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The Heritability of P300 Amplitude in 18 Year-Olds is Robust to Adolescent Alcohol Use

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Abstract

P3 amplitude reduction (P3AR) is associated with adolescent alcohol use (AAU) and highly heritable, suggesting that P3AR may index a genetic predisposition (e.g. an endophenotype) for AAU. However, because P3AR and AAU covary naturally in the population, these observations are also consistent with P3AR reflecting neurotoxic effects of AAU on the developing adolescent brain. In this report, we describe the use of recent advancements in biometric modeling to examine changes in the genetic and environmental contributions to variability in P3 amplitude related to cumulative AAU by late adolescence in a large community-based twin sample. We found that the genetic and environmental contributions to variability in P3 amplitude were unaffected by AAU. This suggests that P3AR indexes risk for alcoholism independent of any deleterious effect of AAU on adolescent brain development.

P300 is Unaffected by Adolescent Alcohol Use

Based on converging evidence, P300 amplitude reduction (P3AR) has been proposed as an endophenotype (Gottesman & Gould, 2003), or, a genetically transmitted risk marker for alcoholism (Hesselbrock, Begleiter, Porjesz, O'Connor, & Bauer, 2001; Iacono, Carlson, Malone, & McGue, 2002). P3AR has been linked to risk for alcoholism across a variety of experimental tasks and in a variety of clinical and epidemiological populations (Carlson, Iacono, & McGue, 2004; Habeych, Charles, Scabassi, Kirisci, & Tarter, 2005; Iacono, Carlson, et al., 2002; Porjesz & Begleiter, 1996; Porjesz et al., 1998), including adolescents at high familial risk for alcoholism at a young age prior to alcohol exposure (Begleiter, Porjesz, Bihari, & Kissin, 1984; Viana-Wackermann, Furtado, Esser, Schmidt, & Laucht, 2007). P300 amplitude has also been found to be heritable (heritability refers to the proportion of variability in P300 amplitude estimated to be due to genes) in twin and family studies (van Beijsterveldt & van Baal, 2002).

In standard biometric models based on twin data, the genetic similarity between members of monozygotic (MZ; 100%) and dizygotic (DZ; 50%) twin pairs can be used to decompose the variance in a trait into three sources: additive genetic effects (that is, the combined effects of individual genes summed over loci, A); shared environmental effects (or the extent to which twins are similar regardless of zygosity because the environment has made them more alike, C); and non-shared environmental effects (or the extent to which members of twin pairs differ, presumably because of differing environmental influences, E; measurement error is

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Informed consent was obtained on all participants of legal age. Informed assent was obtained on all participants under the age of 18 and informed consent was obtained from their parents or guardian.

also contained in E). In the standard univariate “ACE” model, all of the variance in a single trait (V_i) is decomposed into the A, C, and E components. A meta-analysis of P300 amplitude heritability studies (van Beijsterveldt & van Baal, 2002) concluded that the best fitting ACE model indicated that the non-shared environment could be dropped from the model without sacrificing model fit, leaving genes accounting for about 60% of the variability in P300 amplitude and the non-shared environment accounting for the remaining 40%. Heritability, while a necessary requirement for an endophenotype, characterizes the influence of genes across all environments experienced by the participants. Thus, an important step in establishing the clinical utility of P3AR as an endophenotype is demonstrating that the heritability of P3AR does not vary systematically with environmental adversity that could both promote alcoholism and disrupt P300 amplitude development.

Adolescent Alcohol Use

Evidence suggests that adolescent alcohol use (AAU), a robust correlate of adult alcoholism (Anthony & Petronis, 1995; Brown & Tapert, 2004; DeWit, Adlaf, Offord, & Ogborne, 2000), may represent one such deleterious environment. Animal research has related ethanol exposure to neurodevelopmental damage, both in terms of structure (e.g. hippocampus, frontal lobes) and function (e.g. thermoregulation, balance, memory; Brown & Tapert, 2004; Crews, Braun, Hoplight, Switzer, & Knapp, 2000; Hiller-Sturmhofel & Swartzwelder, 2004; White & Swartzwelder, 2004). Additionally, a variety of neurobiological deficits have been reported for human adolescents with alcohol use disorders on neuropsychological measures (Brown, Tapert, Granholm, & Delis, 2000; Moss, Kirisci, Gordon, & Tarter, 1994; Sher, Martin, Wood, & Rutledge, 1997) and in brain structure and function (De Bellis et al., 2000; De Bellis et al., 2005; Tapert et al., 2001). Thus, while P3AR may index genetic risk for alcoholism prior to alcohol exposure (Begleiter et al., 1984; Viana-Wackermann et al., 2007), AAU may also reduce P300 amplitude. This scenario represents an alternative, environmentally based explanation for why P3AR is often associated with risk for alcohol misuse in alcohol exposed adolescents. Evidence that P3AR indexes neurotoxic effects of AAU would challenge the utility of P3AR as an endophenotype given the high frequency of AAU reported in epidemiological surveys (Brown, et al., 2008; Johnston, O’Malley, Bachman, & Schulenberg, 2007).

