Event-Related Potentials in Alcoholic Men, Their High-Risk Male Relatives, and Low-Risk Male Controls

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A total of 217 adult male subjects were evaluated utilizing event-related potentials (ERPs) elicited with two different auditory tasks (Counting and Choice Reaction). Ninety-eight alcoholic subjects from high-density, multigenerational families were evaluated along with 39 first-degree nonalcoholic relatives from the same high-density families. Eighty controls, selected for low density of alcoholism in their extended families, were also studied. Using both conventional and topographic analyses, no significant differences in the amplitude of the P300 component could be found with either of the auditory tasks. No significant differences in amplitude of N250 were seen. The latency of N250 increased with increasing conditional probabilities (0.33, 0.67, and 1.00), a trend that was amplified in the Counting task as compared with the Choice Reaction task. This prolongation in a task not requiring a reaction response (button press) tended to increase the latency more for alcoholics than controls or high-risk nonalcoholic subjects. Age, lifetime, and recent drinking were treated as covariates in all analyses. The absence of P300 amplitude differences between adult high- and low-risk subjects is discussed in the context of the much more reliable differences seen between high- and low-risk children from the same high- and low-density families, when evaluated with the same auditory tasks.

Key Words: ERP, P300, High-Risk, Alcohol Consumption, Family History.

The possibility that the P300 component of the event-related potential (ERP) may have etiological significance for alcoholism has been discussed for over a decade now.1-3 There is long-standing and extensive literature discussing the cognitive impairment seen in chronic alcoholics, starting with the classic work of Victor et al.,4 who described the neuropathological changes seen on autopsy. These subcortical changes were correlated with both memory and attentional deficits. The notion that cognitive changes as reflected in scores on neuropsychological tests administered to alcoholics might be a premorbid condition associated with the etiology rather than simply the consequences of alcohol use was conceptually quite appealing.5 Similarly, the P300 as an electrophysiological index of cognitive function presents the same conceptual dilemma. Are the alterations seen in P300 amplitude and latency a cause or consequence of drinking? Unraveling this puzzle is especially difficult because there are a multitude of factors affecting the emergent waveform, of which P300 is but one component.

Because the P300 component of the ERP is an electroencephalographic index of an individual's capacity to process stimulus information, both P300 amplitude and latency have been studied in samples thought to vary on some neurocognitive, behavioral, or maturational dimension. For example, P300 amplitude and latency vary with the developmental stages of childhood and in accordance with the varying rates of maturation of the auditory and visual modalities during childhood.7,8 In fact, it has been shown that the neural generators of P300 may well be different for each modality.10,11

Also, there is a substantive literature demonstrating a relationship between neuropsychological states, such as Alzheimer's disease, closed-head injury, and other organic brain syndromes and an increased latency of P300.12-16 In contrast to the increased latency seen in association with environmental exposures or organic disease states, decrements in P300 amplitude are more often associated with the presence of psychiatric disorders, as for example when schizophrenic patients are evaluated17-19 or when individuals without evidence of any psychiatric disorder carry an especially high loading for a psychiatric disorder, particularly alcoholism.2,3,20 Thus, it is possible that the amplitude of the P300 may be an inherent characteristic of individuals before they develop psychiatric states that may be related to their vulnerability for incurring these disorders.3

Elucidating the factors responsible for variations in P300, particularly those that might be heritable, is of interest given the observed association between P300 and psychiatric pathology. There is a considerable body of evidence suggesting that brain neuroelectrical activity, whether background electroencephalograms,21-24 averaged sensory-evoked responses,25,26 or ERPs27,28 is heritable.

