Event-Related Potential Characteristics in Children of Alcoholics from High Density Families

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Sons and daughters (ages 8–14) of male alcoholics without psychiatric problems were compared with sons and daughters of controls employing two auditory paradigms to elicit event-related potentials (ERPs). All of the children of alcoholics were from high density families (each father had an average of 3.7 first and second-degree relatives meeting criteria for alcoholism). Subjects were presented with high- and low-pitched tones with global probabilities of 25% and 75% of total trials, respectively. Subjects were instructed to count silently the number of “high” tones (rare targets) but not the number of “low” tones (non-targets) and report the number heard. In a second auditory paradigm (Choice Reaction task), subjects were asked to perform a different motor response to each high or low tone. The amplitude of the P300 component was influenced significantly by event probability (decreased amplitudes were associated with increased event probability). A greater rate of decrease in P300 amplitude occurred among the high risk children as event probability increased. In addition, greater negativity beginning at approximately N250 was observed for both tasks at the frontal electrode for the high risk children as compared to controls. This enhanced frontal negativity is interpreted in terms of a maturational lag hypothesis.

VULNERABILITY to developing alcoholism involves the influences of both genetic and environmental factors.1 Recently a great deal of attention has been focused on event-related brain potentials (ERPs) as possible biological markers for alcoholism risk. It is now well established that chronic alcoholics exhibit deviations in ERP characteristics.2,3 There is considerable consistency in results obtained for the P300 component of the ERP for individuals at risk for alcoholism who are not themselves alcoholic4–7 though there have been reports that failed to find differences between high and low risk groups.8 Using both auditory and visual paradigms, Polich and Bloom9 reported no differences in ERP amplitude or latency as a function of family history of alcoholism. While the electrophysiological paradigms used were similar to those employed in other investigations, the clinical criteria for alcoholism may have differed from other investigations reporting positive results. Polich used family history to define the presence of alcoholism in parents, allowing a single positive symptom to qualify as alcoholism rather than the usual multiple symptom criteria (e.g., Feighner criteria require three out of four symptom categories). Thus, individuals with a single alcoholic relative diagnosed by a single symptom of alcoholism may have resulted in the family history positive group having a rather weak form of familial alcoholism, and increasing the chances of finding no differences between the family history positive and negative groups.

P300 refers to a scalp-positive component, which occurs at approximately 300 msec9,10 and is related to stimulus evaluation whereas components with latencies of 200 msec or less (e.g., P50, N100, P200) tend to be associated primarily with the physical characteristics of the stimuli presented. (N100 amplitude also is related to general levels of attention.)

Differences in ERP characteristics have been reported from this laboratory for adult men from high risk families when contrasted with low risk (control) subjects.11,12 The subject population studied has included only individuals from high density families where multiple cases of alcoholism are found. This variant has an early onset, and is expressed in spite of a favorable environment (the majority of fathers of the alcoholic probands were professional or semi-professional). The variant studied, therefore, is at least as severe as that described by Cloninger and colleagues in their descriptions of the “male-limited” (Type 2) form.13 This form tends to be transmitted from father to son, includes familial sociopathy and tends to be expressed independent of environmental conditions.

While reports concerning family history positive and negative adult young men have been mixed, more consistently positive findings have been noted for minor children.5,7 Residual effects of alcohol consumption might have subtle effects on information processing, though correlations between P300 amplitude and latency with recency of alcohol use have not consistently been found.12 To rule out any confounding effect of alcohol use, testing of the children of subjects previously described12 was initiated. These children represent a third generation for whom ERP data is being collected as part of a large scale pedigree study.

METHODS

Two groups of children were tested: children of alcoholic fathers and children of normal controls. The alcoholic fathers were selected from...
treatment facilities if they had a diagnosis of alcoholism and were free of other DSM-III (Axis I) psychopathology. Families were included only if all first degree relatives met criteria for absence of other psychopathology with one exception: sociopathy was present in less than one third of the families.

Alcoholism was determined through use of a structured interview, which included portions of both the Diagnostic Interview Schedule and the Renard Diagnostic Interview, allowing for determination of whether or not the individual met DSM-III or Feighner Criteria for alcoholism. Additionally, the families of the alcoholic probands were required to meet certain structural characteristics for inclusion: the presence of at least one other alcoholic male sibling, one nonalcoholic male sibling, and at least one parent. For the present study, a child between the ages of 8–16 was also required. Thus, three generations of clinical interview data were available for the families of these children, allowing for determination of the average number of first and second degree alcoholic relatives (3.7) in each family.

