within the same sample of children.

Additionally, previous findings from this laboratory (Hill et al., 1990) included the observation that there was a significantly increased late negativity in the auditory ERP measured by the peak of the N250 component, over anterior regions (Fz and Cz) in the high-risk children as compared to children of low-risk families. The late anterior negativity typically decreases with development (Friedman et al., 1984). Thus, the Hill et al. (1990) finding is of considerable interest, as it suggests that the offspring of alcoholic families might be characterized by a delay in maturational processes. However, the limited sample upon which that earlier report was based did not allow for high- and low-risk groups to be compared by age. Therefore, the developmental lag hypothesis suggested by the earlier work (Hill et al., 1990) was followed up in a new, larger sample of children.

Subjects and Methods

Recruitment of families

A total of 93 children between the ages of 8 and 18 years old participated in the study. The children were drawn from two groups: a high-risk and a low-risk group. In some instances more than one child came from the same nuclear family (7 high-risk families and 15 low-risk families had two children, and three high-risk families had three children). Structured face-to-face clinical interviews were performed for parents of the children and a consensus diagnosis between two clinicians completed using DSM-III (American Psychiatric Association, 1980) and Feighner Criteria (Feighner et al., 1971).

The high-risk group

The high-risk group had an exceptionally high density of alcoholism within the pedigrees from which they were drawn and consisted of 51 children (27 males and 24 females). The high-risk families were part of a larger pedigree study of alcoholism (the Cognitive and Personality Factors Family Study—CPFFS) that includes multiple extended pedigrees with multigenerational alcoholism, largely uncontaminated by other psychopathology (all first-degree relatives were required to be free of DSM-III [Axis I] disorders). These high-risk families had been ascertained through a proband set comprised of a pair of alcoholic brothers, one of whom was in treatment at the time of identification. The presence of alcoholism or other psychopathology was determined for these brothers and all available first-degree relatives through face-to-face interviews (Diagnostic Interview Schedule [DIS]), allowing for DSM-III and Feighner Criteria to be applied. While no restrictions were placed on the families with regard to Axis II disorders, the rate of antisocial personality disorders among fathers of the proband set of brothers that had identified the high-risk pedigrees was extremely low (Hill, 1992).

Because genetic mediation of alcoholism can be expected to be the result of having a constellation of relatives who are alcoholic, not simply whether or not one's parents are alcoholic, children without an alcoholic parent who had a minimum of three second-degree relatives alcoholic were included in the high-risk group. To insure that this strategy did not alter the results obtained, further analyses were performed using data obtained from two subgroups of the high-risk group: (1) high risk, with an alcoholic parent, and (2) high risk, without an alcoholic parent. Results of this analysis were used to confirm that the findings obtained using all 51 high-risk children when contrasted with the 42 low-risk children were comparable.

These high-density target families represent a form of alcoholism that appears to be mediated by a major effect (Aston and Hill, 1990). Of the 51 high-risk children included in this study, 31 came from a family with one alcoholic parent (three had two parents alcoholic). Furthermore, among the relatives for whom reliable diagnostic information was available, it is noted that 8 grandmothers and 35 grandfathers were alcoholic. Of the 143 maternal and paternal uncles available for assessment, 119 were alcoholic. Among the 37 maternal and paternal aunts, 12 were alcoholic. Thus, each child had an average of 4.1 alcoholic relatives. (Calculations were based on the high-risk CPFFS pedigrees only.) Therefore, even those children who did not live with an alcoholic parent had a very high lifetime risk for developing alcoholism.

The low-risk group

The low-risk group (n = 42) had no first- or second-degree relatives with diagnosed alcoholism and consisted of 22 male and 20 female children. The low-risk (control) families from which the children were drawn were also part of the larger study (CPFFS) and included multiple members of pedigrees selected for absence of psychopathology. These families were selected from among volunteers who responded to advertisements in local newspapers soliciting participation in research. Families qualified for participation by having both multiple siblings and at least one parent available for personal interview, as well as absence of psychopathology, including alcoholism, using the face-to-face structured psychiatric interview. The families were selected through a pair of male siblings so that the structural characteristics of the two family types would be similar. All available siblings and parents were interviewed in-person using the DIS to confirm the absence of psychopathology in the designated sibling pair and their first-degree relatives.

In the case of children from low-risk families, one parent, and his/her extended pedigree, was free of all Axis I
psychopathology including alcoholism, as were his/her first-degree relatives. However, the parent “marrying in” might have alcoholism or other psychopathology present. In order that a truly low-risk sample might be studied, cases of this type were excluded from the present analysis. In other words, the control children came from bilineal low-risk pedigrees.