Disentangling the Effects of AAU on P300 amplitude

It is fair to suggest that the natural covariation between P3AR and AAU has hindered explicating the role of AAU in producing P3AR. Adolescents who consume the greatest amounts of alcohol are expected to manifest P3AR, whether P3AR is caused by AAU or is an expression of a genetic risk for AAU. Thus, both the P3AR endophenotype hypothesis and the alcohol exposure hypothesis explain why P300 amplitude is associated with AAU in adolescents with alcohol use histories. However, the two hypotheses diverge as to the expected genetic and environmental (G-E) contributions underlying P300 amplitude at different points along the alcohol exposure continuum. The endophenotype hypothesis, for instance, predicts that P300 amplitude relates to risk for AAU independent of whether the risk for alcohol misuse is manifested or not. In this case, the G-E interplay underlying P300 amplitude would be similar for all individuals regardless of varying AAU histories. With the alcohol exposure hypothesis, an individual’s genetic contribution to P300 amplitude would be moderated by alcohol exposure. For instance, an individual whose genes confer a tendency to have large amplitude P300 would never manifest this genetic propensity if heavy drinking attenuated the normal development of P300 amplitude by altering genetic expression or by poisoning nerve cells.

Recent developments in biometric modeling make possible the examination of how environmental exposure moderates G-E contributions to a phenotype of interest (Johnson,

2007; Purcell, 2002). To illustrate how these methods can be used to address our alternative hypotheses, we can model the contribution of A, C, and E to variability in P3 amplitude as a function of the degree of exposure to alcohol. Figure 1a visually depicts the G-E interplay underlying P300 amplitude in the population corresponding to the endophenotype hypothesis. Based on the results of the van Beijsterveldt and van Baal (2002) meta-analysis, the proportion of variance (Y axis) in P300 amplitude estimated to be due to genes (60%; A) and non-shared environment (40%; E) remains the same for all adolescents no matter how much alcohol they have consumed (as indicated on the X axis).

Conversely, the hypothesis that AAU actively reduces P300 amplitude predicts that the G-E interplay underlying P300 amplitude is contextually dependent on AAU history. This would occur because the contribution of A and E to P300 amplitude variability depends on the extent of alcohol consumption. However, the mechanisms underlying the possible neurotoxicity of alcohol on adolescent brain development are not well established and thus it is not clear how the contribution of A and E to P300 amplitude would vary with the amount of alcohol consumed. It is possible to present idealized plausible alternatives that illustrate how alcohol consumption might moderate G-E interplay. Because increased alcohol consumption is associated with diminished P300 amplitude, it is reasonable to assume that with increasing alcohol consumption, the variability evident in P300 amplitude increases by rendering P300 smaller than it would otherwise be in the absence of alcohol consumption. Hence, both Figures 1b and 1c posit increases in the overall variability in P300 amplitude with increasing alcohol consumption. Figure 1b illustrates what we might expect if alcohol consumption increases the genetic variability in P300 amplitude because the environmental adversity that derives from the effects of alcohol on the adolescent brain alters genetic expression. This figure is consistent with a diathesis-stress model wherein the inherited liability for alcohol misuse indexed by P3AR is enhanced with exposure to the adverse environment. Figure 1c also indicates that variability in P300 amplitude increases with adverse environmental exposure, but here the effect is due entirely to the nonshared environment (e.g., by alcohol poisoning brain cells involved in P300 generation).