A number of studies have reported that when ERP waveforms of two individuals are compared, greater concordance is observed between first-degree relatives than unrelated individuals, with the greatest similarity observed in monozygotic twins.20,27,28 In particular, the later components of the ERP associated with cognitive processing activities, especially the amplitude of the P300 component, show a very high similarity in monozygotic twins, with the correlations ranging between 0.5 and 0.9.27-30 A high degree of similarity in siblings has also been demonstrated,
with correlations ranging between 0.61 and 0.82, depending on the task used to elicit the P300. ERP data from a large family study of alcoholism has been analyzed using segregation analysis to determine possible modes of inheritance of the P300 component, with evidence presented for a major gene controlling the familial similarity in P300 amplitude. ERP differences, especially in the amplitude of the P300 component, have been reported for abstinent male alcoholics when compared with control subjects, although differences have not been found in all studies. On the other hand, adult female alcoholics show profound reductions in P300 amplitude in comparison with age-matched normal controls. Thus, it may be the case that the differences observed between high- and low-risk children for P300 amplitude may well normalize by adulthood for males in contrast to females. At any rate, it is clear that a number of laboratories now have been able to document differences in P300 characteristics between high- and low-risk children. Because we did not see amplitude reduction or prolongation of latency in the rare stimulus condition in male alcoholics previously, the purpose of the present analysis was to determine if abstinent alcoholics or their high-risk relatives would differ from low-risk controls, with respect to either P300 amplitude or latency. The present results represent a greatly expanded sample of 98 alcoholics and 80 controls (previous results were based on 34 alcoholics and 20 controls). To ensure that bias did not enter from confounding effects of other variables, careful histories of current and past health status, as well as exposure to alcohol and drugs, were obtained for all individuals utilized in the analysis.

METHODS

Subjects

A total of 217 male subjects ascertained through a large family study. Cognitive and Personality Family Factors Study (CPFSS), were interviewed to determine lifetime psychiatric status and tested in our laboratories. Individuals studied were determined to be from either high-risk for alcoholism families or low-risk controls. The high-risk families had an especially high density of alcoholism running in their families, such that each alcoholic proband found in a treatment center and included in the present report had an average of 2.9 first-degree relatives who were also alcoholic. The low-risk controls were from families without alcoholism or any psychiatric disorders. Within the high-risk families, some individuals became alcoholic and some did not. As a result, comparisons are reported for alcoholics, nonalcoholics with the same familial loading, and low-risk controls. All subjects in the present report were male and over the age of 21 years.

High-risk families were ascertained through a proband pair of male alcoholics, identified through one member of the pair currently being in treatment at the time of selection. Data were also obtained for nonalcoholic male siblings. Alcoholic brothers were required to meet Feighner et al. criteria for alcoholism, as well as DSM-III criteria for alcohol dependence (DSM-III was the nomenclature in place when the study began). Other axis I diagnoses were ruled out, (i.e., recurrent major depression, schizophrenia, and drug dependence). Nonalcoholic members of high-risk families did not meet even probable criteria for alcoholism, although all were social drinkers.

Low-risk families were selected for absence of psychopathology from among volunteers answering advertisements for subjects to participate in a study of personality and cognition (no criteria for acceptance were advertised). Control probands, along with first-degree relatives, were free of all axis I psychiatric disorders, including alcoholism.

Subjects with major medical diseases unrelated to alcoholism were not included. Only a few of the subjects had a history of epilepsy, stroke, or head injury leading to unconsciousness for >30 min. (Two controls reported injury-related seizure disorders by past history only; one nonalcoholic high-risk brother reported a current seizure disorder. Although many of the alcoholics reported loss of consciousness at some time in their life, only four alcoholics reported being unconscious for an extended period of time. All four cases were childhood coma as a result of injury.) Subjects were asked to refrain from using alcohol or drugs for 48 hr before testing. To supplement and verify self-reports of recent abstinence, a blood sample was obtained on the day of testing and liver enzymes [including SGOT, SGPT, and γ-glutamyltranspeptidase (GGT)] were analyzed. Only one subject included in the analysis had liver enzymes and self-report indicating persistent liver abnormalities (one control had had type C hepatitis). Drug screens were performed to verify self-reports.

Lifetime alcohol consumption, utilizing the format described by Skinner, was determined for all members of the CPFSS study since its inception in 1984. Additionally, a detailed history of all beer, wine, and hard liquor (average, typical and highest) per occasion was assessed for multiple time frames (including 7 and 30 days). In addition, all subjects were questioned about the last time they used alcohol so that number of days dry could also be determined. From these data, derived measures were calculated: Lifetime Consumption in g/kg and Drinking Intensity = Lifetime Consumption/years of heavy drinking (as described by Pfefferbaum et al.). The number of years of heavy drinking was determined by utilizing the subject's current age minus the age of onset of alcoholism.

Clinical Assessment

Clinical assessment included administration of a comprehensive diagnostic interview that included the Diagnostic Interview Schedule (DIS), as well as a current and lifetime history of drinking and drug use. The DIS diagnosis was verified by a second unstructured interview performed by a second clinician, allowing for a consensus diagnosis of presence or absence of alcoholism and other psychiatric disorders using DSM-III criteria (DSM-III was the nomenclature in use at the initiation of the study).