Assessment of prenatal ethanol exposure

Because the mothers of these children could have consumed alcohol during pregnancy, the study design included interviewing all mothers (even those who were social drinkers) about drinking during pregnancy. This information was validated using other information gathered routinely for both parents including current and lifetime drug and alcohol use. Of those mothers who met a lifetime diagnosis of alcoholism, two denied drinking during pregnancy. The third mother drank heavily during the first 3 months of her pregnancy, but ceased drinking when her pregnancy was confirmed.

Bilineal vs. unilineal psychopathology in parents

Due to the design of the larger study from which the sample was drawn, less control could be exerted over the clinical characteristics of the parent “marrying in” to the target (high-risk and low-risk) CPFFS families. Therefore, occasionally it was the case that some children from high-risk families may have had the potential for familial psychopathology other than alcoholism, though this was minimal (9 parents had more than one episode of depression, the majority [n = 7] were “marrying in” parents). In those cases where the parent “marrying in” was alcoholic or had alcoholic relatives, the child’s risk was thereby increased due to the additional relatives of the child being alcoholic through the other parent. The parent “marrying in” to the target family was assessed in the majority of cases by in-person assessment using the structured interview (DIS), though in a few cases family history alone was available. Additionally, family history information for the “marrying in” parent’s side of the family was routinely obtained.

Demographic characteristics

All children included were white with the exception of one child (Oriental mother with white father). Children were matched for age and socioeconomic status of their parents. The mean (± SD) age was 11.26 ± 3.4 for high-risk males and 11.38 ± 3.5 for high-risk females. The mean age for low-risk males was calculated to be 10.77 ± 2.7, while the females averaged 11.85 ± 3.3. For purposes of data analysis, subjects were classified into “prepubescent” (8-12 years) and “postpubescent” (13-18 years). (Although an endocrinological/physical examination was not performed to verify pubertal status, assumptions about pubertal status and the age group to which the child was assigned were highly correlated by verbal report.) The socioeconomic status (SES) of the two groups was determined by employing Hollingshead’s Four Factor Index of Social Status (Hollingshead, 1975). This instrument combines the education and job title of each parent into a summary score which can then be grouped into five levels: V, professional; IV, semiprofessional/technical; III, skilled; II, semiskilled; and I, unskilled.

Somewhat higher socioeconomic status was noted for the low-risk children. The high-risk group of children was drawn from high functioning parents with 39.5% being from homes where parents were in the top two Hollingshead categories (professional or semiprofessional/technical). A greater percentage of low-risk children were from families having this level (62.9%). A chi-square analysis revealed a marginally significant difference between the two groups when children whose parents were from levels I, II and III were grouped, and those from levels IV and V were grouped in a $2 \times 2$ analysis ($\chi^2 = 3.81$, 1 df, $p < .051$). Given the marginally significant differences between the groups with respect to socioeconomic status, statistical analyses were performed with SES as a covariate to determine if SES of the family from which the child was drawn would alter the results obtained. (No alterations in results were seen as a result of SES being used as a covariate.) The lower SES of the families of high-risk children may not be surprising in view of the tendency for alcoholism to result in a downward drift in socioeconomic status of the family. Nevertheless, the differences in SES did not contribute to the results obtained.

Psychiatric assessment of high-risk and low-risk children and their first- and second-degree relatives

An in-person diagnostic assessment was performed for all living and available parents, grandparents, aunts and uncles of these children (more than 80% of relatives) by two trained clinicians who were required to meet a consensus diagnosis. For those relatives not assessed by a face-to-face interview, a minimum of two family history reports was used to arrive at an appropriate family history diagnosis for the second-degree relative who was absent or deceased. A structured interview (Diagnostic Interview Schedule) was performed by a trained interviewer. A second unstructured interview was performed by a master’s- or doctorate-level psychologist to arrive at a best-estimate consensus diagnosis. The DIS allowed for determination of whether or not the adult relative met DSM-III and Feighner Criteria for Axis I pathology. All children were similarly interviewed to determine the presence or absence of clinical syndromes. Each child was assessed using a K-SADS (Orvaschel et al., 1982) interview administered...
by a trained interviewer, followed with an assessment by a 
child psychiatric (third or fourth year) resident. A consen-
sus diagnosis was reached for each child by blind evalua-
tion. Results of our analysis of these children revealed that 
the overall rate of psychopathology among the high-risk 
children was quite similar to the low-risk children (Hill and 
Hruska, 1992). Conduct disorder, including oppositional 
disorder, was similar (5.6% of high risk versus 2.4% of 
low risk). Alcohol abuse was diagnosed in 3.7% of the 
high-risk children and none of the low-risk children.