Although this type of biometric moderation modeling has not been applied to the study of P300 amplitude, it has been applied to the study of other characteristics, and findings have been reported indicating that effects like those depicted in Figures 1b and 1c are reasonable. For instance, Button, Lau, Maughan, and Elay (2008) found that the genetic contribution to adolescent externalizing behavior was increased with environmental adversity (punitive parenting style), a result that is consistent with a diathesis-stress model for the development of externalizing. In a study of adolescent depression, Feinberg, Button, Neiderhiser, Reiss, and Hetherington (2007) reported that with increasing parental negativity, the genetic influence was constant but the nonshared environmental effect on depression increased, a finding similar to that displayed in Figure 1c. Studies such as these demonstrate how traditional heritability estimates may obscure dynamic G-E interplay. They also point to the potential power of moderation models to characterize dynamic G-E interplay among P3AR and AAU, phenomena that naturally covary, to elucidate the mechanisms by which AAU impacts adolescent brain development.

In this report, two nested biometric models that differ only in how cumulative AAU through age 18 is associated with P300 amplitude at age 18 are compared. The first model ("Endophenotype") estimates the G-E influences underlying P300 amplitude as unaffected by AAU, and thus evaluates a version of the idealized model presented in Figure 1a. The second model used in this report ("Alcohol Exposure"), estimates the G-E influences underlying P300 amplitude as *a function of how much alcohol was consumed by each individual*. This model, by quantifying variations in the G-E interplay underlying P300 amplitude as AAU increases, is capable of detecting the types of idealized G-E variations

depicted in Figures 1b and 1c as well as any other variations that might be present. Systematic change in the G-E interplay underlying P300 amplitude cannot be easily explained under the endophenotype hypothesis and, if confirmed, such moderation of P3AR would implicate alcohol consumption per se as a contributor to individual differences in P300 amplitude. In this way, biometric moderation modeling represents a novel and complementary approach to endophenotype validation.

Methods and Materials

Participants were from the Minnesota Twin Family Study (MTFS), an ongoing longitudinal study of a community-based sample of same-sex twins born in the state of Minnesota and their parents (Iacono & McGue, 2002). Participants were originally identified from public birth records and contacted over the phone or by mail. Families were eligible to participate if they lived within a day's drive of our labs and if neither twin had a cognitive or physical handicap that precluded participation. Written informed assent or consent was collected from each participant and their parents or guardian. This study focuses on the younger cohort of twins from the MTFS born between 1977 and 1985 and first assessed between the ages of 10 and 12 years. Almost all participants were Caucasian (98%), consistent with the demographics of Minnesota at the time that this study began. Data from this report comes from the younger cohort's third assessment (ages: males: $M = 18.04$, $SD = 0.66$; females: $M = 18.28$, $SD = 0.71$) as a comprehensive alcohol use assessment was conducted with this cohort for the first time. Our aim was to recruit these participants during their senior year of high school. Zygosity was determined using information from the parents, evaluation by the staff of physical similarity, physical measurements (e.g., fingerprint ridge count), and, when needed, the use of DNA markers. More information about the characteristics of this cohort of twins can be found elsewhere (Blazei, Iacono, & McGue, 2008; Herndon & Iacono, 2005; Johnson, McGue, & Iacono, 2005; McGue, Iacono, Legrand, Malone, & Elkins, 2001).

Psychophysiological Assessment

The "rotated heads" task (Begleiter et al., 1984) was used to record the electroencephalogram (EEG) while participants sat in a darkened room in a high-back padded chair. The background of the computer screen remained black for the length of the session. For 80 trials, a white oval with a "nose" and one "ear" was presented. On 40 trials, the "nose" was pointed up and the "ear" was either on the right or the left side of the oval. On the remaining 40 trials, the "nose" was pointed down with the "ear" on either side. Subjects were instructed to identify which side of the "head" the "ear" was on, using an appropriate response button. An additional 160 trials with just the oval required no behavioral response. Stimuli were presented pseudo-randomly for 98 ms with a random inter-trial interval between 1 and 2 seconds. The horizontal radius of the oval was 4.3 cm and the vertical radius was 5.3 cm. Participants sat 65 cm from the screen. Participants were given practice trials prior to beginning the task.

