P300 Amplitude Decrements in Children from Families of Alcoholic Female Probands

Shirley Y. Hill, Diane Muka, Stuart Steinhauer, and Jeannette Locke

A total of 70 age- and gender-matched children and their relatives who were from families that were either at high or low risk for developing alcoholism were studied and the children evaluated using event-related potential paradigms from both the auditory and visual modalities. The high-risk children were ascertained through families at exceptionally high risk for female alcoholism (half of all female first- and second-degree relatives were alcoholic), while low-risk control families had been selected for absence of alcoholism in first- and second-degree relatives. Results indicate that reduced amplitude of P300 and greater negativity of N250 characterize children from high-risk families when evaluated with an auditory task. The visual task discriminated high- and low-risk groups for male children only, consistent with earlier findings for high-risk families ascertained through a male alcoholic. Further, daughters of alcoholic mothers (biological father nonalcoholic) display significantly lower P300 than matched controls, indicating that transmission of alcoholism risk may be possible from mother to daughter without the necessity of paternal alcoholism.

Key Words: P300, N250, ERP, women, alcoholism, high-risk children

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Introduction

There is increasing recognition of the fact that there may be two types of alcoholism in women. One type appears to have an early onset, is characterized by a higher density of relatives who are alcoholic, and may have a poorer prognosis (Hill and Smith 1991; Lex et al 1991; Glenn and Nixon 1991). If this form is genetically mediated, one might expect biological variation among these women and possibly their offspring. Previously, differences in the P300 component of the event-related potential (ERP) between alcoholic women from high-density families and controls have been reported (Hill and Steinhauer 1993a). If the biological marker (P300 decrement) is predictive of later development of alcoholism, studies of children who have not yet become dependent would be most informative. However, minor children who are relatives of female alcoholics have not previously been characterized using event-related potentials.

The P300 component of the event-related potential 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 (Sutton et al 1965; Donchin 1979). Second, the ERP waveform appears to be under genetic control (Aston and Hill 1990; 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 1993b; Steinhauer and Hill 1993;
Other groups have found moderate support (Elmasian et al. 1982; 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; 1988b).

To date, six studies have employed prepubescent boys (Begleiter et al. 1984; Begleiter et al. 1987; Hill et al. 1990; Hill and Steinhauer 1993b; Whipple et al. 1988; Steinhauer and Hill 1993), while the majority of other studies have utilized young adult college students. Only two studies have contrasted both pre- and postpubescent boys and girls (Hill and Steinhauer 1993b; Steinhauer and Hill 1993). In these studies, P300 decrements were found using both auditory and visual modalities among high-risk as compared to low-risk controls. Previous findings from this laboratory (Hill et al. 1990; Steinhauer and Hill 1993) have also included the observation that there was 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). These findings are of considerable interest, as they suggest that children ascertained through male alcoholic proband families might be characterized by a delay in maturational processes.

Understanding the critical differences in methodologies leading to these divergent results is of considerable interest. The age of the high-risk subjects employed (prepubescent, 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.

Finally, criteria for “high-risk” status has varied considerably across studies. 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 (FHP) 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 (nongenetic) 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 reports from 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 1993b; Steinhauer and Hill 1993).

Because age and gender distributions of the various samples utilized in previous studies may have contributed to the inconsistencies of the reported findings, an age- and gender-matched sample of controls was selected from a larger available data set, making it possible to statistically control for these critical variables. Finally, because important age and gender differences vary by modality (Hill and Steinhauer 1993b; Steinhauer and Hill 1993), it was of interest to study age- and gender-matched groups of high-risk children ascertained through a female alcoholic in comparison with low-risk controls using both an auditory and a visual paradigm. Thus, this study provides the opportunity for comparing results across modalities within the same sample of children selected for their membership in high density for female alcoholism families.

Methods

Subjects

RECRUITMENT OF FAMILIES. A total of 70 children between the ages of 8–18 years participated in the study. The age- and gender-matched 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: six high-risk families and seven low-risk families had two children, and three high-risk families had three children.