Family history data were obtained, where needed, using a semistructured interview developed by one of the authors (S.Y.H.). Validity data have been calculated and specificity found to be >90% (Smith, unpublished Ph.D. dissertation). Familial density of alcoholism was determined in 98% of the cases by direct, in-person interview of the first-degree relative.

As an independent assessment of cognitive functioning, all subjects were administered the Category Test from the Halstead-Reitan Battery.

ERP Procedure

ERPs were elicited with high and low tones presented in an oddball paradigm as previously described. All stimuli were generated by a tone generator controlled by a PDP-11/23 and ERP data collected with the same computer system.

Auditory ERP Procedures. All subjects were given an audiometric screening test of 40 dB HL at 500, 1000, 2000, and 4000 Hz to assess hearing impairment. Two tasks were used to elicit the ERPs: a Counting task and a Choice Reaction task. Subjects were presented with either 800 or 1500 Hz tones (40-msec duration, 2-msec rise/fall time, intensity 70 dB). Randomly generated by computer and presented every 3 sec. (The only restriction was that two high tones could not be presented in a row; subjects were explicitly informed of this restriction.) The overall probability of the high tone was 0.25.

Counting Task. In the Counting task, subjects were asked to simply count the number of high tones they heard and report the total at the end of each of four successive blocks of 80 trials.
**Choice Reaction Task.** In the Choice Reaction task, subjects were asked to press one button (e.g., "button on your left") when they heard a high tone and the other (e.g., "button on your right") when they heard the low tone. Subjects were randomized for which came first (right or left), and each subject alternated on each of the required two successive blocks of 80 trials.

**Electrophysiological Recordings**

ERPs were recorded using Ag/AgCl electrodes placed over the midline frontal, vertex, parietal, and occipital (Fz, Cz, Pz, and Oz, respectively) locations, left and right parietal (P3 and P4) locations, and referred to linked ears, with forehead ground. Ocular artifacts were monitored from an electrode placed beneath the left eye, referred to linked ears. Electrophysiological data were amplified by 20K (10K for the eye channel) using a Grass model 12 Neurodata system, set to a bandwidth of 0.01-30 Hz. A Digital Equipment Corporation PDP-11/23 lab computer sampled each trial for a 1200-msec epoch at 8-msec intervals, beginning with a 200-msec prestimulus baseline. The subject's response, reaction time, and correctness of the response were encoded directly into the online file.

**ERP Data Reduction**

Eye artifacts (blinks or eye movements) greater than ~75 μV were identified online or detected automatically during offline analysis.

**ERP Data Analysis**

Conventional Analyses. After excluding trials with artifacts, three averaged ERPs were computed for each task and labeled by the conditional probability associated with their occurrence implied by the restriction that two high-pitched tones could not occur in succession. Thus, the predictable low tone following each high tone had a conditional probability of 1.00. After any low tone, the likelihood of an unpredictable low tone occurring on the next trial was 0.67, and the complementary conditional probability of a high tone was 0.33 (the latter condition corresponding to the "rare" event in the typical oddball paradigm.

ERP component N100, P200, N250, and P300 were identified offline using a peak detection program. The N100, P200, and N250 components were selected as the maximum deflection at Cz (within the respective predefined time windows 80–136, 136–240, and 200–320 msec). The program searched for maximum positivity of P300 at Cz in an initial window beginning at either 256 msec (or the peak of N250, if later) and extending to 416 msec. Augmented by the analog plots of all ERP data, peak identification was verified visually by at least two experimenters who could extend the search window up to 700 msec. Latency of the peak component (at Cz for N100, P200, and N250, and at Pz for P300) and peak-to-baseline amplitudes for each electrode at these latencies were automatically stored in ASCII files.

Amplitude and latency data were analyzed by ANOVA using repeated measures designs, using the Greenhouse-Geisser correction for degrees of freedom as appropriate.

*Topographical Analysis.* The topographic distribution of P300 for the rare event (0.33 condition) was analyzed among the six available electrode sites using both unadjusted and normalized amplitudes to determine possible differences by risk group. Analyses were restricted to the 0.33 condition, because it represents the most frequently reported "target" condition in high-risk for alcoholism studies. Inconsistencies in the literature with regard to whether or not alcoholics and controls differ in P300 amplitude might be due to differences in the scalp distribution of this component between the two groups. Additionally, our previous work suggests greater N250 negativity in high-risk for alcoholism children. It may be the case that this difference persists into adulthood. Therefore, the scalp distribution of N250, as well as P300, between the two risk groups was tested.