EEG activity was recorded from three channels (P3, PZ, P4) using an electrode cap and linked earlobes as the reference. Sensors placed on the outer canthus and above one eye recorded eye movements (EOG). A ground electrode was placed on the right shin. The electrode impedance was kept below 5 Kohms for EEG and 10 Kohms for the EOG and ground. Data were recorded on a Grass Model 12A Neurodata acquisition system with a $\frac{1}{2}$ amplitude low and high frequency filter settings at .01 Hz and 30 Hz, respectively. The EEG was digitized on line at a rate of 256 samples/second over a 500 ms baseline and a 1500 ms post baseline epoch. A standard eye-blink correction algorithm was applied to remove ocular artifacts from the raw EEG data (Gratton, Coles, & Donchin, 1983) and a 7.5 Hz low pass digital filter was applied to reduce high frequency noise. Analog-to-digital saturation led to repetition of trials.

Consistent with the alcoholism-P3 literature and our past investigations using this task, only data recorded from the PZ electrode during target trials ($N=80$) were considered for this report. These trials were averaged together and baseline corrected for each participant. A computer algorithm calculated the amplitude and latency of the largest positive peak between 280 ms and 600 ms after stimulus onset for each participant's average waveform. Members of twin pairs are the same age and gender, and variability in P300 amplitude related to age or gender can influence apparent twin similarity. Because this may distort the estimates of genetic and environmental influence on a trait (McGue & Bouchard, 1984), we regressed P300 amplitude on age, age², age $\times$ gender, and age² $\times$ gender, and the standardized residuals from these regressions were used in subsequent analyses.

Assessment of Adolescent Alcohol Use

Adolescents underwent a semi-structured interview of alcohol use behaviors using a version of the Substance Abuse Module of the Composite International Diagnostic Interview (Robins, Babor, & Cottler, 1987) that was modified to include questions covering alcohol misuse. One drink was defined as one bottle of beer, one glass of wine, or one ounce of hard liquor. To simplify our analysis, a single AAU factor score was generated using principal components factor analysis from three log10+1-transformed alcohol use outcomes: number of intoxications over the last six years (NumIntox), maximum number of drinks consumed in any one 24 hour period over the last six years (MaxCon), and the average number of drinks consumed on each drinking occasion during heaviest period of drinking over the last six years (HeavyAve). These outcomes were selected because they capture both frequency and intensity of alcohol use. The three alcohol use outcomes loaded between 0.889 and 0.973 on the largest factor (accounting for 88.2% of the total variance), supporting the use of a single composite variable as a measure of AAU. Prior to transformation, all three variables were winsorized down to the 95th percentile, where the top 5% of extreme values were replaced with the value at the 95th percentile. This was done to reduce skew but preserve the meaningfulness of extreme alcohol use behavior. The 95th percentile was 100 for NumIntox, 24 for MaxCon, and 12 for HeavyAve. The transformed alcohol use variables were also subjected to age and age² correction (McGue & Bouchard, 1984). The overall AAU factor score distribution was standardized to a mean of 0 and a standard deviation of 1. Larger factor scores indicated greater alcohol use; zero reflected the sample's mean factor score. Descriptive statistics for the three alcohol use variables (winsorized but untransformed) are provided in Table 1. 973 participants (312 male MZs; 313 female DZs; 166 male DZs; 182 female DZs) had P300 amplitude data available for inclusion in this study. This sample was comprised of members of 321 male twin pairs (216 MZ pairs) and 345 female twin pairs (210 MZs). 466 (70%) twin pairs had P300 amplitude and AAU data for both twins, while 200 twin pairs had at least one member missing P300 amplitude, AAU data, or both.

For various reasons, more subjects completed the alcohol use assessment than completed the ERP task (e.g., because the assessment was conducted by phone for those unable to schedule an in-person visit). Of the 973 participants with P300 amplitude data, 7 participants did not complete the alcohol use history assessment. To test for possible bias in alcohol use estimates at age 18 introduced by missing ERP data, alcohol use factor scores were compared between those with ($n=966$, $M=0.01$, $SD=1.01$) and without ($n=300$; $M=-0.04$, $SD=0.96$) P300 amplitude data in an independent samples t-test. The results suggest that there was no difference in AAU between those with and without P300 amplitude data at age 18 ($t(1264)=-0.72$, $p=0.48$).