The high-risk families were part of a larger study of alcoholism in women which includes assessment of all available first-degree relatives to determine both clinical status and variation on a number of neurobiological indicators of risk (The Biological Risk Factors Family Study—BRFFS). These high-risk “target” families had been ascertained through a proband set comprised of an alcoholic woman currently in treatment for alcoholism and one other first-degree relative who was also alcoholic. Families with at least two alcoholic sisters were given preference for entry into the study (75% of cases). The presence or absence of alcoholism or other psychopathology was determined for the proband alcoholic woman and all available first-degree relatives through face-to-face interviews (Diagnostic Interview Schedule [DIS]), allowing for DSM-III (APA 1987) and Feighner Criteria to be applied.

The low-risk (control) families from which the low-risk children were drawn were also part of a larger study and included multiple members of pedigrees selected for absence of psychopathology. These families were selected through a volunteer who responded to advertisements in local newspapers soliciting participation in research. Thus, while volunteer bias might enter in for the initial responder,
there was no reason to believe that the other family members who were studied would have a volunteer bias, as they were recruited by the research team. Families qualified for participation by having both multiple siblings and at least one parent available for personal interview. Using the structured face-to-face psychiatric interview made it possible to equate the groups for psychopathology as much as possible and to eliminate alcoholism from among the first- and second-degree relatives of the index case.

THE HIGH-RISK GROUP. The high-risk group consisted of 35 children (15 males and 20 females) drawn from high-risk pedigrees exhibiting an exceptionally high density of alcoholism (3.9 alcoholic first- and second-degree relatives per family or 45.2% of known relatives). Of the known alcoholic relatives, an equal proportion were female (48% of female relatives were alcoholic; 49% of male relatives were alcoholic). With the male to female ratio running 2:1 in the general population (Kessler et al 1994), this is clearly a higher rate of female alcoholism.

Twenty-seven children had an alcoholic mother. Fifteen of these also had an alcoholic father. 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; therefore, eight children were included who did not have an alcoholic mother, though in five cases the father was alcoholic. These fathers were either men who married into the “target” pedigrees or were brothers of the female proband alcoholics. Also, the eight children without an alcoholic mother came from target families with multiple cases of female alcoholism (17 aunts and 3 grandmothers). The three children with neither parent alcoholic had multiple alcoholic second-degree relatives (an average of three second-degree relatives alcoholic).

THE LOW-RISK GROUP. The low-risk group (n = 35) had no first- or second-degree relatives with diagnosed alcoholism and consisted of 15 male and 20 female children. These children, chosen to be age- and gender-matched to the high-risk group of children, were a subsample from the larger study of families.

ASSESSMENT OF PRENATAL ETHANOL EXPOSURE. Because the mothers of these children (both high- and low-risk) 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. Twenty-seven children from the high-risk group had an alcoholic mother. Mothers of 11 high-risk children reported drinking during pregnancy (two used marihuana as well), while mothers of 24 children denied drinking or use of street drugs during pregnancy.

PSYCHIATRIC ASSESSMENT OF FIRST- AND SECOND-DEGREE RELATIVES OF THE CHILD. An in-person diagnostic assessment was performed for all living and available parents, grandparents, aunts, and uncles of these children (more than 60% 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 were 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 an M.A.- or Ph.D.-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.

BILINEAL VERSUS 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) families. In the case of children from low-risk families, the parent from the target family and his/her extended pedigree had minimal Axis I psychopathology (alcoholism was excluded in the individual and in his/her first-degree relatives). This was done to match the level of psychopathology to that of the high-risk group as much as possible. The high-risk group had been selected for the purpose of identifying markers of risk for alcoholism and not general psychopathology. However, the parent “marrying in” might be alcoholic or exhibit major psychopathology (e.g., schizophrenia or recurrent depression). 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 for alcoholism pedigrees.

Similarly, it was the case that some children from high-risk families may have had the potential for familial psychopathology other than alcoholism. An effort was made to keep major psychopathology to a minimum in order to match controls and make it possible to study a vulnerability diathesis more specific to alcoholism. Drug dependence is clearly common among alcoholics and was a contaminating factor for these alcoholic women, with 34.3% showing secondary drug dependence. Cases were included only if the drug dependence was secondary to the alcoholism using the primary/secondary distinction of the St. Louis Group, in
which the disorder with the earlier onset is considered primary. Additionally, about a third of the women experienced a major depressive episode; however, a minority of these had more than one episode (5.7%), and in no case was the alcoholism secondary to affective disorder. Some women also met criteria for anxiety disorder (8.6%).

