Am. J. Hum. Genet. 54:658-668, 1994

Prolonged P300 Latency in Children with the D_2 Dopamine Receptor A1 Allele

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Summary

Previous studies have indicated the presence of a hereditary component in the generation of the P300, or P3, a late positive component of the event-related potential. Moreover, the dopaminergic system has been implicated in the P3. In the present study, 98 healthy Caucasian boys, mean age of 12.5 years and of above-average intelligence, were studied. The sample was composed of 32 sons of active alcoholic (SAA) fathers, 36 sons of recovering alcoholic (SRA) fathers, and 30 sons of social drinker (SSD) fathers, with none of them having yet begun to consume alcohol or other drugs. *Taq*I A D_2 dopamine receptor alleles (A1 and A2) were determined. A significant difference in the frequency of the A1 allele was found among these three groups of boys, with the SAA group having the highest A1 allele frequency (.313), followed by the SRA (.139) and the SSD (.133) groups. The relationship of the A1 and A2 alleles to P3 amplitude and latency was also determined. The results showed no significant difference in P3 amplitude between boys with the A1 and A2 allele. However, P3 latency was significantly longer in the total sample of boys with the A1 allele compared with those carrying the A2 allele. These findings suggest that polymorphism of the D_2 dopamine receptor gene is an important determinant of P3 latency.

Introduction

Characteristics of the P300

The P300, or P3, component of the event-related potential (ERP) is a late positive waveform elicited during stimulus discrimination (Sutton et al. 1965). The P3 occurs in response to rare targets in a stream of more-frequent stimuli and is believed to reflect cognitive events of information processing (Donchin and Coles 1988). P3 amplitude is controlled by the task relevance of the eliciting events and is inversely proportional to the subjective probability of the stimulus (Ritter and Vaughan 1969; Courchesne et al. 1975; Squires et al. 1977*a*), whereas P3 latency depends on the duration of stimulus evaluation (Kutas et al. 1977; Donchin 1984) and is increased by task difficulty (Friedman et al. 1975; Gomer et al. 1976; Squires et al. 1977*b*).

Relatively little is known about the precise neural structures involved in the generation of the P3. However, growing evidence suggests that there are multiple cortical generators of the P3 that are to some extent modality specific (Knight et al. 1989; Johnson 1993; Polich and Squire 1993).

Heritability of the P300

Several studies have addressed the issue of heritability of the P3 in humans. MZ twins showed P3 latencies that were significantly more alike than those of unrelated matched control subjects (Surwillo 1980; Polich and Burns 1987). In another study comparing MZ and DZ twins (Rogers and Deary 1991), the within-pair similarity of P3 latency was significantly greater in MZ than in DZ twins. However, in this same study, within-pair similarity of P3 amplitude was not significantly greater in MZ than in DZ twins. These findings support the view that P3 latency has an important genetic component.

P300 in Normative and Clinical Populations

It has been found that P3 latency decreases in children during maturation (Courchesne 1984; Howard and Polich 1985), whereas increased latency of this endogenous component has been observed in the aged

Received August 10, 1993; accepted for publication December 7, 1993.

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0002-9297/94/5404-0010$02.00

(Goodin et al. 1978; Brown et al. 1983; Picton et al. 1984), as well as in dementia (Goodin et al. 1979; Syndulko et al. 1982; Neshige et al. 1988), Parkinson disease (Hansch et al. 1982; O'Donnell et al. 1987; Stanzione et al. 1991), multiple sclerosis (Newton et al. 1989), and other organic brain dysfunctions (Papanicolaou et al. 1984; Morrow et al. 1992). P3 characteristics have also been studied in alcoholics and their children; however, the findings have not always been consistent. Thus, in alcoholics, increased latency (Pfefferbaum et al. 1979; Polich 1984; Steinhauer et al. 1987), decreased amplitude (Porjesz and Begleiter 1981; Ciesielski et al. 1985) of the P3, or both (Porjesz and Begleiter 1985; Pfefferbaum et al. 1987) have been described. Similarly, children of alcoholics were reported to display increased latency (Schmidt and Neville 1985; Steinhauer et al. 1987; Hill et al. 1988), decreased amplitude (Begleiter et al. 1984; O'Connor et al. 1986), increased latency and decreased amplitude (Elmasian et al. 1982; Whipple et al. 1988, 1991; Hill et al. 1990), or no changes in either of these two P3 measures (Polich and Bloom 1987, 1988; Polich et al. 1988). A recent review (Berman and Noble, in press) summarizes the extant studies of and controversy about the P3 as a putative marker for alcoholism.