Health, medications and drug use screening

Each child was assessed using a structured health ques-
tionnaire to rule out possible group differences in head 
trauma, high fever or loss of consciousness. No differ-
ences were found. Also, any medications that could alter 
EEG findings were noted and children were dropped from 
the analysis if they were on any psychoactive drugs.

On the day of testing, each child was asked to consume 
a normal breakfast but without caffeine. Before testing be-
gan, a pre-protocol interview was administered to verify 
that the child had complied with instructions concerning 
not using alcohol or drugs in the 48 hours preceding test-
ing. All ERP assessments were performed before lunch to 
control for any possible diurnal variation. The majority of 
children both high risk and low risk were assessed during 
the summer months (81%) to control for any possible sea-
sonal variation. Additionally, each child submitted a urine 
sample for drug screening. All children included were free 
of drugs and alcohol at the time of testing.

Auditory event-related potentials procedures

Each child performed two tasks during which auditory 
ERPs were recorded. Subjects were given an audioscope 
screening test of 20 dBHL at frequencies of 500, 1,000, 
2,000 and 4,000 Hz. Results indicated that hearing was 
not impaired in any of the subjects.

The experiments consisted of a simple counting task 
(CT) followed by a choice reaction time task (RT), which 
have been employed previously (Hill et al., 1990; Stein-
hauer et al., 1987). Both are modified versions of the typ-
ical oddball paradigm. For both tasks, the subjects sat in a 
sound-attenuated, darkened room, and listened to “high” 
(1,500 Hz) and “low-pitched” (800 Hz) tones, presented 
every 3 seconds through a loudspeaker placed in front of 
the subject. Prior to testing, subjects were required to iden-
tify “high” and “low” tones in order to ensure pitch differ-
entiation. Tones were 40 msec in duration with an abrupt 
(2 msec) rise and fall time, at an intensity of 70 dBA 
(Edmont-Wilson sound level meter, model 60-510). High 
and low tones were randomly generated by computer so that 
the overall probability of a high (infrequent) tone would 
be 0.25. The only restriction on the random tone sequence 
was that two high tones could not occur in succession.

All subjects were told at the onset of testing that (1) the 
first tone that they would hear on each block of trials 
would be a low tone, (2) there would be fewer high tones 
than low tones and (3) two high tones would never occur 
in a row. To be sure that the task was understood, each 
subject was asked which tone would be heard after a high 
tone. All subjects responded correctly that a low tone 
would follow. Thus, a low tone, when preceded by a high 
tone, was a totally predictable event (“certain”), having a 
conditional probability of 1.00. After the occurrence of 
any low tone, either a high tone or another low tone could 
follow. Two low tones in succession, occurring on two-
thirds of these trials, carried a conditional probability of 
.67, while a high tone occurring after a low tone (rare 
event) occurred one-third of the possible times (condi-
tional probability of .33).

For the counting task, the subject was asked to count 
silently the number of “high” (infrequent) tones heard, 
and to report the total at the end of the block. In the 
choice reaction task, subjects pressed one button when a 
high tone was heard and another button when a low tone 
occurred, alternating with each subject as to whether the 
left button first corresponded to a high tone or a low tone. 
On the second choice reaction block, the subject was re-
quired to do the opposite. Responses were automatically 
encoded to determine accuracy.

For both the counting and choice reaction tasks, sub-
jects were asked to perform two blocks of 80 trials each. 
Each error-free block resulted in a reward of 25¢; 10¢ was 
given for each block with 1-2 errors (3 errors = no re-
ward). Blocks with six or more errors were excluded from 
the analysis. During the choice reaction task, all trials 
performed incorrectly were also discarded. ERPs were re-
corded using SensorMedic Ag/AgCl electrodes filled with 
Grass electrode paste, placed at midline frontal, vertex, 
parietal and occipital locations (Fz, Cz, Pz, Oz), as well 
as left and right parietal sites (P3, P4). All active elec-
trodes were referred to linked ears, with a forehead 
ground. Eye movement and blink artifacts were recorded 
by an additional electrode located under the left eye which 
also was referred to linked ears. All data were monitored 
online by an oscilloscope, and all trials affected by eye 
artifact (exceeding approximately 50 μv) were coded for 
exclusion. Data were digitized for 1,200 msec at 125 Hz, 
beginning 200 msec prior to stimulus onset, by a PDP 11/ 
23 computer system and stored on magnetic media.