Data were normalized according to the procedure described by McCarthy and Wood.54 For each group, the root mean square (RMS) value of the group mean amplitude across electrodes was calculated separately for each task. A scaling factor was calculated to set the resulting vector length equal across each group. In brief, a scaling factor was calculated as the ratio of the RMS value derived from the control group means divided by the RMS value for each of the contrasting group means (one factor for alcoholic males, and a separate factor for their nonalcoholic brothers). Each individual's data were then scaled by the appropriate factor before performing the ANOVAs. Scaled data were only used for topographical analyses of P300 and N250 to explore topography as described here; all other amplitude analyses were based on unscaled data.

**RESULTS**

**Category Errors**

A one-way ANOVA of Category errors revealed no significant differences between risk groups (alcoholic males, nonalcoholic brothers of alcoholics, and control males). This suggests that the groups were well matched for overall neuropsychological performance.

**Sociodemographic Status**

As may be noted in Table 1, the mean Hollingshead scores for alcoholics and their nonalcoholic brothers were similar, with average scores falling in level III (skilled occupations). Controls, on average, fell within the level IV classification (technical and semiprofessional).

**Familial Density of Alcoholism**

As may be seen in Table 1, the average number of first-degree alcoholic relatives (determined by in-person interview) was 2.9 for the high-risk subjects. This is a greater density of alcoholism than is usually seen in studies in which family history-positive and family history-negative individuals are contrasted. Moreover, studies reporting familial density have typically utilized family history report rather than direct face-to-face interviews. Family history has notoriously low sensitivity when a single informant is used.46,47 Because the familial density of alcoholism in this study is greater than that seen in many studies, the severity of alcoholism is greater than that usually reported, with a mean age of onset, according to the Feighner et al.42 criteria, of 20.9 years.

**Drinking History**

As may be seen in Table 1, the average alcoholic male drank 18,654 g/kg over his lifetime. This compares with 15,040 g/kg reported by Pfefferbaum and colleagues.53 For comparison, "drinking intensity," as described by Pfefferbaum et al.,35 was calculated using the first alcohol problem to determine the onset of alcoholic drinking. Drinking intensity was found to be 1.073 g/kg/yr for the alcoholic group and is somewhat higher than that reported by Pfefferbaum et al. Also, as may be seen in Table 1, approximately two-thirds of the alcoholic subjects studied were not abstinent in the month preceding testing.

The possible effects of alcohol withdrawal on P300 were tested utilizing the extensive drinking history available for
Table 1. Sample Characteristics: Means (ses)

<table>
<thead>
<tr>
<th></th>
<th>Alcoholic males (n = 98)</th>
<th>Nonalcoholic brothers (n = 39)</th>
<th>Control males (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.3</td>
<td>41.1</td>
<td>44.4</td>
</tr>
<tr>
<td>Education</td>
<td>13.0</td>
<td>13.4</td>
<td>14.7</td>
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<tr>
<td>Socioeconomic status</td>
<td>36.1</td>
<td>39.1</td>
<td>46.2</td>
</tr>
<tr>
<td>No. of first-degree relatives alcoholic</td>
<td>2.9</td>
<td>2.9</td>
<td>0</td>
</tr>
</tbody>
</table>
| Lifetime alcohol consumption (g/kg)/
  (kg)†                     | 18,654                   | 3,076                         | 2,302                  |
| Age of onset of
  alcoholic drinking†      | 20.9                     | —                             | —                      |
| Drinking intensity (g/kg/ y)†| 1,073                    | —                             | —                      |
| No. of drinks past 7 days | 13.0                     | 5.0                           | 3.1                    |
| No. of drinks past 30 days| 23.2                     | 29.6                          | 15.6                   |
| % of subjects abstinent
  in past 30 days          | 35.7                     | 10.3                          | 20.0                   |
| % of subjects abstinent
  in past 60 days          | 19.4                     | 10.3                          | 16.3                   |
| % of subjects abstinent
  in past 90 days          | 11.2                     | 10.3                          | 12.5                   |
| Category test errors      | 53.3                     | 54.8                          | 52.2                   |

* Data available for 199 subjects.
† Age of onset of first alcoholism problem by Feighner criteria.
‡ Drinking intensity was calculated by dividing the number of lifetime drinks by the subject’s total years of alcoholic drinking (current age minus the age of onset of alcoholism). Drinks were first converted to grams using 31.5 g/drink and adjusted for body weight in kilograms.