Control families were selected from among volunteers who responded to an advertisement in local newspapers, which did not indicate that alcoholism was the focus of the study. Adult male social drinkers, who had at least one male sibling and parents available for clinical interview and testing, were selected. In these families, no first or second degree relative met criteria for Axis I psychopathology including alcoholism. All of the mothers from both the affected and control families were free of alcoholism and other psychopathology by family report (mothers were not interviewed directly).

Twenty-two Caucasian children between the ages of 8 and 14 were tested. None of the parents or paternal grandparents (who were interviewed) were foreign born. Therefore, the ethnic background for all of the children was quite homogeneous—all were third generation Americans. The socioeconomic status (SES) of the two groups were determined by employing Hollingshead’s Four Factor Index of Social Status. Using this instrument, socioeconomic status was determined for both fathers and paternal grandfathers of these children. Overall, the families were quite similar with respect to socioeconomic status. Because alcoholism tends to produce downward drift in SES, it might be possible to see lower SES in families of alcoholics than in control families as a result. Scores were first calculated for paternal grandfathers. The mean SES was 37.50 ± 14.7 for fathers of the alcoholic whose child was tested, and 40.2 ± 14.3 for the grandfathers of the control case whose child was assessed (a non-significant difference). When the fathers of the children were compared, the alcoholic fathers were found to be somewhat lower (SES = 36.9 ± 4.9) than the control fathers (45.1 ± 11.9), though this difference was non-significant. Scores of 30–40 are typical for individuals who are employed in skilled, clerical or sales occupations, whereas scores falling in the 40–54 range are typical for individuals who occupy technical and semi-professional jobs. There were seven girls and four boys in each group.

The mean age (±SE) was 10.63 (±1.86) for the children of the affected fathers and 10.81 (±2.48) for controls. The mean age of the fathers in the two groups did not differ (mean age = 37.38 ± 0.49 for the affected group and 37.88 ± 0.42 for the controls). The education of the fathers did differ, however (t(14) = 2.44, p = 0.03), with the affected fathers averaging 12.88 years (se = 0.07) and the controls 14.63 (se = 0.13). All of the children were in the appropriate grade in school for their chronological age. In order to assess the neuropsychological functioning of these children, all were administered the children’s version of the Category test from the Halstead-Reitan Battery.

PROCEDURE

The ERP assessment consisted of two successive tasks: a Counting task and a Choice Reaction task. Both involved a modified version of the oddball paradigm commonly employed in other ERP studies. Subjects were presented with high-pitched tones (1500 Hz) or low-pitched tones (800 Hz). The tones were presented at 70 decibels (Edmont-Wilson, Model 60-510, Sound Level Meter) and were 40 msec in duration, with an abrupt (2 μsec) rise and fall time through a speaker placed in front of the subject who sat in a sound-attenuated chamber in a darkened room. One tone was presented every 3 seconds for a total of 50 tones (trials) per block.

Subjects were first asked to identify sample tones as “high” or “low” in pitch. The subject was then instructed to count silently the number of “high” tones (rare targets) but not the number of “low tones” (frequent non-targets) at the end of each block. Subjects were told that there would be fewer high than low tones, and that there would never be two high tones in succession. To determine whether or not subjects understood the paradigm, each subject was asked before recording commenced to relate what would occur next if a high tone was heard. All subjects reported that the next tone would be a low tone.

The sequence of tones was generated randomly by computer so that the overall probability of a rare high tone was 0.25. The random sequence was restricted only by the requirement, as noted above, that no two high tones occur in succession. This procedure permitted the identification of frequent tones that were highly predictable—they followed the occurrence of the rare high tone. Between 17 and 23 high tones occurred during each block.

A total of six blocks of trials were given each subject, four Counting blocks followed by two Choice Reaction blocks. Approximately 2 minutes was given for rest between blocks. The decision to present the two tasks in the same order to all subjects rather than counterbalance or randomize the tasks was guided by the assumption that the Counting task could not follow the Choice Reaction task without possibly contaminating the evaluation of the non-target. That is, the Choice Reaction task conditions the subject to associate a motor response to both high and low tones. During the Counting task, subjects silently counted the number of high tones, reporting the number of targets heard at the end of each block. In the Choice Reaction task, two blocks of trials were given in which the subject did not count tones but instead was asked to make a motor response to indicate what he or she had heard. On one block, subjects were instructed to press a button with their left thumb if a high tone was heard and with their right thumb if a low tone was heard. The correct response was reversed during the other block of trials so that a high tone signified a right press and a low tone a left thumb press. On the first block, a high tone was associated with a left thumb press for half of the subjects, the other half with the right thumb. During averaging of this task, the few trials indicating errors were omitted.