Artifact free trials for each task were averaged for each 
condition and electrode. At least two raters, blind to each 
subject's family history, identified the ERP components 
(N100, P200, N250, P300) using an interactive computer 
algorithm which chose the maximal amplitude for a given 
component (at Cz for N100, P200 and N250; at Pz for 
P300) within a predefined latency window (N100: 80-136
msec; P200: 136-240 msec; N250: 200-320 msec; P300: 264-424 msec). Components found outside the expected latency range were identified by consensus among raters, and the computer was adjusted to select this latency. This is of particular importance since component latencies are typically longer in children than in adults, and are decreased for older children. Peak amplitude was computed as the deviation from the median voltage during the 200 msec prestimulus baseline, using the same time point for all electrode sites. Latency and amplitude data were automatically extracted and stored in ASCII files for subsequent analysis.

Results

Behavioral analysis

Performance was analyzed by ANOVA (BMDP2V) with respect to age (which was dichotomized into 2 levels: 8-12 years and 13-18 years) and gender of high- and low-risk groups. Mean error rates by group ranged from 0.40 to 1.82 errors (over 160 trials) in the counting task, and from 0.38 to 3.89 errors for the choice reaction task. There were no significant performance differences due to familial risk group or gender for either task, but there was a significant effect of age, with fewer errors for the older compared to younger children ($F = 7.9, 1/85$ df, $p = .006$). Thus, behavioral accuracy was extremely similar between the groups, regardless of familial risk group or gender, while modest age effects were observed.

ERP analyses-amplitudes

Amplitude data were first analyzed by ANOVA (BMDP2V, followed by post hoc Newman-Keuls tests at the .05 level) for differences between familial risk group (F), gender (G), age (A), task (T), probability (P) (.33, .67, 1.0), and electrode (E) (6 locations). Where appropriate, Greenhouse-Geisser epsilon correction factors were used to reduce degrees of freedom. Given the large number of factors, analyses are reported only for main effects, and first- and second-order interactions involving familial risk group, gender, age, task and probability. For differences among electrode sites, only main effects and first-order interactions are reported. Grand mean ERPs across subjects are shown separately for the high- and low-risk groups at all electrode sites as a function of event probability for the counting task (Figure 1) and choice reaction task (Figure 2). Particular ERP components vary in latency with increasing age, especially P300, so that in the grand averages, it is not always clear whether an early or late P300, or both, represent age variation. Separate components N100 through P300 are labeled for Cz, and P300 is also labeled at Pz (see Figure 3).

P300 Amplitude

The initial focus of data analysis was on the P300 component of the ERP. The first overall analysis performed was a six-factor analysis of variance (ANOVA) using Familial risk group (F) \times Task (T) \times Probability (P) \times Gender (G) \times Age (A) \times Electrode site (E). A number of significant main effects were observed. Of most interest for the aims of this study, children from high-risk families had smaller P300 amplitudes than low-risk children (group: $F = 4.08, 1/85$ df, $p = .047$). P300 was smaller across groups for younger (12 and below) than older (13 and above) children (age: $F = 20.0, 1/85$ df, $p < .0001$). P300 was larger in amplitude for decreasing event probability (probability: $F = 245.8, 2/131$ df, $p < .0001$; amplitude for the .33 condition was significantly larger than for .67 or 1.00), and varied across electrodes ($F = 239.3, 3/244$ df, $p < .0001$). Maximum amplitude for these tasks was at Pz, which differed significantly from all other electrodes (see Figures 1 and 2). Adjacent midline electrodes were also significantly different from each other (mean amplitude in μV: Fz = -5.4; Cz = 3.7; Pz = 13.4; Oz = 10.0), but left and right parietal amplitudes (P3 = 7.4; P4 = 8.2) did not differ.

Electrode site

There was an interaction of Age \times Electrode site, reflecting greater negativity for younger than older subjects at the anterior Fz and Cz electrodes ($F = 7.4, 3/244$ df, $p = .0001$). Additionally, a Task \times Electrode effect was seen due to larger differences between tasks being observed at the Pz electrode in comparison to anterior locations ($F = 8.8, 3/247$ df, $p < .0001$). Similarly, a Probability \times Electrode interaction was found, reflecting significantly larger differences between probability conditions at Pz than at other sites ($F = 32.9, 4/377$ df, $p < .0001$). A Task \times Probability interaction was also found ($F = 4.5, 2/161$ df, $p = .014$). A trend for a Familial risk group \times Age interaction ($p = .062$) was also noted.

