Visual P300s in Long-Term Abstinent Chronic Alcoholics

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Background: Evidence of reduced P3b amplitudes in chronic alcoholics and individuals at risk for developing alcoholism suggest that the P3b may be an endophenotypic marker for alcoholism. If this is the case, then long-term abstinent alcoholics (LTAAs) should exhibit reduced P3b amplitudes. Thus far, P3b studies on chronic alcoholics have focused primarily on samples with relatively short-term abstinence (less than 15 months). This study examines the amplitude and latency of the P3b and P3a event-related brain electrical components in LTAAs compared with normal controls (NCs) and whether these measures are related to alcohol use and other subject variables.

Methods: Electroencephalographs (EEGs) were recorded on 48 LTAAs (mean abstinence = 6.7 years) compared with 48 age-matched and gender-matched NCs during a visual P300 experiment consisting of standard, target, and rare nontarget conditions. This paradigm elicited the P3b (target condition) and the P3a (rare nontarget condition) components.

Results: Long-term abstinent alcoholics had reduced P3b amplitudes and increased P3b latencies in comparison with NCs. Long-term abstinent alcoholics also exhibited delayed P3a components, but no P3a amplitude reductions. Alcohol use variables, a family history of alcohol problems, and the duration of alcohol abstinence were not associated with any amplitude or latency variables.

Conclusions: Even after very prolonged abstinence, reduced P3b amplitudes are present in chronic alcoholics and are not associated with any family history or alcohol use variables. These results provide equivocal support for reduced P3b amplitude being an endophenotypic marker for alcoholism, but are also consistent with P3b being affected by a threshold of alcohol abuse, with the effect not resolving over long periods of abstinence.

Key Words: P300, P3a, P3b, Long-Term Abstinence, Alcoholism, Genetic Predisposition.
reviewing the literature, we have come across only 2 studies of individuals with more than 1 year of sobriety (Glenn et al., 1994; Porjesz and Begleiter, 1985) and only the latter could be appropriately considered a study on very long-term abstinence. Glenn et al. investigated the P3b in alcohol-dependent individuals completing treatment and retested the sample 14 months later. Porjesz and Begleiter studied a sample of chronic alcoholics in Alcoholics Anonymous with 3 to 10 years of sobriety. Both studies found visual P3b amplitude reductions in their abstinent alcoholics compared with controls.

There is more than 1 type of P300 event-related brain electrical potential. In contrast to P3b, which is involved in response production to target stimuli, there is a less posterior P3a component, which reflects the brain-orienting response to unexpected nontarget stimuli (larger to novel rare nontargets). Evidence for P3a abnormalities in alcoholics and high-risk individuals is at present equivocal. Some studies have observed reduced P3a amplitudes (with no latency delays) in chronic alcoholics (Hada et al., 2000; Rodriguez Holguin et al., 1999a), while we have reported delayed latency with no amplitude reductions (Biggins et al., 1995). Investigations of alcoholism high-risk samples have also produced varying results. High-risk males evidenced reduced P3a amplitudes compared with low-risk males in auditory (Hada et al., 2001) and visual paradigms (Rodriguez Holguin et al., 1999b). However, an earlier study found no difference in P3a amplitude, but delayed P3a latency in high-risk compared with low-risk samples (Rodriguez Holguin et al., 1998).

In this article, we examined the visual P3b and P3a components in long-term abstinent alcoholics (LTAAs) compared with age-matched and gender-matched light/nondrinking normal controls (NCs). We examined the degree to which this comparison was modulated by the family history of alcoholism, the severity of alcohol abuse, the presence and severity of comorbid psychiatric disorders, tests of cognitive function, and duration of abstinence.

METHODS

Subjects

All participants were recruited from respondents to flyer postings, mailings, newspaper advertisements, Internet postings, and referrals from other research participants. The subject sample was composed of LTAAs and age-matched and gender-matched NCs. An LTA and an NC were matched if they were the same gender and had ages within 5 years of each other. Most (all but 5) LTAAs were matched to an NC who was less than 3 years older or younger. The mean age difference between a matched LTA and NC was 0.43 years for females and 1.91 years for males. Each experimental group consisted of 23 females and 25 males aged 35 to 58 (mean = 46.3, SD = 6.8). The inclusion criteria for the LTA group were meeting lifetime DSM-IV-R (American Psychiatric Association, 2000) criteria for alcohol dependence and being abstinent for at least 6 months. Normal control participants responded to advertisements for light/nondrinkers and could not have met lifetime criteria for alcohol abuse or dependence.