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 through family history information. In addition, for 25% of these parents, a consensus diagnosis was obtained based on both the structured interview (DIS) and a follow-up interview by a second clinician.

**DEMOGRAPHIC CHARACTERISTICS.** Most children included were Caucasian (one child came from racially mixed parentage). Children were matched for age, gender, and socioeconomic status of their parents. The mean age (± SD) was 11.34 ± 2.7 for high-risk children and 11.29 ± 2.9 for low-risk children. 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 occupation 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. The high-risk group of children was drawn from high-functioning parents, with 48.6% 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 (71.4%). 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 in a 2 x 2 analysis against those from levels IV and V ($X^2 = 3.81, df = 1, p < .05$).

**HEALTH, MEDICATIONS, AND DRUG USE SCREENING.** Each child was assessed using a structured health questionnaire to rule out possible group differences in head trauma, high fever, or loss of consciousness. No differences were found. Also, any medications that could alter EEG findings were noted. Two high-risk children were on psychoactive medication (Ritalin® [methylphenidate hydrochloride] and desipramine); all others were free of psychoactive drugs. Data for the two children on psychoactive drugs were examined and found to be in the normal range.

On the day of testing, each child was asked to consume a normal breakfast but without caffeine. Before testing began, a preprotocol interview was administered to verify that the child had complied with instructions concerning not using alcohol or drugs in the 48 hours preceding testing. All ERP assessments were performed before lunch to control for any possible diurnal 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.

**Event-Related Potentials**

**AUDITORY PROCEDURE.** 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, 1000, 2000, and 4000 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; Steinhauser et al 1987). Both tasks are modified versions of the typical oddball paradigm. For both tasks, the subjects sat in a darkened, sound-attenuated room and listened to “high” (1500 Hz) and “low”-pitched (800 Hz) tones, presented every 3 seconds through a speaker placed in front of the subject. Prior to testing, subjects were required to identify “high” and “low” tones to ensure pitch differentiation. 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 (conditional 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 required to do the opposite. Responses were automatically encoded to determine accuracy.

For both the Counting and Choice Reaction tasks, subjects were asked to perform two blocks of 80 trials each. Each error-free block resulted in a reward of $0.25; $0.10 was given for each block with one or two errors (three errors—no reward). Blocks with six or more errors were excluded from the analysis. During the Choice Reaction task, all trials performed incorrectly were also discarded.

**VISUAL PROCEDURE.** The visual event-related potential task employed was patterned after the procedure utilized by Begleiter et al (1984). A Digital Equipment Corporation PDP-11/23 lab computer was slaved to coincide with the video output of an Atari 130 computer which executed a BASIC program to present the stimuli at 33 msec duration, with intrtrial interval varying randomly between 2.25 and 4 sec. Stimuli were displayed on a Magnavox RGB Monitor 80, placed 132 cm from the subject, subtending a visual angle of 3.8°. The monitor was set to the default green mode, resembling the oscilloscope display used by Begleiter et al (1984). Five stimuli were randomly presented. One view, the nontarget stimulus, was a simple circle to which the subject was instructed not to respond (blank condition). There were four possible aerial views of target stimuli, a representation of a head with a nose and only one ear. The subject was instructed to press the button which corresponded to the depicted ear. The easy condition occurred when the nose was oriented upward and the ear (right or left) was on the same side as the button depressed. In the hard condition, the nose was oriented downward and the subject was required to spatially rotate the head in order to respond correctly. Thus, in the hard condition, the ear was depicted on the opposite side of the head as the button pressed.

A standard set of instructions was read to each child. If additional clarification was needed, directions were amplified by the experimenter. The child was first shown a picture of each of the stimuli and asked to make the correct response to each head (target) stimulus. Next, each stimulus was presented on the video monitor at a slow pace using a long-exposure duration (3200 msec). Once the child was performing correctly (usually less than 10 trials), the visual display duration was decreased to the 33 msec exposure time of the main experiment for several additional practice trials. (The children were encouraged to respond quickly but, more importantly, to respond accurately.) Two blocks of 120 trials were presented to the subjects. Of the 240 total trials, 160 were blank (nontargets), 40 were easy-condition targets (20 right, 20 left), and 40 were hard-condition targets (20 right, 20 left).