It is not clear why these differences in the literature exist in alcoholics and their children. This diversity may stem, in part, from variations in task demands and subject populations (Whipple et al. 1991). However, even when similar tasks are used, the P3 component varies greatly across individuals (Brown et al. 1983; Polich et al. 1983; Polich 1984, 1986; Sklare and Lynn 1984). It is possible that hereditary factors (vide supra) may additionally contribute to individual differences in P3 morphology in alcoholics and their children.

Many studies on the P3 have treated alcoholism as if it were a single discrete entity. However, Jellinek (1960) has emphasized the distinction between alcoholics who had persistent alcohol-seeking behaviors (i.e., "inability to abstain entirely") and others who could abstain from alcohol for prolonged periods. More recently, Cloninger (1987), through empirical studies, has found that alcohol-seeking behaviors, among other behaviors and personality traits, distinguishes type 1 and type 2 alcoholics. Type 1, with necessary environmental provocative factors and hereditary background (i.e., "milieu limited"), is found in both males and females and is characterized by an ability to abstain. Type 2, a more severe form of alcoholism, with a necessary hereditary background but irrespective of environmental provocative factors (i.e., "male limited"), is found primarily in men and is characterized by an inability to abstain. If P3 characteristics vary in these two types of alcoholics, then it is possible that the divergent P3 findings in alcoholics and their children found in the literature may, in part, be related to the various admixtures of type 1 and 2 alcoholics studied.

P300 and the Dopaminergic System

There is growing evidence to suggest the involvement of the dopaminergic system in the generation of the P3. In normal subjects, the administration of haloperidol, a D_2 dopamine receptor blocker, caused a significant prolongation of latency but not changes in amplitude of the P3 (Stanzione et al. 1990). In Parkinson disease, an affliction marked by a widespread destruction of dopaminergic fibers at the cortical and subcortical levels (Scatton et al. 1982; Cortés et al. 1989), the prolonged P3 latency found in these patients (Hansch et al. 1982; Stanzione et al. 1991) is significantly shortened by the administration of the dopamine precursor, L-Dopa (Starkstein et al. 1989; Stanzione et al. 1991). Moreover, in human aging, where increased latency of the P3 has been found (Goodin et al. 1978; Brown et al. 1983; Picton et al. 1984), postmortem (Severson et al. 1982; Seeman et al. 1987) and positron-emission-tomography (Wong et al. 1984) studies have shown a progressive decline with age in the number of D_2 dopamine receptors. While other neurochemical systems are not excluded in P3 wave characteristics and are most likely involved, the combined data suggest an important role for the dopaminergic system, particularly the number of D_2 dopamine receptors, in P3 latency.

If the dopaminergic system is involved in the generation of the P3 and if hereditary factors contribute to this late component of the ERP, the question raised herein is whether a particular molecular genetic component involving the dopaminergic system is associated with the phenotypic expression of P3 latency and amplitude. The present study addresses this issue by examining the relationship of the *Taq*I A D_2 dopamine receptor (DRD2) alleles to P3 morphology in three groups of young boys (sons of nonabstaining and abstaining alcoholics and sons of nonalcoholic social drinkers) before they have begun to consume alcohol and other psychoactive agents.

Subjects, Material, and Methods

Subjects

Subjects were 98 10–14-year-old Caucasian (non-Hispanic) sons of active alcoholic (SAA) fathers, sons of recovering alcoholic (SRA) fathers, and sons of social drinker (SSD) fathers. These boys were recruited by dis-

tributing flyers to elementary and junior high schools in the Los Angeles area. Interested parents who called the UCLA Alcohol Research Center were given more detailed information over the telephone, and a brief initial screening interview was conducted with the parent who made the first contact. During this initial screening, the drinking status of the father and family history of alcoholism were obtained. In addition, information was gathered as to the son's history of a learning disability, color blindness, head injury, loss of consciousness, medical or psychiatric disorder, and alcohol and other drug use. Further information was gathered regarding the mother's drinking pattern during her pregnancy with the subject.

After the initial screening, more in-depth information was obtained from potential families. During this interview, parents were asked to give a list of all known biological relatives with a history or current status of alcoholism, drug abuse, or major psychiatric disorders. They were also asked to describe their present and past patterns of alcohol and drug use and, if recovering alcoholics, the period of their abstinence. To verify the parents' alcohol use history, they were asked to choose three individuals who could provide confidential collateral information on this issue. Furthermore, a request was made to provide copies of medical records from treatment facilities on the alcoholic fathers, if treatment for alcoholism was received by them.