Exclusion criteria for both groups were as follows: (1) positive diagnoses for schizophrenia or schizophreniform; (2) history of drug dependence other than nicotine or caffeine; (3) significant history of head trauma or cranial surgery; (4) history of diabetes, stroke, or hypertension that required medical intervention or of other significant neurological disease; (5) laboratory evidence of hepatic disease; (6) clinical evidence of Wernicke–Korsakoff syndrome; and (7) current substance abuse other than caffeine or nicotine.

Initial participant screening was conducted by phone interview, which assessed alcohol use/dependence, use/dependence of other drugs, medical history, and mental-health history. Participants who met all inclusion criteria and no medical or substance-related exclusion criteria were to complete a total of 4 sessions that included clinical, neuropsychological, electrophysiological, and neuroimaging assessments. During the first session, alcohol use history was assessed using the lifetime follow-back methodology of the Lifetime Drinking History questionnaire (Skinner and Allen, 1982; Sobell and Sobell, 1990). This provided alcohol use variables for lifetime use, peak use phases, and the duration of abstinence. Abstinence periods were not verified outside of the questionnaire through relatives or medical records, which is a limitation to these methods. However, the lifetime follow-back methodology has been shown to have excellent reliability in test–retest comparisons and a high diagnostic power for alcoholism (Skinner and Sheu, 1982; Sobell et al., 1988). The density of a family history of alcohol problems was assessed using the family history drinking questionnaire (Mann et al., 1985). The family history density (FHD) measure was the proportion of first-degree relatives who had alcohol problems. Although corroboration of these measures from relatives was not sought, the FHD measure has been shown to have test–retest reliability and overall accuracy (Stoltenberg et al., 1998; Vogel-Sprott et al., 1985).

Psychiatric diagnoses were established using the computerized diagnostic interview schedule (cDIS; Robins et al., 1998). Participants were examined for a total of 6 anxiety disorders (social phobia, agoraphobia, panic disorder, PTSD, obsessive disorder, and compulsive disorder), 3 mood disorders (depression, dysthymia, and mania), and 2 externalizing disorders (conduct disorder and antisocial personality disorder). All positive diagnoses were termed “lifetime” diagnoses, while disorders that were present within the past 12 months were termed “current.” Symptom counts for all positive symptoms (regardless of diagnosis) were also obtained for each disorder, using the DSM-IV-R (American Psychiatric Association, 2000) for symptom guidelines. The diagnoses (Di Scalfani et al., 2006) and symptom count data (Fein et al., 2006a) are the subject of other manuscripts where we report that the LTAAs have a higher prevalence of diagnosed psychiatric disorders than NCs and that the psychiatric illness in the LTAAs is present in subdiagnostic threshold psychiatric symptoms and in psychological measures of anxiety and mood disturbance and deviance proneness.

The neuropsychological assessment took place on the second laboratory day and were grouped into 9 domains: Attention, Verbal Fluency, Abstraction/Cognitive Flexibility, Psychomotor, Immediate Memory, Delayed Memory, Reaction Time, Spatial Processing, and Auditory Working Memory. Attention domain scores were based on scores from the color condition of the Stroop task (Golden, 1978) and attention subtests from the MicroCog Assessment of Cognitive Functioning (standard version) (Powell et al., 1993). Verbal fluency was determined by scores from the American version of the Nelson Adult Reading Test (AMNART; Grober and Sliwinski, 1991) and the Controlled Oral Word Association Test (COWAT) (Benton and Hamsher, 1983). Abstraction/Cognitive Flexibility tests included the Short Category Test (booklet format) (Wetzel and Boll, 1987), Trail Making Test B (Reitan and Wolfson, 1985), and Analogies and Math Object tests from the MicroCog. The Psychomotor group was composed of Trail Making Test A and the Symbol Digit
During the course of the study, 3 EEG acquisition systems (with 2 different amplifiers) were used. The first 2 were a 32-channel system $(n=7)$ and a 40-channel system $(n=8)$ that were acquired using the NuAmps (Compumedics Neuroscan Inc., El Paso, TX) single-ended, 32-40-channel amplifier and Scan 4.2 Acquisition Software (Compumedics Neuroscan Inc.). The NuAmps amplifier had a fixed range of $\pm 130 \, \mu V$ sampled with a 22-bit A/D converter where the least significant bit was 0.062 $\mu V$. The third system was a 64-channel system $(n=8)$, which used the SynAmps2 (Compumedics Neuroscan Inc.) amplifier and Scan 4.3 Acquisition Software (Compumedics Neuroscan Inc.). The SynAmps2 amplifier had a fixed range of $\pm 333 \, \mu V$ sampled with a 24-bit A/D converter where the least significant bit was 0.019 $\mu V$. Only electrode sites FCz and Pz, common to all 3 systems, were examined in this current report. The reference was the right ear for all recordings. The ground was 4 cm above the nasion for the 32-channel and 40-channel caps and 8 cm above the nasion for 64-channel caps. Electrode impedances were maintained below 10 k$\Omega$. The sampling rate was 250 samples/s, and activity was recorded for approximately 6.5 minutes. Vertical eye movements were recorded by electrodes above and below the left eye for later reduction of ocular artifact. To ensure that between-amplifier comparisons were valid, data from control participants whose data were collected using the different amplifier systems (NuAmps, SynAmps2) were examined and revealed no differences associated with the different acquisition amplifiers. Additionally, the ERP results reported below were replicated in a within-amplifier analysis, using only the participants whose data were collected using the NuAmps amplifier.