**ELECTROPHYSIOLOGICAL RECORDING AND PEAK DETECTION.** ERPs were recorded using SensorMedic Ag/AgCl electrodes 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 electrodes 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 (exceedingly approximately 50 μV) were coded for exclusion. Data were digitized for 1200 msec at 125 Hz, beginning 20 msec prior to stimulus onset, and stored on magnetic media. Artifact-free trials for each task were averaged for each condition and electrode.

ERP components (N100, P200, N250, P300) were identified 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). For the visual task, the P300 latency window was extended if necessary. Components found outside the expected latency range were verified by consensus among two raters blind to each subject’s family history, 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 peak to baseline amplitude data were automatically extracted and stored in ASCII files for subsequent analysis.

**Results**

**Event-Related Potentials**

Analyses were performed using an analysis of variance (ANOVA) (BMDP2V) to reveal statistically significant differences between risk groups (high-risk or low-risk children) for both the auditory and visual tasks. Where appropriate, Greenhouse-Geisser corrections were employed.

**Behavioral Measures**

Reaction times and errors from the visual task, as well as total number of errors from the auditory task, were statistically analyzed using t tests (BMDP3D) to determine differences in performance between the high-risk and low-risk groups. There were no differences among the risk groups in performance for either task. Also, no differences were seen by gender.

**Auditory Task**

**P300 AMPLITUDE AND LATENCY.** Amplitude data for the auditory task were analyzed to determine differences due to risk group (high-risk or low-risk), task (Counting or
Table 1. ANOVA Summary—ERP Amplitude by Component Elicited During the Auditory Task
(70 Age- and Gender-Matched Children)

<table>
<thead>
<tr>
<th>Condition</th>
<th>P300 (Pz)</th>
<th>N250 (Cz)</th>
<th>P200 (Cz)</th>
<th>N100 (Cz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>F df p</td>
<td>F df p</td>
<td>F df p</td>
<td>F df p</td>
</tr>
<tr>
<td>Group x task</td>
<td>6.47 1.68 NS</td>
<td>4.08 1.68 .025</td>
<td>6.29 1.68 .0001</td>
<td>1.35 1.68 NS</td>
</tr>
<tr>
<td>Task</td>
<td>5.10 1.68 NS</td>
<td>3.31 1.67 .073</td>
<td>1.04 1.68 NS</td>
<td>5.28 1.68 .0001</td>
</tr>
<tr>
<td>Probability</td>
<td>160.51 2.102 .0001</td>
<td>3.92 1.68 NS</td>
<td>6.90 2.112 .006</td>
<td>1.35 1.68 NS</td>
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<tr>
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<td>70.68 1.68 NS</td>
<td>13.04 1.68 .0006</td>
<td>4.08 1.68 .0001</td>
<td>1.35 1.68 NS</td>
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<td>Group</td>
<td>1.68 NS</td>
<td>1.68 NS</td>
<td>1.68 NS</td>
<td>1.68 NS</td>
</tr>
</tbody>
</table>

ANOVA = analysis of variance; ERP = event-related potential; NS = not significant.

Choice Reaction task), and probability (.33, .67, and 1.0 conditional probabilities). Since P300 has been determined to be significantly larger at the midline parietal electrode (Pz) in these paradigms, all analyses involving P300 were conducted at Pz (Table 1). SES values were used as covariables to determine if the 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.

Overall, there was a trend for the high-risk children to have lower amplitudes than the low-risk children (F = 3.10, df = 1.68, p = .08) and a significant group by task interaction (F = 6.47, df = 1.68, p = .013). This was largely due to the fact that there were no significant differences to report for the Counting task other than probability effects (F = 106.38, df = 2.116, p < .0001). Therefore, all further analyses for amplitude from the auditory modality are confined to the Choice Reaction task.