The Structured Clinical Interview for the *Diagnostic and Statistical Manual III-Revised* (DSM-III-R) (SCID) (Spitzer et al. 1989) for alcohol dependence was administered to establish the presence or absence of alcoholism in the parents. The SCID was also used to determine the presence or absence of other drug abuse and psychiatric disorders, if these problems were reported by the parents.

Information regarding the son's alcohol and other drug use history was obtained briefly from his parents during the initial screening. More detailed information regarding the son's alcohol and other drug use history was gathered from a modified version of the Jessor and Jessor questionnaire (Jessor and Jessor 1977) during the son's first appointment. In addition, the son's pediatrician was contacted for verification of his medical and psychiatric history and current status.

Inclusion/exclusion criteria were as follows: (1) Sons of alcoholics must have fathers who satisfy DSM-III-R criteria for alcohol dependence and also have at least one other first- or second-degree relative who is reported to be an alcoholic. This group may include mothers who have a history of alcoholism, but their drinking during pregnancy with their sons must not have exceeded 4 drinks/wk. (2) Sons of nonalcoholic social drinker fathers must have mothers who do not have a history of alcoholism and no other first-, second-, or third-degree relative who is reported to be an alcoholic. The mothers' drinking during pregnancy with their sons must not have exceeded 4 drinks/wk. (3) Sons were excluded if they had parents with a history of major psychiatric disorders (e.g., schizophrenia or affective disorders) unassociated with their drinking. (4) Sons must have no history of alcohol and other drug use and no current use of psychoactive medications. (5) Sons must have no history of serious head injury, significant loss of consciousness (for more than 5 min), learning disability, medical illness, or major psychiatric disorders. (6) Sons must have no hearing or visual impairment (corrected to normal vision acceptable) or color blindness. (7) Parents must be willing to allow the researchers to contact relatives or significant others to verify their past and present drinking patterns, treatment history, and the sons' medical status. (8) Sons must be willing to donate urine samples to be tested for alcohol and illicit drug levels. They must also agree to have breath samples analyzed for alcohol prior to neuropsychological and neurophysiological testing.

The research protocol had the approval of the UCLA Human Subject Protection Committee. All subjects (sons and parents) gave informed consent and were monetarily compensated for their participation.

Procedures

The boys were tested individually in the laboratory on two different sessions. In the first session, the Wechsler Intelligence Scale for Children-Revised (WISC-R) (Wechsler 1974) was administered, and Full Scale IQ was calculated. In the second session, the boys were neurophysiologically tested as described below. During each test session, breath samples were analyzed with an Alco-Sensor III (Intoximeters) to verify that the subject had not recently consumed alcohol. No boy was found to have alcohol in his system. Moreover, during each of the neuropsychological and neurophysiological sessions, urine samples were collected and screened for eight different classes of drugs, including ethanol, amphetamines, benzodiazepines, phencyclidine, barbiturates, cocaine, opiates, and tetrahydrocannabinol. None of the 98 boys tested ethanol positive or other-drug positive during any of the test sessions.

ERPs were recorded with an Electrocap from Fz, Cz, Pz, Fp1, Fp2, F7, F8, T3, T4, T5, T6, C3, C4, P3, P4, 01, and 02 sites of the international 10–20 electrode placement system referred to nosetip. In addition, vertical and horizontal channels of the electrooculogram

(EOG) were recorded with a pair of bipolar montages from electrodes situated, respectively, above and below the right eye and on the two outer canthi. Data acquisition and stimulus delivery were controled by two IBM-compatible microcomputers operating as a SCAN EEG/EP workstation (Neuroscan). Electroencephalogram (EEG) and EOG channels were amplified (bandpass = 0.1–50 Hz) and digitized at a rate of 232 Hz for 1,100 ms, beginning 100 ms prior to stimulus delivery.

Two different versions of a continuous performance task (CPT) using colored shapes were utilized. In one version (CPT_{10}), stimuli were circles, triangles, and squares centered on a CTX color monitor in blue, green, red, or violet against a black background for 100 ms (interstimulus interval = 2 s, 2 blocks of 150 stimuli). Each shape had a single white digit at its center. Twenty percent of the items were repetitions of the immediately preceding stimulus and were designated as targets. Since targets had to be identical to the preceding stimulus, they had to be the same on all three features—shape, color, and the identity of the digit. Since the 3 shapes, 4 colors, and 10 digits appeared equally often, the probability of a shape match among nontargets was $^1/_3$, the probability of a color match was $^1/_4$, and the probability of a digit match was $^1/_{10}$. In the second version of the CPT (CPT_4), only the digits 1–4 were displayed, and a fourth shape was added (a four-lobed clover), so that the probability of a nontarget matching on any single feature was $^1/_4$. In addition, block size was increased from 150 to 160 stimuli. Otherwise, there were no differences between the two CPTs. Twenty-one subjects received only the CPT_{10}, 35 received only CPT_4, and 42 subjects received both.