Electroencephalography recordings were processed offline using the Edit program in Scan 4.3 (Compumedics Neuroscan Inc.). Artifacts from eye movements were removed using the ocular artifact reduction algorithm (Artcor procedure) in Scan 4.3. Data were then band-pass filtered between 0.5 and 15 Hz using a zero-phase lag filter at 48 dB/octave. Stimulus-locked epochs were created from all instances of each of the 3 experimental conditions and were baseline corrected using the 100-ms prestimulus interval. Any epochs with out of range voltages ($\pm 75 \, \mu V$) were rejected as artifacts and excluded from further processing. To extract the P3a and P3b, epochs with correct behavioral responses for the rare nontarget and target conditions were averaged separately using the Average procedure, producing 1 average waveform per condition per subject. Peak amplitudes 250 to 550 ms poststimulus were identified using the Peakdetection procedure. Peak amplitudes (and their corresponding latencies) resulting from the rare nontarget condition at electrode FCz and for the target condition at electrode Pz were used for statistical analysis. Topographic maps were created using the 2-D mapping function from Scan 4.3, which implements global interpolation to determine voltage values between electrode sites. These maps were based on the 10 to 20 positioning system. Intersected with the layout of the 40-channel recordings, this consisted of the following electrode sites: AFz, AF4, FP1, FP2, AF3, POz, F7, F3, Fz, F4, F8, FT7, FC3, FCz, FC4, FT8, T3, C3, Cz, C4, T4, TP7, CP3, CPz, CP4, TP8, A2, T5, P3, Pz, P4, T6, A1, O1, Oz, O2, FC1, FC2, and VEOG (originally sites VOEGU and VEOGL). Only subjects whose data were recorded with 40-and 64-channel caps were included in the topographic maps. Extraneous leads from 64-channel data were omitted. None of the data from original electrode sites were weighted or changed in any way to create this uniformed layout.

**RESULTS**

**Demographics and Subject Variables**

Table 1 shows demographic, alcohol use, and clinical measures for the LTAA and NC groups. As anticipated, total lifetime and peak alcohol consumption was dramatically greater in LTAA compared with NCs. On average, LTAA drank over 20 times the alcohol dose of NCs. Long-term abstinent alcoholics also had a density of first-degree relatives with alcohol problems that was over 3 times that of NCs [$I_{(1.92)} = 33.66$, $p = 0.000$]. The range for FHD values in the LTAA group was 0.00 to 1.00, while the NC group had a range of 0.00 to 0.67. Figure 1 shows this distribution by group and gender. Within the LTAA group, the duration of alcohol abstinence ranged from 0.47 to 21.26 years (see Fig. 2).

**ERP Results**

The LTAA group showed reduced amplitudes and increased latencies for the P3b component when compared with their age-matched and gender-matched controls (see Table 2). Group membership accounted for 19.5% of the
variance for P3b amplitude \( F(1, 46) = 11.157, p = 0.002 \) and 9.7% of the variance for P3b latency \( F(1, 46) = 4.953, p = 0.031 \). Long-term abstinent alcoholics also exhibited delays in the P3a component, with the group effect accounting for 22.2% of the variance \( F(1, 46) = 13.161, p = 0.001 \) for P3a latency. However, no reduction in P3a amplitude was found \( F(1, 46) = 0.258, p = 0.614 \). No significant gender effects on amplitude or latency were observed for either component. The topographical maps in Fig. 3 show that the parietal P3b maxima in the LTAA sample occur later than in the NC sample and are less pronounced. The P3a had a frontal maximum, with LTAA amplitudes reaching the same frontal intensity as those of NCS, but occurring later. Figure 4 illustrates average waveform differences between LTAA and NCS.