Latency data from the auditory task were analyzed at the Pz electrode to determine effects of risk group, task, and probability. P300 latency was found to decrease with increasing conditional probability (F = 1.44, df = 2.22, p = .0011). No latency differences by risk group status were observed for either auditory task.

Average ERP waveforms from the Choice Reaction task are plotted by group in Figure 1. A group by probability analysis, restricted to results obtained for the Choice Reaction data, was performed for P300 amplitude (Table 2). A significant group effect was found, with the amplitude for the high-risk group being 4 μV lower than for the low-risk group (F = 6.52, df = 1.68, p = .013) (Table 3). A significant probability effect was also found (F = 114.7, df = 2.109, p = .001). Limiting the analysis to each probability indicated that the high-risk children displayed significantly smaller P300 amplitudes than low-risk children for both the .33 and .67 conditions of the Choice Reaction task (.33 condition: F = 5.54, df = 1.68, p = .022; .67 condition: F = 5.15, df = 1.68, p = .027; 1.00 condition: F = 3.95, df = 1.68, p = .051).

Because the results might have been due to the joint influence of having both a biological mother and father who were alcoholic, subsequent analyses were restricted to those 11 female children who had an alcoholic mother only (children with an alcoholic father were excluded). Note that a total of 12 children had only an alcoholic mother; only one was male, precluding a gender by group analysis. A group by probability analysis was performed for these high-risk children and their matched controls. Because female children from alcoholic mothers displayed reduced amplitude, a significant group effect was seen for P300 amplitude (F = 4.52, df = 1.20, p = .046). Additionally, a probability effect was seen due to the expected effect of the increasing conditional probability on decreased amplitude (F = 61.18, df = 2.29, p = .0001). Both may be seen in Figure 2. This group effect was significant for the target condition (.33) (t = 2.87, df = 20, p = .010) and the .67 condition (t = 2.22, df = 20, p = .038) due to the high-risk daughters of alcoholic mothers displaying lower P300 amplitude than the low-risk daughters of control mothers.

Prenatal Drinking. In order to control for prenatal alcohol exposure, the 11 high-risk children whose mothers drank during pregnancy (and their corresponding 11 controls) were removed from the analysis. Group differences for Choice Reaction task amplitude for the .33 and .67 conditions remained even in this smaller sample of 24 children of abstinent mothers when compared with their age- and gender-matched controls (.33 condition: F = 6.21, df = 1.46, p = .016; .67 condition: F = 4.46, df = 1.46, p = .04).

N250 Amplitude and Latency. Data for the N250 component were analyzed at the midline vertex electrode site (Cz). Group differences were not found for the Counting task, consistent with the results for P300 amplitude. However, significant differences were found for the Choice Reaction task (Table 2). Using a group by probability analysis, a significant group effect was found due to the high-risk children having greater negativity than the low-risk children (F = 3.98, df = 1.68, p = .050). When N250 amplitude was analyzed using age as a covariate, group differences became more pronounced for the Choice Reaction task (F = 5.54,
df = 1.67, $p = .022$). A significant probability by group interaction was also seen ($F = 3.98, df = 2.115, p = .038$) due to high-risk children having greater negativity for all conditions, especially the .67 and 1.0 conditions.

The P300 amplitude appears to be consistently less positive and N250 appears more negative among our high-risk children in comparison to low-risk controls. Because P300 shows the greatest difference between risk groups in the target condition, P300 in the .33 condition (Choice Reaction task) was analyzed by risk group using the N250 amplitude at Pz as a covariate. The reported P300 risk group difference ($p = .022$) became more significant ($F = 7.84, df = 1.67, p = .007$), suggesting that the P300 and N250 components in the target condition are independent, though related, predictors of risk group status.

N250 latency data from the auditory task were analyzed at midline vertex. N250 latency increased with increasing conditional probability ($F = 23.55, df = 2.129, p < .0001$).

**EARLY COMPONENTS.** ANOVAs were performed, and no group differences were found, for both N100 and P200 amplitudes (see Table 1). Early component auditory laten-

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**Figure 1.** Grand average event-related potential (ERP) waveforms from the Choice Reaction task by risk group (high-risk: $n = 35$; low-risk: $n = 35$) for the target (.33) condition. Note that positivity is drawn as a downward deflection.