At the end of each block subjects were reinforced for correct performance with a reward of $0.25 for no errors, $0.10 for one or two errors, and no reward for three or more errors. Prior to the experiment a criterion was set to exclude all blocks of trials in which more than four errors occurred. Because both tasks are quite simple, all of the children performed with very few errors. For the Counting task, no subject made more than two errors per block. In the Choice Reaction task only one subject made as many as three errors in one block.

PSYCHOPHYSIOLOGICAL RECORDING

The electroencephalogram was recorded using Ag/AgCl electrodes filled with Grass electrode paste, which adhered to midline frontal, vertex, parietal and occipital locations (Fz, Cz, Pz, O2) and modified left and right parietal (P3 and P4) locations, referred to linked ears, and with forehead as ground, using a bandpass of 0.01 to 30 Hz. Digitized samples were obtained every 8 msec for a 1200 msec epoch beginning 200 msec prior to stimulus onset. An additional electrode, placed under the left eye and referred to linked ears, was used to record eye movement and blink artifacts. Changes in the eye artifact channel and all data channels were monitored online by oscilloscope; changes of approximately 50 μV or greater in the eye channel, which also could be detected as influencing the Fz recording, were coded on the keyboard for automatic exclusion from averaging. Approximately 12.5% of trials were excluded, with no differences in number of trials excluded between high risk and control groups. All recording, storage and data analysis was performed by digital computer.
While oddball tasks typically are analyzed according to rare vs. frequent events, there are clear effects based on sequences of stimuli.\textsuperscript{17,18} Thus, the data could be segregated into the two conditions of infrequent high tones (25% of all trials) and frequent low tones (75% of all trials). However, trials in which low tones occurred could also be analyzed with respect to whether or not this trial was preceded by a low tone or a high tone. Because subjects were explicitly told that two high tones would never follow each other, when a high tone occurred the subject could be certain that the next trial was one in which a low tone would occur. Therefore, data were analyzed by segregating stimuli according to their second order sequence, which is the conditional probability of each event established by the event on the prior trial. High tones were labeled as Targets (T) and low tones as Non-Targets (NT) in the Counting task. The same terminology is used for the Choice Reaction task though all tones were actually task relevant, requiring a key press. When a Non-Target was preceded by a Target, then its occurrence was predictable since two targets never could occur in succession. Thus, for this type of Non-target (labeled NT/t), the conditional probability was equal to 1.00. However, once a Non-Target had occurred, then the next trial was a target one-third of the time (labeled T/nt, $p = 0.33$; these are exactly the same trials that would represent a global probability of 25%, which corresponds most closely with the rare event in those analyses that used only the rare/frequent distinction), and a non-target two-thirds of the time (conditional probability for NT/at = 0.67). Averages labeled by these three conditional probabilities (0.33, 0.67, 1.00) were calculated for each subject for each task. By looking at all three conditions, it is possible to determine whether the subjects are merely responding to the global probability of the stimulus (0.25/0.75) or to the conditional probability. Previous results with adult populations demonstrate responsiveness to the conditional probabilities for both P300\textsuperscript{19,19} and pupillary dilation.\textsuperscript{20} Therefore, it did not seem justified to restrict the analyses to only the rare event. The mean number of artifact free trials used to compute average ERPs for the 0.33 and 0.67 conditions was 70 in the Counting task and 35 for the Choice Reaction task; for the 0.67 condition, there was a mean of 129 trials in the Counting task and 71 trials in the Choice Reaction task.

**ANALYSIS OF ERP DATA**

ERP components were identified using a computer algorithm which initially identified the largest peak (positive or negative, depending on component polarity) within a predefined time window, and at the electrode for which the component has generally been determined to be largest in amplitude in these paradigms. Initial latency windows were searched between 80–136 msec at Cz for N100, 136–240 msec at Cz for P200, 200–320 msec at Cz for N250, and 264–424 msec at Pz for P300. Two raters, blind to the family or diagnosis of the subject, verified the selection of the peak, or corrected the point selected after agreeing that a peak out of the normal range had occurred. This was particularly critical where small amplitude P300s had occurred, such as in the 1.00 condition for some subjects, and because P300 often occurs with a latency greater than 400 msec in children. Where peaks were difficult to identify, or several peaks similar in amplitude occurred, the first such peak was always selected. For each average ERP, the number of individual trials, latency of each component, and amplitudes at each electrode for the same latency (relative to a baseline defined as the median voltage for the 200 msec prestimulus period) were automatically written to a separate data file for statistical analysis. (The mean observed latencies for these components across subjects and tasks were N107, P180, N257 and P376.)