Covariate analyses revealed that neither alcohol use variables nor duration of abstinence or FHD had significant effects on P3a or P3b. Figure 5 illustrates the lack of relationship between duration of abstinence and both P3a and P3b amplitude. An examination of the covariate effects of psychiatric comorbidity (diagnosis and symptom counts) also did not reveal any significant relationships.

<table>
<thead>
<tr>
<th>Variables</th>
<th>LTAA</th>
<th>NC</th>
<th>Effect sizes (% of variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
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<tr>
<td>Age (y)</td>
<td>48.4 ± 6.6</td>
<td>45.4 ± 7.1</td>
<td>48.0 ± 6.6</td>
</tr>
<tr>
<td>Years of education</td>
<td>15.5 ± 2.4</td>
<td>15.5 ± 2.0</td>
<td>16.0 ± 1.9</td>
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<tr>
<td>Alcohol use variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age started drinking</td>
<td>16.1 ± 2.8</td>
<td>14.9 ± 3.0</td>
<td>20.4 ± 4.9</td>
</tr>
<tr>
<td>Duration of abstinence (y)</td>
<td>6.5 ± 5.7</td>
<td>6.9 ± 6.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of use (y)</td>
<td>23.8 ± 8.2</td>
<td>21.5 ± 8.9</td>
<td>24.5 ± 10.8</td>
</tr>
<tr>
<td>Average dose (drinks/mo)</td>
<td>130.9 ± 78.9</td>
<td>168.2 ± 127.0</td>
<td>6.8 ± 7.6</td>
</tr>
<tr>
<td>Duration of peak use (y)</td>
<td>8.0 ± 6.9</td>
<td>4.9 ± 5.0</td>
<td>9.5 ± 9.4</td>
</tr>
<tr>
<td>Peak dose (drinks/mo)</td>
<td>269.6 ± 205.1</td>
<td>321.5 ± 191.3</td>
<td>16.7 ± 22.4</td>
</tr>
<tr>
<td>FHD( ^a )</td>
<td>0.4 ± 0.3</td>
<td>0.4 ± 0.3</td>
<td>0.2 ± 0.2</td>
</tr>
<tr>
<td>Clinical diagnoses( ^c )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mood disorder(s)</td>
<td>12 (52.2%)</td>
<td>16 (64.0%)</td>
<td>14 (60.9%)</td>
</tr>
<tr>
<td>Anxiety disorder(s)</td>
<td>9 (39.1%)</td>
<td>9 (36.0%)</td>
<td>4 (17.4%)</td>
</tr>
<tr>
<td>Externalizing disorder(s)</td>
<td>3 (13.0%)</td>
<td>10 (40.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Demographic and alcohol measures are reported as mean ± standard deviation.

Significance levels for group comparisons of alcohol use variables are not valid as alcohol use was part of the group selection criteria.

Proportion of first-degree relatives who are problem drinkers. Proportions were normalized by arcsine transform before statistical analysis.

Clinical measures are reported as number of participants (percent of participant group).

Effect is significant:

\( p < 0.05; \quad ** p < 0.01; \quad *** p < 0.001. \)

LTAA, long-term abstinent alcoholics; NC, normal control.

![Fig. 1. Family history density (FHD) scatter plot by group and gender. FHD is the proportion of first-degree relatives with alcohol problems. Individuals are family history positive if they have at least 1 relative with alcohol problems (i.e., FHD > 0). 43 LTAA, 23 NCS. Family history–negative individuals have FHD values of zero (5 LTAA, 25 NCS). Horizontal bars indicate mean FHDs. LTAA, long-term abstinent alcoholics; NC, normal control.](image1)

![Fig. 2. Duration of abstinence scatter plot by gender. Horizontal bars indicate mean abstinence.](image2)
Among all neuropsychological variables, only 2 potential covariates had significant effects (uncorrected for multiple comparisons). When corrected for multiple comparisons, there were no significant covariates.