To evaluate further the extent of chronic drinking on the ERP, a blood sample was taken on the morning of testing to determine liver enzyme levels. Five of the 98 alcoholic subjects displayed elevated GGTP levels (more than twice the normal value). Of these, one case reported accidental liver trauma. None of these five individuals showed evidence of chronic liver injury, because their SGPT and SGOT levels were not elevated. GGTP was within the normal range for all of the nonalcoholic brothers. However, 1 of the 80 control subjects had an elevated GGTP level, traceable to type C hepatitis.

ERPs

Analyses were performed using an ANOVA (BMDP 2V) to reveal statistically significant differences between the three risk groups for both the Counting task and Choice Reaction task. Average ERP waveforms for the 0.33 condition for each risk group are shown in Fig. 1. The total number of single trials analyzed in the control, alcoholic, and nonalcoholic groups was 5,349, 6,373, and 2,390, respectively, for the Counting task. For the Choice Reaction task, the total was 2,975, 3,528, and 1,428 for the controls, alcoholics, and nonalcoholics, respectively.

P300 Amplitude

P300 amplitude data were analyzed to evaluate the main effects and interactions of four factors: risk group, task, probability, and electrode location. Only the first- and second-order interactions significant to this study are reported. Across electrodes, P300 amplitude was most positive at Pz ($F = 73.46, df = 2.507, p < 0.0001$) and therefore, further analyses were restricted to the Pz electrode.

* Group Effects. No significant differences were seen involving risk group, with mean amplitudes at Pz (collapsed across task and probability) of 8.29 μV, 8.12 μV, and 7.87 μV for the alcoholics, nonalcoholics, and controls, respectively.

* Age Effects of P300 Amplitude by Group. An analysis of covariance was performed to control for the possible effects of age on the P300 component. Although significant as a covariate ($p < 0.0001$), covarying age had minimal influence on P300 amplitude by risk group.

* Drinking History and P300 Amplitude by Group. The number of days abstinent before testing was used as an independent factor and found to be nonsignificant. Similarly, the group by days abstinent interaction was not significant. Additionally, an analysis of covariance was performed using the amount of alcoholic beverages (beer, wine, and liquor) consumed in the past 7 and 30 days, as well as days dry (see Table 1 for mean values) to determine drinking effects on the ERP. The alcoholic group exhibited the greatest amount of drinking during the previous week and month.
before testing. However, covarying these drinking variables had a minimal effect on group differences.

**Task and Probability Effects.** Amplitude was seen to decrease with increasing conditional probability ($F = 444.99$, $df = 2.329$, $p < 0.0001$). Across all subjects, mean P300 amplitudes across electrodes were $9.54 \mu V$, $4.18 \mu V$, and $2.71 \mu V$, respectively, for the 0.33, 0.67, and 1.00 conditional probability conditions in the Counting task, and 9.94 $\mu V$, 5.50 $\mu V$, and 2.51 $\mu V$ in the Choice Reaction task. The Choice Reaction task elicited overall higher amplitudes than the Counting task ($F = 4.24$, $df = 1.214$, $p = 0.041$).

**P300 Latency**

*Group Effects.* Latency data for P300 were subjected to a three-factor ANOVA using risk group, task, and probability. A significant group effect was found for P300 latency due to the fact that alcoholics and their nonalcoholic brothers showed somewhat shorter latency than controls, the maximum difference (between controls and nonalcoholic brothers) being 12 msec on average, representing 1½ sampling points ($F = 3.97$, $df = 2.214$, $p = 0.020$). Covarying recent drinking in the past 7 and 30 days decreased the significance of this group difference ($F = 3.08$, $df = 2.212$, $p = 0.048$). Age, when used as a covariate, removed the significant latency differences by group. As a further assurance that latency differences between groups were not missed because of slight differences in age of the two groups, all scores were age-regressed and the ANOVA repeated with age-regressed values. Again, latency did not differ with group membership.