The P300 showed the typical scalp distribution particularly for these tasks (midline parietal maximum for all groups and conditions). Therefore, further analyses of P300 amplitude were limited to the Pz electrode. Larger amplitudes were associated with being older (age: $F = 4.3, 1/85$ df, $p = .041$), responding to the less frequent (.33) conditional probability (probability: $F = 236.2, 2/125$ df, $p < .0001$) and with performance of the choice reaction task ($F = 8.3, 1/85$ df, $p = .005$), a task which requires a motor response.

There was an interaction of Task \times Probability ($F = 3.6, 2/168$ df, $p = .031$): In the counting task, the .33 condition was significantly larger in amplitude than the .67 and 1.00 conditions, while for the choice reaction task, all conditions differed significantly (.33 > .67 >
Figure 1. Grand mean ERPs at all recording sites as a function of the conditional probability of events during the counting task. Waveforms are presented separately for high-risk (n = 51) and low-risk (n = 42) groups. Positivity is drawn as a downward deflection.

Figure 2. Grand mean ERPs for the choice reaction task, grouped as in Figure 1.
In the choice reaction task, P300 amplitude at Pz was larger for the older than younger low-risk children. In contrast, there was little change in P300 amplitude at Pz for the high-risk group by age (Figure 4). Note that the lower P300 amplitudes at all other sites did show increasing amplitudes for the older compared to younger high-risk children.

For purposes of clarification, an age regression was performed for amplitude of P300 (choice reaction task) over the age range 8-18 years (girls: $r = -.002, p = NS$; boys: $r = .39, p = .006$). When the age regression is broken down by risk status, it becomes clear that the high-risk boys are lagging behind the low-risk boys, while similar slopes for high-risk and low-risk girls are evident (see Figure 5a,b,c,d).

Most studies using an oddball paradigm limit their critical analyses to only the rare stimulus condition. For comparability with such studies, the previous analysis (Familial risk group × Gender × Age × Task) was repeated using only the Pz data for the .33 probability condition (the probability that a target follows a nontarget),
the infrequent high tone, at Pz. The Familial risk group \times Age interaction remained \((F = 4.1, 1/85 \text{ df}, p = .046)\). Thus, the interaction of age with familial risk group status was found both in the condition typically studied by most investigators (the infrequent event), as well as across all conditions. ERPs are superimposed for high- and low-risk children in Figure 3 for the infrequent (.33 probability) condition. The groups have been separated on the basis of age and of gender. Only the midline data at frontal, vertex and parietal locations, and EOG artifact channel, are shown.

Gender effects

Two further analyses were performed \((F \times T \times P \times A)\) in which the data were restricted to males only or to females only. When analyzed separately on the basis of gender, P300 amplitude was observed to be significantly reduced for male high-risk children compared to male low-risk children \((F = 6.0, 1/45 \text{ df}, p = .019)\). No significant differences were observed among females.

Age and gender effects on P300

The finding of a Familial risk group \times Age interaction was followed up by separate analyses using all conditions at Pz for the young and older age groups \((F \times G \times T \times P)\). Among the older children, the effect of reduced P300 amplitude was significant for high-risk compared to low-risk children \((F = 8.0, 1/24 \text{ df}, p = .009)\). The younger children did not show this difference.

Separate analyses were then conducted for the four groups identified on the basis of age and of gender. There was a significant familial risk group effect for older male children \((F = 7.7, 1/11 \text{ df}, p = .018)\). No differences were seen in any of the other three groups (prepubertal boys and girls or postpubertal girls). Mean amplitudes and latencies of P300 for the infrequent probability stimulus for each task are shown by risk status, age and gender in Table 1.

In summary, P300 reduction among high-risk children was more prominent among older than younger children. When separated by gender and age, the difference due to risk status was seen most strongly for 13-18 year old males. This was due to the fact that the amplitude of P300 starts out lower in 8 year old boys than 8 year old girls. As a result, boys show more augmentation of auditory P300 with age than do girls. Developmentally, the boys lag behind the girls. When risk status is added to the equation, one finds that high-risk status confers a greater lag for boys than girls because they have not fully matured to young adult (age 18) levels.