To determine how AAU scores related to the construct of pathological alcohol use, we compared alcohol use composite scores between those with and without a lifetime history of a Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association, 1994) alcohol use disorder (AUD; abuse or dependence). The

lifetime presence of a DSM-IV AUD at this or prior assessments was established using a best-estimate approach that incorporates both child self-reported AUD symptoms and parental report on the child (see Iacono, Carlson, Taylor, Elkins, & McGue, 1999, for interviewing and diagnostic procedures). Using this approach, 15.9% ($n=154$) of adolescents met definite criteria for DSM-IV alcohol abuse, 7.3% ($n=73$) of adolescents met definite or probable (definite minus 1 symptom) criteria for alcohol dependence, and 17.3% ($n=167$) of adolescents met criteria for either abuse or dependence. AAU scores at age 18 were about 1.5 SD higher in those with an AUD ($n=167$, $M=1.25$, $SD=0.58$) compared to those who never met criteria ($n=799$, $M=-0.24$, $SD=0.88$), $F(1,964)=440.99$, $p<.001$. These results also confirm that a significant fraction of the youths in this study were problem drinkers.

Biometric Analyses

Biometric modeling was used to evaluate the genetic and environmental moderation of P300 amplitude by AAU scores. The bivariate models used in this study (Purcell, 2002) examine the association between P300 amplitude and AAU (see Figure 3). The two models (depicted in Figure 3) are nested: the “Endophenotype” model is a structurally reduced version of the “Alcohol Exposure” model that can be created by constraining all 6 of the moderating parameters (e.g. the β_X parameters) to 0. In the first model (“Endophenotype”), the genetic and environmental influences that shape P300 amplitude do not change according to how much alcohol an individual consumes. First, the variance that P300 amplitude and AAU share in common was decomposed into proportions attributable to genetic (a_{21}), shared environmental (c_{21}) and nonshared environmental (e_{21}) influences. Then, the residual variance unique to P300 amplitude was decomposed into genetic (a_{11}), shared environmental (c_{11}) and nonshared environmental (e_{11}) influences. The second, more complex model (“Alcohol Exposure”) is similar to the first, simpler model in nearly all regards except that the six A, C, E components shared and unique to P300 amplitude were expressed as linear functions of AAU scores. As such, the genetic and environmental influences that shape P300 amplitude were allowed to change with (be moderated by) AAU scores. For example, the shared genetic component between the moderator alcohol use (M) and P300 amplitude was expressed in the linear form $a_{21} + \beta_{Xa21}M$, where a_{21} is the parameter for common genetic influence on the P300 amplitude, β_{Xa21} is a regression coefficient estimated from the data, and M is the level of AAU (expressed as a z-score in this report).

Biometric models were fit to the raw data using full information maximum likelihood as implemented in the Mx software system (Neale, Boker, Xie, & Maes, 1999), an “all data” estimation procedure that corrects for potential statistical biases due to missing data. Four indices produced by Mx were used to evaluate model fit: (1) the likelihood-ratio test (-2LL); computed as the difference in -2 log-likelihood values between models tested, with 6 degrees of freedom corresponding to the 6 moderating parameters constrained to 0 in the first model, against the χ distribution; (2) the Akaike Information Criterion (Akaike, 1987); (3) the Bayesian Information Criteria (BIC; Raftery, 1995); and (4) Draper’s Information Criterion (DIC; Draper, 1995). AIC recognizes both accuracy of model fit and parsimony. BIC is conceptually similar to AIC but penalizes more for model complexity. DIC is similar to both BIC and AIC, but is thought to provide a better balance between parsimony and fit (Markon & Krueger, 2004). In all cases, the model with the lowest value of the fit statistics is preferred.

Evidence that P300 is affected by varying levels of AAU is provided if the second Alcohol Exposure model fits the data better than the Endophenotype model. If the Alcohol Exposure model provides a significantly better fit, the affected model parameters can clarify some of the processes underlying the impact of AAU on P300 amplitude expression. Alternatively,

evidence that AAU plays a minimal role in P300 amplitude expression is provided if the Alcohol Exposure model does not improve fit to the data.