*Task and Probability Effects.* Similarly, a difference in latency associated with task (Counting = 344.3; Choice Reaction = 348.1 msec) representing a change of <1 sampling point was also found ($F = 3.92$, $df = 1.214$, $p = 0.049$). Corresponding to the results found for P300 amplitude, the latency of P300 was seen to decrease with increasing conditional probability (356.40, 345.60, and 336.63 msec for 0.33, 0.67, and 1.00 conditions, respectively) ($F = 38.97$, $df = 2.386$, $p < 0.0001$). Additionally, a task by probability interaction ($F = 22.52$, $df = 2.420$, $p < 0.0001$) reached statistical significance due to a slight increase in latency for the 0.67 condition in the Choice Reaction task.
The analysis was then reevaluated separately by task. Of particular interest was the latency difference observed between high-risk subjects in the Choice Reaction task. Nonalcoholic males exhibited the shortest P300 latency (341.1 msec), followed by their alcoholic brothers (345.1 msec). Control males showed a significantly longer latency at 358.2 msec ($F = 5.53, df = 2,214, p = 0.005$). Removing the effects of age accounted for only some of the variation between risk groups; a significant latency difference remained between groups ($F = 3.23, df = 2,213, p = 0.042$). However, it should be pointed out that this represents only 2 sampling points and may be of minimal significance.

**Topographical Analysis: Scalp Distribution of P300 and N250**

There were no differences in scalp topography for the rare, infrequent tone among groups for P300 or N250 using either unadjusted amplitudes or normalized data.

**Other ERP Components (N100, P200, and N250)**

All other components (N100, P200, and N250) were analyzed at the Cz electrode. Only first-order interactions are reported here for these early components.

**N100 Amplitude.** Significantly greater negativity was observed for N100 among controls ($-12.4 \mu V$) and alcoholic men ($-11.4 \mu V$) than was seen for the nonalcoholic brothers of alcoholics ($-9.98 \mu V$) ($F = 4.89, df = 2,214, p = 0.008$).

**P200 Amplitude.** There were no group effects associated with P200 amplitude. P200 was significantly more positive for the Counting task ($6.19 \mu V$) than for the Choice Reaction task ($2.49 \mu V$) ($F = 253.2, df = 1,214, p < 0.0001$). P200 amplitude became more positive with increasing probabilities ($3.67, 4.30$, and $5.05 \mu V$ for $0.33, 0.67$, and $1.0$ conditions, respectively) ($F = 23.42, df = 2,359, p < 0.0001$).

**N250 Amplitude.** The amplitude of the N250 component was observed to be less negative in the Counting task ($0.18 \mu V$) than in the Choice Reaction task ($-3.30 \mu V$) ($F = 238.8, df = 1,214, p < 0.0001$), which parallels the finding for P200. Although N250 varied over a $2.4 \mu V$ range among the three probability conditions ($F = 45.99, df = 2,322, p < 0.0001$), the amplitude of the N250 component did not appear to be linearly related to event probability. A task by group interaction ($F = 6.20, df = 2,214, p = 0.002$) was found due to a greater increase in negativity for the Choice Reaction task in comparison with the Counting task for controls ($4.46 \mu V$) compared with both the nonalcoholic ($3.10 \mu V$) and alcoholic ($2.88 \mu V$) groups. Similarly, a greater range of amplitude was observed in association with event probability for controls when compared with both the alcoholic and nonalcoholic males (probability by group interaction: $F = 3.45, df = 4,322, p = 0.017$). Thus, the high-risk subjects appeared to modulate the amplitude of N250 in response to changing probability conditions less than the controls.

**N100 and P200 Latencies.** Although N100 and P200 latencies showed significant but minor differences ($<1$ sampling point difference) associated with task and probability conditions, no significant differences by group membership were seen.

**N250 Latency.** For the Counting task, N250 latency (255.6 msec) was longer than for the Choice Reaction task (246.9 msec) ($F = 36.21, df = 1,214, p < 0.0001$). Additionally, N250 latency increased with increasing event probabilities ($241.3, 250.5$, and $262.0$ msec for $0.33, 0.67$, and $1.0$, respectively) ($F = 92.04, df = 2,394, p < 0.001$). Prolongation of N250 for the Counting task, as compared with the Choice Reaction task, was greatest for the alcoholic males (13 msec) as compared with controls (7 msec) and nonalcoholic brothers (5 msec), and resulted in a significant task by group interaction ($F = 3.63, df = 2,214, p = 0.028$). It should be noted that this statistically significant difference is based on only an interval of $\sim 2$ sampling points (each sampling point is 8 msec), so that its importance should be viewed cautiously. Similarly, a significant group by probability interaction ($F = 5.44, df = 4,394, p = 0.0004$) was related to differences in latencies between groups that were no $>14$ msec at any probability level.