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**Table 2.** ANOVA Summary—ERP Amplitude Elicited During the Choice Reaction Task (70 Age- and Gender-Matched Children)

<table>
<thead>
<tr>
<th>Condition</th>
<th>P300 (Pz)</th>
<th>N250 (Cz)</th>
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<tr>
<td></td>
<td>$F$</td>
<td>$df$</td>
</tr>
<tr>
<td>Group</td>
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</tr>
<tr>
<td>Probability</td>
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<tr>
<td>Group x probability</td>
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<td>2.109</td>
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<tr>
<td>Group covariate = age</td>
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<td>1.67</td>
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</table>

ANOVA = analysis of variance; ERP = event-related potential
Table 3. Means ± SD of P300 and N250 Components—Choice Reaction Task (70 Children)

<table>
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<th>P300 (µV)</th>
<th>N250 (µV)</th>
</tr>
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<tbody>
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<td>(msec)</td>
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</tr>
<tr>
<td>.33</td>
<td>361.60</td>
<td>376.00</td>
</tr>
<tr>
<td></td>
<td>(63.1)</td>
<td>(70.5)</td>
</tr>
<tr>
<td>.67</td>
<td>349.94</td>
<td>364.80</td>
</tr>
<tr>
<td></td>
<td>(63.9)</td>
<td>(78.3)</td>
</tr>
<tr>
<td>1.0</td>
<td>352.46</td>
<td>352.46</td>
</tr>
<tr>
<td></td>
<td>(64.6)</td>
<td>(78.0)</td>
</tr>
</tbody>
</table>

The early components (N100 and P200) were significantly affected by conditional probability (N100: p = .0008; P200: p < .0001). No group effects or interactions were present.

Visual Task

AMPLITUDE. P300 amplitude did not differ by risk group for the overall sample of 68 children studied (two children with invalid data were removed from the analysis). However, as expected, there were significant differences due to the stimulus condition presented (hard, easy or blank) as a result of the higher amplitude seen for the target conditions (hard and easy) in comparison to the blank (non-target) condition (F = 203.10, df = 2,102, p < .0001). A similar condition effect was seen for N250 amplitude due to the blank condition eliciting the smallest N250 amplitude (F = 6.34, df = 2.129, p = .003).

An analysis by risk group status was performed separately for male and female children. This decision was based on our previous results showing risk group differences in visual P300 amplitude by gender (Hill and Steinhauber 1993b). For the sons, the group effect was significant for the hard condition (t = 2.17, df = 26, p = .039), though not the easy condition (t = 1.30, df = 26, NS). Grand average ERP waveforms for males can be seen in Figure 3. For daughters, the group effect for both the hard and easy conditions was not significant. In view of the fact that equal performance was seen by gender, the visual P300 risk group differences seen for boys but not girls suggest that there are electrophysiological differences by gender that cannot be attributed to gender differences in performance.

For the early components, only a stimulus condition effect was seen for P200 amplitude (p < .0001). There were no risk group differences found for P200 or N100 amplitude.

LATENCY. As expected, the latency of P300 varied with the stimulus condition administered (F = 44.76, df = 2.106, p < .0001), with the blank condition having the shortest latency. No risk group differences were found for latency of P300 (492 msec for high-risk and 504 msec for low-risk). Analysis of covariance (age as covariate) showed a group effect.
Discussion

The present results demonstrate that alcoholism in women, particularly the familial form seen in our selected high-density families, may have neurobiological underpinnings. There was a trend for high-risk children overall to have lower P300 amplitude. This effect was most clearly seen when results were analyzed for the Choice Reaction task, in which a significant group effect was seen overall, as well as for each of the conditional probabilities. The superiority of the auditory Choice Reaction task in uncovering risk group differences has been shown in previous studies from this laboratory (Hill et al. 1990; Steinhauer and Hill 1993b). Auditory tasks appear to be more reliable than visual ones in eliciting high/low-risk group differences in mixed gender samples. This is undoubtedly due to the fact that the visual and auditory modalities appear to mature at different rates by gender. We note from our previous work (Hill et al. 1993) that irrespective of risk group status, the developmental course for the visual P300 amplitude in girls is to decrease over time from childhood to adolescence (8–18 years), while boys tend to show an increase over the same age range. The visual system appears to mature earlier in girls so that, having reached their peak amplitude before the age of 8 years, a steady decline ensues until at least early adulthood. In contrast, the auditory system shows an increase in P300 amplitude until young adulthood for both girls and boys (Steinhauer and Hill 1993b). Thus, if the P300 amplitude is indexing a developmental delay for high-risk children, it may be manifest most clearly with auditory tasks when female children are employed and evident for boys using either visual or auditory tasks.