Results

Figure 2 illustrates the well-known association between P3AR and alcoholism risk in a plot that presents the grand mean waveforms separately for adolescents in the top and bottom deciles of AAU. The A (genetic), C (shared environmental), and E (non-shared environmental) estimates for AAU were 0.39 (95% confidence interval [CI]: 0.22, 0.58), 0.35 (95% CI: 0.17, 0.53), and 0.26 (95% CI: 0.22, 0.30), respectively, all of which were statistically significant. The A and E estimates for P300 amplitude were 0.63 (95% CI: 0.44, 0.75) and 0.36 (95% CI: 0.31, 0.42), respectively, and both were statistically significant. The C estimate, 0.01 (95% CI: 0.00, 0.18), was not significantly different from 0 and remained 0.01 throughout the moderation analysis. These values are consistent with those reported previously (van Beijsterveldt & van Baal, 2002). Approximately 3% of the variance in P300 amplitude was due to genetic covariance with AAU, whereas only 1% of the variance in P300 amplitude was due to shared environmental covariance. The genetic correlation was -0.23 (r_A ; 95% CI: -0.54 , 0.00) and the nonshared environmental correlation was -0.06 (r_E ; 95% CI: -0.17 , 0.00).

Testing for Moderation

The ACE estimates for P300 amplitude reported above (A:0.63; E:0.36) represent the genetic and environmental contributions to P300 amplitude on average across all levels of AAU in our sample. Figure 4a visually presents the ACE estimates for P300 amplitude as calculated from the Endophenotype model. The Alcohol Exposure model, on the other hand, produces estimates of the G-E influences on P300 amplitude as a function of AAU. To the extent that such changes are present, the Alcohol Exposure model would provide a significantly better fit to the data than the Endophenotype model. As indicated in top portion of table 2, the -2LL, AIC, BIC, and DIC comparisons all fail to support the Alcohol Exposure model as providing the better fit to the observed data, suggesting that the genetic and environmental influences on P300 amplitude do not vary with AAU. Figure 4b visually presents the ACE estimates across the range of AAU scores as calculated in the Alcohol Exposure model. As shown, the sum of the ACE estimates slightly decreased from low to high levels of AAU, due mostly to a decrease in the non-shared environmental contribution to P300 ("E"). That the results support a lack of G-E variability as a function of AAU is consistent with the endophenotype hypothesis.

Discussion

Two competing possibilities emerge from the literature regarding the role of AAU in the expression of adolescent P300 amplitude. On one hand, P300 amplitude has been proposed as an endophenotype, or genetically transmitted biological marker, for alcoholism. This theory predicts that P300 amplitude relates to risk for alcohol misuse due to common genetic processes independent of manifested AAU. On the other hand, evidence suggests that neurodevelopment is sensitive to AAU and P300 amplitude reduction may index the neurotoxic effects of alcohol on adolescent brain development. This theory predicts that the G-E influences underlying P300 amplitude would change as AAU increases. If so, then the clinical utility of P300 amplitude as an alcoholism endophenotype would vary in populations where AAU is prevalent. Additionally, the mechanisms by which alcohol use impacts adolescent brain development would be better understood. To address these possibilities, we utilized recently developed biometric methods capable of quantifying variability in the G-E interplay underlying P300 amplitude in a large epidemiological twin

sample. We reasoned that increasing levels of early alcohol use would disrupt G-E interplay underlying P300 amplitude if AAU related to P3AR differently as a function of AAU.

Our results provided no evidence that AAU moderated the G-E interplay underlying P300 amplitude. In particular, no effective change in genetic variance across the range of AAU was estimated even when the model allowed for moderating effects of AAU to be present. The robustness of the G-E interplay underlying P300 amplitude supports P3AR as an endophenotype for alcoholism risk, rather than a biological marker sensitive to the effects of AAU. While it is impossible to conclude that AAU is benign in regards to P300 amplitude, these results suggest that AAU is not systematically reducing P300 amplitude enough to compromise the clinical utility of P300 amplitude as an alcoholism endophenotype in this unselected community sample of adolescents.

Limitations

There are a number of limitations to this study. Our results may not easily generalize to clinical populations of interest because we examined an epidemiological sample. For instance, perhaps moderation is present in adolescents with greater amounts of AAU, such as those found in chemical dependency treatment programs. On one hand, we observed a large range of AAU in our study, including both alcohol-naïve adolescents as well as those who reported consuming many drinks in a single day and a history of multiple intoxications. Indeed, 57.6% of the males and 49.2% of the females in our sample reported drinking to intoxication and many have been intoxicated more than once. Additionally, 17.3% of our adolescents met criteria for a DSM-IV AUD diagnosis. On the other hand, alcohol is a recognized neurotoxin. Alcohol in sufficient quantities would likely permanently reduce P300 amplitude. The results of this study, however, suggest that this threshold is not reached frequently enough to challenge the validity of P300 amplitude as an endophenotype when applied to community populations. Similarly, the results of this study may not be easily generalized to populations selected for particularly early alcohol consumption, as only 1.7% of our sample reported an intoxication by age 11. Additionally, this study only examined AAU up to approximately age 18. Our results may have been different had we examined the effects of cumulative alcohol use on P300 amplitude at an older age.