**DISCUSSION**

If auditory P300 amplitude is a biological marker of alcoholism risk in children indexing a neurobiological developmental delay, then it would be expected to normalize by adulthood in most individuals. As a result, large differences would be expected between high- and low-risk children and minimal to no significant differences between high- and low-risk adults. How then can we explain observed differences between abstinent alcoholics and controls previously reported? First, there are as many negative reports concerning P300 amplitude differences between alcoholics and controls $^{12,20,36,37}$ as there are positive ones. $^{33-35}$ P300 latency differences have been shown in previous studies of alcoholics compared with controls, $^{20,33}$ although not in all studies, $^{34}$ including the present one. Whereas a 12-msec difference in latency was noted between controls when compared with alcoholics and their nonalcoholic brothers, this group difference was no longer significant when age was used as a covariate. Another possibility might be that some aspect of the task (e.g., difficulty, length of intertrial interval, and probability of targets) might have contributed to the negative result. We view these explanations as quite unlikely. First, the task difficulty, auditory reaction time paradigm, is similar to that employed in other investigations in which differences between alcoholics and controls were reported. $^{33}$ In fact, the amplitude of the P300 response for alcoholics in that study was quite similar to the present one (both $\sim 8 \mu V$). The difference between the two studies appears to be in the larger P300 amplitude reported for
controls (largely hospital staff) in that study compared with the present one. This suggests that particular characteristics of the alcoholics studied may have contributed to the alcoholic versus control differences previously reported for P300 amplitude and/or latency.

Among these might be markedly lower socioeconomic status or lesser educational attainment (individuals might be less test wise) or an older age at testing, factors that might be especially cogent for the chronic alcohol abuser for whom alcohol may accelerate the aging process. Previously reported prolongation of latency in chronic alcoholics may have been due to the fact that chronic alcoholics, with longer histories of drinking, tend to be older. There is a well-known relationship between P300 latency and age\textsuperscript{48} in which the P300 latency increases with advanced age. Moreover, chronic alcohol use is associated with poorer neuropsychological test performance, quite possibly due to accelerated aging.\textsuperscript{39} Note, for example, that the 27 male alcoholics studied by Pfefferbaum and colleagues\textsuperscript{33} averaged 40 years of age, with a range extending to 60 years and were drawn from among men enrolled in a Veterans Administration treatment program. This may be contrasted with the present report in which male alcoholics averaged 36 years of age and were largely employed in skilled levels of occupation (mean SES of 36). Moreover, controls and nonalcoholic brothers in the present study were, on average, reasonably well matched for age to these alcoholics. Nevertheless, latency effects that were seen were entirely attributable to age.

A second important point to note is that some investigators have assumed that whatever vulnerability factor P300 represents in children has similar explanatory power for young adults.\textsuperscript{50} Thus, it is viewed as an index of genetic vulnerability that persists into adulthood. However, Polich et al.,\textsuperscript{51} in the metaanalytic review, included 10 published studies that concerned nonalcoholic high-risk individuals over the age of 18.\textsuperscript{50,52-60} Interestingly, only four of these studies reported significant differences between high-risk, nonalcoholic, young adults and controls for P300 amplitude.\textsuperscript{50,53,55,60} Those showing significant differences did so using visual tasks and/or utilizing alcohol administration to uncover differences. There are no studies utilizing an auditory task that have revealed differences between high-risk, nonalcoholic, young adults and low-risk controls in a baseline condition (no alcohol administration). Thus, the present results for the 39 nonalcoholic high-risk subjects who did not differ from controls appear to have a precedent in the studies outlined above.

Others have assumed that observed differences are due to lifetime or recent drinking alone, with genetic vulnerability being a comparatively weaker explanation. If this is the case, alcoholics would be expected to show P300 amplitude decrements as a result of long-standing chronic alcohol consumption leading to neuropathological changes that P300 would index. However, when the careful histories of alcohol use, both lifetime and recent, were used as covariates in the present analysis, there were no appreciable effects on the results. A recent report by Pfefferbaum and colleagues\textsuperscript{33} finds amplitude of P300 related to a family history of alcoholism, but not to the alcoholic's lifetime drinking history.

With respect to the relationship between family history of alcoholism and P300, our results are negative. The present sample was purposely chosen to exhibit maximal familial loading by design (minimum requirement of two male alcoholic probands/family). This familial loading would appear to be greater than that typically studied in which a single relative may be all that is required to gain entry into the family history-positive group.