Auditory and visual P300 amplitudes

Some of the children upon which the current report is based also participated in a visual event-related potential assessment (Hill and Steinhauer, 1993). In order to understand the development of P300 amplitude across modalities, and with respect to gender and age, an ANOVA was completed using the auditory P300 data in the .33 condition for the choice reaction task in comparison with the visual task-hard condition (see Hill and Steinhauer, 1993). Results of that analysis showed significant Gender \times Familial risk group effects \((F = 5.20, 1/76 \text{ df}, p = .0254)\) and a significant Age \times Gender interaction \((F = 7.85, 1/76 \text{ df}, p = .0065)\). Additionally, there was a significant main effect of task \((F = 90.24, 1/76 \text{ df}, p < .001)\) and a significant Task \times Age effect \((F = 7.11, 1/76 \text{ df}, p = .0094)\). These results indicate that there are highly significant differences in the amplitude of P300 developmentally as a function of the task modality. Moreover, it suggests that these age-related differences in the development of P300 amplitude as a function of the modality tested occur at different rates by gender. It is the development of a mature P300 which appears to be affected by risk status.

As may be seen in Figure 5a-d, the age-regression lines for the auditory task indicate a relatively flat slope for control females in contrast to the slope obtained for control males. This is largely due to the fact that control boys start at a lower amplitude and rise to higher levels than do girls. Examination of high-risk boys relative to low-risk boys clearly shows that familial risk group brings about a reduction in the slope of the P300/age-regression line for boys. The developmental lag in the auditory modality appears to be most evident in the latter years (13-18 years) as the slopes diverge more dramatically. When the slope of the age-regression line is compared for high- and low-
risk males using the visual task, it becomes clear that they are most divergent in the early adolescent years (see Figure 6a,b), as previously reported (Hill and Steinhauer, 1993).

Earlier ERP components

Analyses for the N100, P200 and N250 components followed a similar factorial design as for P300. Differences in scalp distribution are summarized along with main effects occurring across electrodes. Further analyses focus upon specific electrode locations of interest.

N100. N100 was most negative at Cz and slightly smaller at Fz, both of which were significantly different from all other sites ($F = 91.6, 2/204$ df, $p < .0001$).

N100 showed a significant effect of age with greater negativity observed for the older than the younger children ($F = 4.5, 1/85$ df, $p = .037$).

P200. P200 was most positive at Cz and nearly as large at Pz, both of which were more positive than all other sites ($F = 145.3, 3/278$ df, $p < .0001$). P200 at Cz showed no differences by group or gender.

N250. N250 showed a strong anterior maximum ($F = 257.2, 3/238$ df, $p < .0001$; Fz > Cz > Pz and Oz > P3 and P4; Newman-Keuls, $p < .05$). N250 was more negative for younger children than older children ($F = 25.7, 1/85$ df, $p < .0001$) and varied by probability ($F = 10.2, 2/151$ df, $p = .0001$). Because N250 was observed to be largest overall at Fz in these children, but tends to show a more central maximum among adults, N250 was exam-
TABLE 1. P300 amplitude and latency in response to the .33 complex probability condition (target follows nontarget)

<table>
<thead>
<tr>
<th>Age</th>
<th>Amplitude [µV]</th>
<th>Latency [msec]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Counting</td>
<td>Reaction time</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIGH-RISK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal Male (n = 19)</td>
<td>9.37 ± 1.5</td>
<td>18.01 ± 9.0</td>
</tr>
<tr>
<td>Female (n = 17)</td>
<td>9.41 ± 1.6</td>
<td>20.85 ± 9.9</td>
</tr>
<tr>
<td>Postpubertal Male (n = 8)</td>
<td>15.75 ± 2.1</td>
<td>16.83 ± 7.7</td>
</tr>
<tr>
<td>Female (n = 7)</td>
<td>16.14 ± 1.3</td>
<td>19.08 ± 5.1</td>
</tr>
<tr>
<td>LOW-RISK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepubertal Male (n = 17)</td>
<td>9.59 ± 1.5</td>
<td>19.72 ± 11.4</td>
</tr>
<tr>
<td>Female (n = 12)</td>
<td>9.58 ± 1.4</td>
<td>16.01 ± 7.5</td>
</tr>
<tr>
<td>Postpubertal Male (n = 5)</td>
<td>14.80 ± 1.9</td>
<td>28.26 ± 11.6</td>
</tr>
<tr>
<td>Female (n = 8)</td>
<td>15.25 ± 2.1</td>
<td>22.90 ± 8.4</td>
</tr>
</tbody>
</table>

Increasing age was strongly associated with decreasing negativity at each site (both p < .0001).