We recorded P300 amplitude from a single electrode during the rotated heads task (Begleiter et al., 1984) in a manner consistent with many published reports that support P300 amplitude as an endophenotype for alcoholism risk (Begleiter et al., 1984; Carlson, et al., 2004; Carlson, Katsanis, Iacono, & Mertz, 1999). This approach may fail to capture subtle ways in which AAU alters the adolescent brain. Important insights into how AAU alters adolescent brain development might be gained by examining the robustness of P300 amplitude elicited by other tasks, P300 amplitude measured from other parts of the scalp, or psychological or neurobiological processes other than P300 amplitude.

It is also possible that a lack of statistical power led to the lack of statistical significance in moderation. However, our results indicated that there was little overall moderation in P300 amplitude across the range of AAU. Improved sample size would not be expected to introduce practically meaningful moderation, particularly not in the genetic contribution to P300 amplitude. Perhaps increased power would have revealed that the decline in the E parameter reflects a true phenomenon. To our knowledge, no study has been conducted to support or refute this effect. Furthermore, this study examined P300 amplitude and AAU cross-sectionally. Examining the covariation of these traits longitudinally is required to identify subtle AAU effects specific to neurodevelopmental trajectories. Although we did not investigate gender differences in this report, in preliminary analyses we examined potential gender differences in how AAU might alter P300 amplitude, but moderation was

not indicated for either gender. Future research with larger samples of both genders might discover the presence of gender differences in how AAU relates to P300 amplitude.

Finally, this study does not provide insight into the psychophysiological mechanisms underlying P3AR. P300 amplitude has been proposed as an index of the neuroelectrical activity underlying “context updating” (Donchin, 1981), but it is unclear how a specific deficit in context updating translates to risk for AAU. This may not be correct level of analysis needed to relate P300 amplitude to AAU. Polich (2007) hypothesized that P300 amplitude indexes neuronal inhibition associated with attention allocation and memory updating, a function attributed to working memory. No study to our knowledge has examined whether working memory deficits mediate the association between P300 amplitude and risk for AAU. Others have proposed that P3AR reflects a disinhibitory process in the brain associated with excess central nervous system excitability that is alleviated by the ingestion of alcohol (Begleiter & Porjesz, 1999). Although behavior genetic research has the potential to provide leads regarding the underpinnings of P3AR, additional research is required to characterize the specific (or global) psychophysiological deficits associated with reduced P300 amplitude, and why these deficits relate to AAU.

Conclusion

The results of this report fit the notion that the P3AR-alcoholism risk association cannot be accounted for by the deleterious effects of AAU. Previous studies have demonstrated that preexisting alcohol use is not necessary for P3AR by examining alcohol naïve adolescents (Begleiter et al., 1984; Berman, Whipple, Fitch, & Noble, 1993; Hill, Muka, Steinhauer, & Locke, 1995; Polich, Pollock, & Bloom, 1994) as early as age 8 (Viana-Wackermann et al., 2007). Longitudinal designs have demonstrated that P3AR predates the future development of alcohol use behaviors even in alcohol-naïve adolescents (Berman, et al., 1993). This study contributes to the literature by suggesting that, in this community sample of adolescent twins, neither P300 amplitude nor its association with AAU is modified by AAU. The finding that P3AR correlates with, but is not caused by, AAU is also generally consistent with the notion that P300 amplitude is an endophenotype for a broad range of disinhibited behavior, of which AAU is just one expression (Carlson et al., 1999; Hicks, et al., 2007; Patrick, et al., 2006). Adolescence is a critical period for brain development (Giedd, 2004), and is often when initial alcohol exposure occurs. Therefore, understanding the impact of AAU on adolescent brain development can help differentiate the effects of AAU from preexisting differences in brain structures or functions that relate to risk for alcohol misuse. Although the present study did not find that AAU affected the heritability of P3AR, additional research is warranted to understand in what ways the adolescent brain may be impacted by AAU.