**DISCUSSION**

The central finding of this study is reduced visual P3b amplitude in 35 to 58-year-old LTAAs. Our data indicate that reduced P3b amplitude, which has repeatedly been reported in alcoholics receiving treatment and in short-term abstinent alcoholics (Glenn et al., 1994, 1996; Suresh et al., 2003), is present in prolonged abstinence, upward of 6 years. We found no association between P3b amplitude and severity of alcohol abuse, duration of abstinence, or the density of the family history of alcohol problems. These findings are consistent with reduced P3b amplitude being the result of alcohol abuse beyond a threshold that is passed in all our LTAAs, and this effect is not ameliorated with abstinence from alcohol, even over considerable periods of time. It is also somewhat consistent with reduced P3b amplitude being an antecedent of alcohol abuse (i.e., an endophenotypic marker). The lack of an association with severity of abuse and with duration of abstinence is consistent with it being a vulnerability marker. The lack of an association with the density of the family history of alcoholism or with the presence of a lifetime diagnosis of antisocial personality disorder is not consistent with reduced P3b amplitude being a vulnerability marker as previous research suggests that such

### Table 2. P300 Amplitude and Latency Measures by Group and Gender

<table>
<thead>
<tr>
<th></th>
<th>LTAA</th>
<th>NC</th>
<th>Effect sizes (% of variance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Female (n = 23)</td>
<td>Male (n = 25)</td>
<td>Female (n = 23)</td>
</tr>
<tr>
<td>P3b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (µV)</td>
<td>6.9 ± 3.1</td>
<td>6.3 ± 2.2</td>
<td>8.8 ± 3.0</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>393.6 ± 53.0</td>
<td>405.9 ± 40.6</td>
<td>377.9 ± 37.0</td>
</tr>
<tr>
<td>P3a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude (µV)</td>
<td>7.8 ± 2.6</td>
<td>6.7 ± 2.6</td>
<td>6.6 ± 2.0</td>
</tr>
<tr>
<td>Latency (ms)</td>
<td>393.0 ± 25.4</td>
<td>411.8 ± 35.1</td>
<td>376.2 ± 37.2</td>
</tr>
</tbody>
</table>

Statistical analysis: Repeated measures (matched pairs: LTAA vs NC) analysis of variance. Group and group-by-sex effects are within matched pairs and sex effect is between matched pairs. P300 measures are reported as mean ± standard deviation. Effect is significant: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

LTAAs, long-term abstinent alcoholics; NC, normal control.

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**Fig. 3.** Topographical snapshots at 32-ms intervals for the P3b (top) and the P3a (bottom) components (n = 89, excluding individuals recorded with the 32 electrode montage). In normal controls (NCs), the P3b is maximal over electrode Pz after presentation of the target stimulus. Long-term abstinent alcoholics exhibit delayed and less pronounced activity when compared with the NC group. The P3a is maximal over electrodes FCz and Fz after presentation of the rare nontarget stimulus. The P3a is delayed in the LTAA group, but their peak voltages are not significantly different.
associations should be present for vulnerability markers (Chassin et al., 1999; Finn and Hall, 2004; Finn et al., 2000; Nurnberger et al., 2004). It is possible that the lack of association between FHD and P3b amplitude is the result of FHD being a poor substitute for true alcoholism as a vulnerability marker in the samples studied. Only a percentage of individuals with a positive family history for alcohol problems have inherited the vulnerability to alcoholism. If individuals with a positive family history have not become alcoholic by their mid 40s (i.e., NCs with a positive family history for alcoholism), it is unlikely that they have inherited that vulnerability. Moreover, all but 5 of the LTAAAs had a positive family history for alcoholism (i.e., at least 1 first-degree relative with an alcohol problem), so there may not have been much variance in the LTAA group in inherited alcoholism vulnerability. We do not have any such arguments pertaining to the lack of association of P3b amplitude with the lifetime diagnosis of ASPD. In summary, our results are not as supportive of reduced P3b being entirely an endophenotypic marker as we had expected. They are as consistent with reduced P3b being a result of chronic alcohol abuse.

P3a amplitude reductions were not found in our LTAA sample, providing evidence against the hypothesis that reduced P3a amplitude is an endophenotype for alcoholism. Current evidence also points in this direction, as diminished P3a amplitudes are not consistently found in alcoholics or samples at risk for alcoholism. Both P3b and P3a latency were delayed in the LTAA sample, and these delays were not associated with any alcohol use or other subject variables.

In summary, we provide evidence that chronic alcoholics exhibit P3b amplitude reductions that persist over years of abstinence. Our data do not unequivocally support the hypothesis that P3b abnormalities in alcoholics are solely...
due an inherited genetic vulnerability to develop alcoholism. Rather, they are even more consistent with persistent reduced P3b amplitude resulting from a threshold effect of chronic alcohol abuse. In addition, the latency delays in both P3b and P3a are likely to be the result of chronic alcohol abuse because similar delays have not consistently been reported in high-risk samples. These findings raise questions as to what impact these amplitude reductions and delays have on brain function and how the abstinent alcoholics deals with these impacts while maintaining abstinence.

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