The purpose of the present study was to replicate previous results from our own laboratory\textsuperscript{20} for adult male alcoholic subjects, which indicated no differences in amplitude and unremarkable differences in latency [only group differences were found for the nontarget (0.67, 1.00) conditions]. Utilizing the same auditory oddball paradigm previously used, but with an expanded sample, the previously reported latency differences were not replicated. With a greatly expanded sample, the effects of age on P300 latency could be analyzed. ANOVA, covarying out age, resulted in no group differences in any condition.

Second, our hypothesis was that recent drinking of alcohol might present a neurotoxic effect upon P300 so that alcoholics who were abstinent longer might be more similar to controls. A previous report linking P300 and alcoholism in adults discounted drinking history (either lifetime number of drinks or drinking intensity) as a predictor.\textsuperscript{32} The present study was designed to test not only the effects of lifetime history and intensity of alcohol use, but also any possible effects of recent drinking in the past week or month (7- and 30-day drinking). The lifetime quantity of alcohol consumed by subjects in the present report was similar to that found by Pfefferbaum et al.\textsuperscript{30} In fact, the reported drinking intensity of 660 g/kg/yr in the Pfefferbaum et al. report appears to be lower than that seen for the present report (1,073 g/kg/yr). Further, to test the possible effects of abstinence in alcoholics on the brain electrophysiological characteristics, the number of days dry was used as a covariate in the analyses.

Our results indicate that auditory P300 does not differentiate alcoholics from nonalcoholics. This is true when both the alcoholics and nonalcoholics are both high-risk for alcoholism (alcoholic and nonalcoholic brothers comparison) or when high-risk alcoholics are compared with low-risk controls. Because this study has an exceptionally large number of alcoholic subjects and well-matched controls, we view these findings as quite robust, although of course one can never prove the null hypothesis.

We believe that P300 does differentiate children at risk for developing alcoholism, as evidenced by our previous work utilizing both the auditory paradigm used in the present analysis\textsuperscript{5,41} and utilizing a visual paradigm.\textsuperscript{9} Overall, we have demonstrated that approximately one-third of
high-risk minor children will display P300 amplitude reduction (1–2 μV below the mean of the entire group). P300 amplitudes are considerably larger among children than adults. In spite of this, large differences in P300 amplitudes were observed between these high- and low-risk children (see Fig. 2 and Steinhauser and Hill). To illustrate the strength of this association, Fig. 2, based on data from Steinhauser and Hill, illustrates the clear differences between high- and low-risk pubertal boys utilizing the same auditory modified oddball paradigm utilized in the present report. The difference in amplitude was ~12 μV. Moreover, these high-risk children, who have an average of 4.1 alcoholic relatives, are the sons of high-risk parents. The low-risk children are the sons of control parents. Therefore, the same degree of genetic loading was effective in both parents and sons. In fact, the children studied in that report (abstracted in Fig. 2) are the children from the same families upon which the present report is based. Exactly the same paradigm and stimulus conditions were used to record ERPs in the children’s report and the present report. Therefore, failure to find differences among the male adult members from high- and low-risk families cannot be due to insufficient familial loading in the high-risk group. Rather, we hypothesize that a proportion of high-risk children show the P300 abnormality that is associated with later development of alcoholism. The P300 abnormality normalizes by adulthood, although the alcohol abuse persists.

Currently, we are following these children to determine if those with P300 abnormalities are especially vulnerable to succumbing to alcoholism as adults through our long-term follow-up of minor children. However, we believe that this abnormality is normalized by adulthood in males so that high-risk males will not vary from low-risk ones with respect to P300 amplitude when they reach maturity at 18–20 years of age. Similarly, high-risk alcoholics will not differ from controls, unless, of course, the combined effects of age, poor health, and chronic drinking lead to neuropathological changes that might be picked up as latency changes in P300. In contrast, young, adult, high-risk males who are healthy and high functioning will not display P300 deficits, even if they are not only high-risk by genetic background but also alcoholic as well.

In summary, auditory P300 is a remarkably robust marker of risk for later development of alcoholism when used as a screening methodology in childhood. However, not all children carry the marker, and of those who do, normalization probably occurs sometime around the onset of young adulthood in males. P300 appears not to be influenced by lifetime or recent drinking. We cannot explain our failure to find differences in adults on the basis of familial density of alcoholism, because the density observed in this series is much higher than that utilized in most other studies of family history-positive and family history-negative individuals in which only a single first-degree relative is required for entry into the family history-positive group. Although the density of familial loading for alcoholism has a direct relationship to the proportion of children who will display P300 abnormalities, the density of alcoholism is unrelated to P300 status in adult subjects.

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