At Fz, a Familial risk group x Age x Gender interaction was seen (F = 4.4, 1/85 df, p = .04) (see Figure 7). This was due to significantly decreasing N250 negativity with increasing age for both male and female high-risk groups. There was a significant decrease in negativity for the female low-risk group, but a nonsignificant decrease in negativity with age for low-risk males. At Cz, there was also greater negativity for female than male subjects (F = 4.8, 1/85 df, p = .031). There was greater negativity in the choice reaction task than for counting (F = 35.9, 1/85 df, p < .0001).

Late Negativity N250. The N250 findings obtained suggested that there would be an increased late negativity associated with maturation that might be greater for the high-risk than the low-risk children. Inspection of waveform differences between the risk groups (Figure 3) illustrates the fact that the most consistent effects observed were at the Cz and Fz electrodes, as had been noted in an earlier report (Hill et al., 1990).

Subsequently, the negativity difference was explored by integrating the activity over a 120 msec epoch beginning 24 msec prior to the peak of the average N250 component. These calculations examined an identical time window across subjects. As expected, an effect of increasing age associated with reduced negativity was seen (F = 17.13, 1/85 df, p = .0001). Of more interest, there was a significant main effect for familial risk group with greater negativity being seen for the high-risk than for the low-risk children (F = 4.0, 1/85 df, p = .048). Further analyses focusing on the effects of familial risk group and gender, separately by each age group, were conducted at Fz and Cz.

For younger children, at Fz, there was a clear difference due to gender, with females showing greater negativity (F = 5.4, 1/61 df, p = .023). There was an interaction of Familial risk group x Gender x Task (F = 4.9, 1/61 df, p = .031). Among the younger children, high-risk boys...
showed the greatest increase in negativity in the choice reaction task compared to the counting task.

For older children, there was a Familial risk group × Gender interaction at Fz (F = 4.4, 1/24 df, p = .049). There was a greater difference by gender among low-risk children than between high-risk children. At Cz, there was a trend (p = .058) for a familial risk group difference: high-risk children had greater negativity than low-risk children, regardless of gender.

The rate of decrease in negativity associated with maturation was examined more carefully through correlations of age with the integrated N250 negativity at Fz, Cz and Pz (across tasks and probability conditions) for each group. For low-risk children, correlations were significant at all sites (Fz: r = -.58, p < .001; Cz: r = -.63, p < .001; Pz: r = .45, p = .003). For high-risk children, the decrease in negativity with age was significant at Fz (r = -.47, p < .001), but not at Cz (r = -.27) or Pz (r = -.13). Comparing between groups, there was a significantly greater rate of decreasing negativity with age for low-risk compared to high-risk children at Cz (p < .05), but not at Fz or Pz (Figure 8).

In summary, risk status conferred a difference in anterior negativity, especially over Cz. The possibility that a reduced rate of decreasing anterior negativity in high-risk children might have been responsible for the P300 differences observed between risk groups was considered. Thus, the influence of the integrated N250 negativity on P300 amplitude was examined by analysis of covariance. However, covarying Fz or Cz negativity from P300 amplitude (at Pz) did not eliminate differences in P300 by risk status for postpubescent males.

### ERP Analyses-Latencies

Analysis of latencies for the N100, P200 and N250 components utilized the peak latency in each condition observed at the Cz electrode, while for P300, the latency at the Pz electrode was used. Latency variations were analyzed by ANOVA according to five factors: familial risk group, gender, age, task and probability.

**Latency of N100 and P200.** Main effects for probability in the N100 and P200 components, though statistically significant, occurred over a range that was less than one sampling point (8 msec), and are therefore not considered meaningful. No other factors were significant for N100. P200 showed only a slightly shorter peak for females (180 msec) than males (192 msec) (F = 9.4, 1/85 df, p = .029).

**Latency of N250.** N250 varied according to probability, with increasing latency associated with increasing event probability, but over a range of only 11 msec (F = 17.6, 2/159 df, p < .0001). There were also significant interactions involving Gender × Probability (F = 3.2, 2/159 df, p = .047), Familial risk group × Age × Task (F = 6.5, 1/85 df, p = .013) and Gender × Age × Probability (F = 3.8 2/159 df, p = .028). When the groups were broken down by age and gender, the only significant finding was a Familial risk group × Task interaction for the young male group (F = 6.8, 1/34 df, p = .013). High-risk, prepubescent males showed decreased latencies compared to low-risk males in the counting task, but longer latencies in the choice reaction task.

**Latency of P300.** P300 latency was decreased for older compared to younger children (F = 20.1, 1/85 df, p <
.0001), and was increased as event probability decreased ($F = 32.2, 2/116 df, p < .0001$). Mean latencies for the .33, .67 and 1.00 probability conditions, respectively, were 391, 366 and 358 msec; latency for the .33 condition was significantly increased over the .67 and 1.00 conditions. An interaction of Gender × Age × Probability was also noted ($F = 3.9, 2/116 df, p = .039$).