Due to the assortative mating for alcoholism that is seen among spouses of alcoholics, it is not surprising that of the 27 children with an alcoholic mother, 14 of them also had an alcoholic father. Therefore, it might be argued that the deficits seen in children of alcoholic parentage might be due to the children having two alcoholic parents, rather than to any specific effects of having an alcoholic mother. Therefore, results were analyzed for the children with only an alcoholic mother finding that P300 amplitude was reduced in these children as well. Moreover, restricting the analysis to only girls reveals a transmission of alcoholism vulnerability from mother to daughter that can be detected in the P300 amplitude.

Studies of children of alcoholic mothers may be complicated by the fact that some mothers will have consumed
alcohol or other drugs during pregnancy. Therefore, systematic analysis of prenatal alcohol consumption was assessed for all mothers, allowing for testing of the separate effects of the familial diathesis factor and the possible effects of prenatal exposure. Analysis of results for mothers who did not drink during pregnancy when compared to controls (who uniformly drank less than 1 drink per day or were abstinent) demonstrated that the familial diathesis factor was significant in the absence of prenatal exposure to alcohol.

The results obtained for the N250 component are interesting in that they replicate those found for children of male alcoholic probands (Hill et al. 1990; Steinhauer and Hill 1993). Children from high-risk families have greater negativity of the N250 component than children from low-risk families. These findings are of interest because of the association between N250 and development. Friedman and colleagues (1984) have shown that N250 becomes less negative as children mature. Thus, the higher negativity of the high-risk children suggests some developmental delay in this regard. In view of the fact that we found that P300 differences between risk groups became more significant when the N250 amplitude at parietal was used as a covariate, we speculate that both N250 and P300 are separate indicators of developmental delay, possibly with differing slopes across adolescence, but each indexing risk for later development of alcoholism. Recently, we followed a subsample of 20 children from our larger longitudinal follow-up (both girls and boys) who were initially tested at a mean age of 10 years and retested approximately 8 years later (Hill et al., in press). This analysis revealed a significant reduction in P300 among children in the high-risk group relative to the low-risk group at both points in time. Moreover, significantly more of the high-risk children had already developed alcohol problems by age 18, the presence of which was correlated with amplitude of auditory P300.

The results for the visual ERP paradigm by risk group were not significant overall. However, when analyses were restricted to the male children of high-risk mothers and age- and gender-matched controls, P300 amplitude was significantly reduced in high-risk boys. This finding is a replication of previous results for male children of male alcoholics (Hill and Steinhauer 1993b). Thus, it is tentatively concluded that the visual paradigm may be a more sensitive indicator of cognitive processing deficits in males, whether they are offspring of alcoholic men or alcoholic women. Because of the gender distribution for children with an alcoholic mother only versus those who had both an alcoholic mother and an alcoholic father, it was not possible to test the separate effects of one parent versus two in the production of P300 amplitude decrements in the visual modality. However, it is noteworthy that the effect is replicated across studies in samples of high-density male alcoholism families as well as high-density female alcoholism families.

In summary, the present results demonstrate that children of mothers drawn from high-density families (i.e., half of all known first- and second-degree female relatives were alcoholic) display decrements in P300 amplitude in both auditory and visual modalities. Consistent with previous results for children of male alcoholics, the visual modality paradigm appears to discriminate risk groups best for male children, while the auditory modality is a better modality for picking up risk group differences for female children. Moreover, a clear demonstration of transmission of alcoholism risk from mothers to minor female children has been shown that cannot be accounted for by either prenatal exposure to alcohol or parentage by an alcoholic father.

References


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Hollingshead AB (1975): Four factor index of social status, unpublished.