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Figure 1. Gene-Environment Interplay in P300 Amplitude by Source of Variance

Plausible exemplars of moderation of P300 amplitude by adolescent alcohol use (AAU). The X-axis represents the range, from low to high, of AAU. The Y-axis represents variance in P300 amplitude accounted for by each model component at that level of AAU. In Figure 1a, the two lines represent variance components: A = genetic, E = non-shared environment; drawn to scale based on the results of a meta-analysis of P300 amplitude heritability studies (van Beijsterveldt & van Baal, 2002) and from our lab (Yoon, Iacono, Malone, & McGue, 2006), where approximately 60% of the variability in P300 amplitude was estimated to be due to A and approximately 40% due to E. Although moderation may also be present in C, the shared environment, if exposure to alcohol creates similarity between twins for P300

amplitude regardless of genetic similarity. C is not included in this figure because the meta-analysis indicated that P300 amplitude variability could be explained solely by contributions from A and E and to ease presentation. Figure 1b represents an example of moderation of the genetic influences to P300 amplitude across the range of AAU. Figure 1c represents an example of moderation of the non-shared environmental influence to P300 amplitude across the range of AAU. Consistent with the definition of heritability as the relative influence of genes on average across all environments experienced by participants, the estimated influence of A and E are approximately equal ($A=60\%$, $E=40\%$) at the midpoint of the X-axis of each figure.

Figure 2. Grand mean waveforms at PZ in adolescents at age 18 by top and bottom deciles on AAU

Figure 3. Path diagram of a biometric moderator model with adolescent alcohol use (AAU) moderating the genetic and environmental influences on P300 amplitude at age 18 (P300)
 Each of the paths impacting P300 is a linear function that combines an overall coefficient separate from the moderator variable (e.g., a_{21}) with the product of a coefficient that indexes the moderation of P300 by AAU on that path (β_{xa21}) multiplied by the level of the moderator (M). The endophenotype model is simply the moderator model with the six moderation terms (β_x) set to zero. A=genetic variance, C=shared environmental variance, E=nonshared environmental variance.

Figure 4. Plot of ACE components for P300 amplitude in the Endophenotype model (left) and the Alcohol Exposure model (right)

The X-axis is marked at 5 z-score units of adolescent alcohol use to help ease presentation. The three lines represent variance components: A = genetic, C = shared environment, E = non-shared environment. The Y axis is percent of variance in P300 amplitude accounted for by that component at that level of adolescent alcohol use (AAU). The left panel contains the ACE components as calculated in the Endophenotype model across the range of AAU. The right panel plots the ACE estimate for P300 amplitude from the Alcohol Exposure model across the range of AAU.

Table 1

Adolescent Alcohol Use Outcomes.

	Intox		Maxcon		AveHeavy	
	Males	Females	Males	Females	Males	Females
% with value ≥ 1	57.6	49.2	74.7	68.3	74.7	68.3
1 st quartile ^a	3.0	2.0	6.0	4.0	3.0	2.0
Median ^a	10.0	5.0	12.0	6.0	6.0	4.0
3 rd quartile ^a	25.0	15.0	20.0	10.0	8.0	5.0

Note. This table contains descriptive statistics for the winsorized raw data from 474 males and 492 females; Intox=Lifetime Number of Intoxications; Maxcon= Maximum number of drinks consumed in a 24 hour period; AveHeavy = Average Amount Consumed During Heaviest Period; % with value ≥ 1 = Percent of participants in the sample endorsing at least one of that alcohol use indicator;

^a = These descriptive statistics are for adolescents who report at least one of that alcohol use indicator.

Table 2

Model Fit Statistics

	-2LL	df	$\Delta\chi^2$	Δdf	<i>p</i>	AIC	BIC	DIC
Endophenotype Model	5538.66	2142				1254.7	-4083.6	-2115.2
Alcohol Exposure Model	5535.12	2136	3.54	6	>.05 ^a	1263.1	-4066.1	-2103.3

Note. -2LL = -2 log likelihood; df = degrees of freedom; AIC = Akaike's Information Criterion; BIC = Bayesian Information Criterion; DIC = Draper's Information Criterion; Active Effects Model = Active Effects of Adolescent Alcohol Use on P300 model;

^a = significance of difference in the goodness of fit between the Endophenotype Model and Alcohol Exposure Model.