**RESULTS**

Amplitude data were subjected to analysis of variance with repeated measures over event probability (three levels) and electrode location (six levels) for each task condition. Where appropriate, degrees of freedom were adjusted by the Greenhouse-Geisser epsilon correction factor. Interactions between event probability and family type were examined for simple main effects at each of the three probability levels. (None of the three-way interactions were significant, and, therefore, are not discussed.) Latencies were treated similarly using only event probability as the repeated measures factor. N100 and P200 showed maximum amplitudes at the midline vertex (Cz) electrode. N250 showed the greatest negativity at the midline frontal location (Fz) (even though the peak had been identified on the basis of the Cz maximum response). P300 was most positive at the midline parietal location (Pz). Averaged waveforms across the subject groups are presented in Figs. 1 and 2. Components have been labeled in Fig. 1 for the 0.33 condition at the Cz location, where the distinctiveness of the components is clear even in the grand average.

**Amplitudes**

Significant differences in amplitude across different scalp locations (ANOVA, $p < 0.0001$) were seen for all components in both tasks (detailed analyses of the main electrode effects are omitted).

In order to determine if children of alcoholics and controls differed in their ability to maintain focused attention, it was of interest to examine the amplitude of N100. No differences in N100 were seen between the two groups of children as a function of family history of alcoholism. However, a trend for greater negativity was seen in the high risk children at the vertex electrode. A main effect for Probability was found for N100 in the Choice Reaction task ($F(2,40) = 3.49, p = .0402$). Greater negativity occurred in both the 0.33 and 1.00 conditions (conditions in which the tone was physically different than that on the previous trial) than in the 0.67 condition.

Analysis of the P200 data revealed no differences between groups. However, a main effect for probability in the Choice Reaction task ($F(2,37) = 3.84, p = 0.033$) was related to increasing positivity associated with increasing event probability. Interactions between probability x electrode for both the Counting ($F(5,96) = 5.39, p = 0.0002$) and Choice Reaction tasks ($F(5,92) = 4.46, p = .0015$) reflected a reduction in positivity for the 0.33 condition compared to the 0.67 and 1.00 conditions, with the greatest difference occurring at the vertex electrode.

It was of interest to analyze possible differences in N250 between groups because of reported differences among children and adults.\textsuperscript{21} (ANOVA summaries for N250 and P300 main effects and second order interactions may be seen in Tables 1–4; probabilities indicated below refer to tests listed in these tables). The N250 component showed a main effect for probability ($p = 0.0182$) and an interaction between probability x electrode ($p = 0.0373$) in the Counting task (Fig. 3) as a result of greater negativity in
the 0.67 condition compared to the 0.33 and 1.00 conditions.

In general, greater negativity beginning at approximately N250 can be observed in Fig. 1 and 2 at the frontal electrode for the high risk children as compared to controls. Similar negativity for the high risk children also appears at vertex in the Choice Reaction waveforms. A significant interaction between family type x electrode was seen for the Counting task (p = 0.0083), which reflected the increased negativity for the high risk children at frontal and vertex locations. A similar trend (p < 0.09) was observed for the Choice Reaction task as well (Fig. 4).

Interactions between family type x probability also were seen in both tasks for N250. Controls were characterized by highest negativity in the 0.33 condition while high risk subjects showed the least negativity in the 0.33 condition. The findings for the Counting task were investigated further by running an ANOVA using only the data at the frontal electrode in this task. A main effect for Probability (F(2,34) = 6.84, p = 0.005) reflected the findings described above across all electrodes. Of greater interest was a significant interaction between family type x probability (F(2,34) = 6.43, p = 0.006); simple main effects indicated greater negativity (p < 0.001) for high risk than control children in both the 0.67 and 1.00 probability conditions.

The P300 component was increased in amplitude with decreasing event Probability for both tasks (p < 0.0001) in both groups (Figs. 5 and 6). This relationship between event probability and P300 was expected based on previous work. The interaction between probability x electrode (p < 0.0001) reflects the greater differences among probability conditions at the Pz electrode compared to other locations.

A number of significant interactions with family type were observed for the Counting task. The interaction between family type x electrode (p = 0.0457) was the result of greater negativity occurring in the more anterior scalp locations, with significantly greater negativity (p < 0.05, simple effects) being observed at the frontal electrode for the high risk children compared to controls. This appeared to be a prolongation of the negativity observed initially with the N250 component for the high risk group.

A greater rate of decrease in P300 amplitude among the high risk children as event probability increased resulted in a significant interaction between family type x probability in the Counting task (p = 0.0129). In the 0.33 condition, the high risk children actually showed larger mean P300 amplitudes than controls, but amplitudes for controls were more positive than for the high risk group in the 0.67 and 1.00 conditions.