**Discussion**

The question of whether or not P300 should be considered a diathesis marker for alcoholism is of considerable interest. The answer to this question currently centers on assessing the factors that have led to inconsistent findings across laboratories. The present study emphasizes the importance of examining maturational changes in P300 when searching for differences between high- and low-risk groups. Developmental differences by modality and gender must be taken into account in order that the diathesis variable can be explored adequately.

Two aspects of event-related potential activity were diminished in this sample of high-risk children. First, the amplitude of the P300 component was reduced in the high-risk children. Second, decreasing anterior negativity with increasing age, a phenomenon which is normally observed during development, was less marked among the high-risk children compared to low-risk children.

The present study revealed a reduction in P300 amplitude across all stimulus probabilities, unlike our earlier report, which involved a smaller sample of children between the ages of 8 and 14 years (Hill et al., 1990). In that study, P300 amplitude did not differ between groups for the rare condition (.33 probability), and was actually larger in amplitude for the high-risk children in one of the two tasks (counting task). In the present study, differences between high- and low-risk groups were more pronounced for the older children (13-18 years old) and for males. The study by Begleiter et al. (1987) utilized an auditory paradigm in boys 7-15 years, finding decreased P200 and P300 amplitudes among high-risk boys, relative to a control group, but no differences in latency between groups for any of the ERP components. Whipple et al. (1988) also employed young boys, evaluating the ERP response to auditory stimuli, finding results that were similar to the present ones, as well as those of Begleiter et al. (1987).

While the present results are consistent with other laboratories that have evaluated children in an auditory paradigm, the results were not consistent with our recently reported results utilizing a visual paradigm, at least with respect to age groups that maximally show the amplitude reduction associated with risk for alcoholism. In the visual modality, we had reported that high-risk boys show a P300 amplitude reduction primarily during the prepubertal years (8-13), rather than in the later portion of adolescence (13-18) (Hill and Steinhauser, 1993). In contrast, the present results showed an overall reduction in P300 amplitude in high-risk children, which was significantly more pronounced among the late adolescent children. Comparison of a group of children that had received both visual and auditory evaluations revealed some intriguing results. The present data are consistent with reports by Courchesne and Yeung-Courchesne (1988), who have reported that the amplitude of the auditory P300 (A/Pcz/300, after Courchesne, 1983) increases over the age range of 5-16 years, while the visual P300 (P3b; Courchesne et al., unpublished) appears to reach a maximum earlier (6-8 years) and decline thereafter to adult levels. While the components are not strictly the same, these data provide an opportunity to compare maturation of two modalities during childhood. Age regression of the P300 component for the auditory modality in our children also showed a steady increase with age, such that among normal boys the slope was +2.42. In contrast, normal boys in the present sample, evaluated with a visual paradigm, showed very little increase in P300 with age so that the slope of the age regression was virtually flat. These results would be expected if the visual P300 peaks at around age 6-8 (Courchesne and Yeung-Courchesne, 1988). High-risk status alters this function so that high-risk boys appear to lag behind their low-risk counterparts. The age at which this is most apparent varies by modality because of differences in when the peak amplitude of P300 occurs. Therefore, it is concluded that both visual and auditory P300 amplitudes may each be considered a marker for alcoholism risk, but only among children for whom maturation of these systems has not yet occurred. Whether or not this developmental delay is resolved by early adulthood is unknown.

In addition to the results for P300 suggesting a developmental delay among high-risk children, the results obtained for anterior negativity for these children also has suggested a possible delay. This is consistent with our earlier report which was based on an independent high-risk sample utilizing the same auditory paradigm (Hill et al., 1990). Friedman et al. (1984) were able to demonstrate a marked effect of age on a negative process at frontal regions in 70 children aged 11 to 18. The negativity began with N250 and was prolonged into the slow wave portion of the response, a region of activity that often overlaps with P300. This negativity showed a marked decrease (i.e., greater positivity) with age.

In summary, multiple aspects of late processing (P300 and anterior negativity) show evidence of delayed maturational development in children who are at exceptionally high risk for developing alcoholism. We suspect these results may be seen only in an immature nervous system and are highly dependent on differential rates of development in girls and boys. It is unclear whether findings for familial risk groups would have been so compelling without purposeful inclusion of children from highly selected pedigrees where the genetic risk was unusually high.
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