Each stimulus subtended between 3.6 and 4.9 degrees of visual angle both vertically and horizontally, with the central digit subtending 1.2 degrees vertically. Subjects were trained in a practice block to make a speeded button press with the left or right index finger (counterbalanced across subjects) by pressing the left button of a three-button "mouse" pointing device (Logitech) in response to targets. Reaction times (RT) were accepted only between 200 and 1,600 ms poststimulus. Digitized EEG was stored on hard disk and digital audio tape for off-line analysis. Separate ERP average waveforms were collected for targets and easy nontargets (nontargets not matching on any feature—shape, color, or digit). Only stimuli eliciting the correct behavioral responses (i.e., Go or NoGo) were included in the averages. EEG was corrected, by linear regression, for intrusion of vertical EOG artifact (Semlitsch et al. 1986). Trials containing uncorrectable sources of artifact were eliminated from the averages through visual inspection, without knowledge of family history group or genotype. P3 was measured in the waveform elicited by targets at the Pz electrode as the point of maximum voltage between 300 and 600 ms poststimulus. The latency and amplitude (relative to prestimulus baseline) of this point were stored for statistical analysis, along with percentage of correct target presses and the associated RT. Prior to peak measurement, all averages were low-pass digitally filtered at 15 Hz.

DNA Analysis

Genomic DNA was extracted from the blood samples (Olds 1986) and subsequently used as template for PCR (Saiki et al. 1988). The primers 5014 and 971 were used to amplify a 310-bp fragment spanning the polymorphic *Taq*I A site of the DRD2 gene (Grandy et al. 1989). The sequence of the 5014 primer was 5′-CCgtcgaCCCTTCCTGAGTGTCATCA-3′ and for the 971 primer was 5′-CCgtcgaCGGCTGGCCAAGTTGTCTA-3′ (lowercase letters code for *Sal*I site). The primer sequences were provided by David K. Grandy, and they were synthesized by Oligos Etc. (Wilsonville, OR).

Amplification was carried out in 100-μl reactions using 1 μg of genomic DNA and 2.5 units of Ampli*Taq* DNA polymerase (Perkin Elmer) in a standard reaction cocktail containing 200 μM of each of the four dNTPs, 1 mM $MgCl_2$, and the recommended buffer provided by the manufacturer (Perkin Elmer). After an initial denaturation step at 94°C for 5 min, DNA was amplified in three-step cycles as follows: denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, using the Perkin Elmer GeneAmp 9600 thermocycler. After 35 cycles, a final extension step at 72°C for 5 min was used.

A 10-μl aliquot was removed and analyzed by gel electrophoresis in a 2.5% agarose gel containing ethidium bromide and was visualized under UV light. The expected 310-bp fragments were visualized with minimal background. Approximately 500 ng of the DNA was digested with 5 units of *Taq*I restriction enzyme (Boehringer-Mannheim Biochemical) at 65°C for 2 h. The resulting products were analyzed by agarose gel electrophoresis as described above. The A1/A2 genotype is revealed by three fragments of 310 bp, 180 bp, and 130 bp; the A2/A2 genotype is indicated by two fragments of 180 bp and 130 bp; and the A1/A1 genotype is shown by the uncleaved 310-bp fragment.

Statistical Analysis

A χ^2 test was used to assess whether there was a significant difference in the frequency of the A1 and the A2 allele among the three groups of sons. Possible dif-

Table 1

***Taq*I A DRD2 Genotypes and Allelic Frequency in Sons of Active Alcoholic, Recovered Alcoholic, and Social Drinker Fathers**

	No. with Genotype			Allelic Frequency		
Group (*n*)	A1/A1	A1/A2	A2/A2	A1	A2	Significance
SAA (32)	3	14	15	.313	.687	$\chi^2 = 8.56$; $P = .01$
SRA (36)	1	8	27	.139	.861	
SSD (30)	0	8	22	.133	.867	

ferences in age, educational level, and IQ between group and allelic types (A1 = A1/A1 or A1/A2 genotypes; A2 = A2/A2 genotype only) were investigated with group (SAA, SRA, and SSD) by allele (A1 and A2) ANOVAs (SAS Institute 1989). The effect of allele, group, and their interaction on P3 amplitude, P3 latency, target accuracy, and RT were assessed by separate applications of a mixed linear model ANCOVA (SAS Institute 1992). This type of generalized ANCOVA allows assessment of both fixed and random effects (Harville 1977). Age, IQ, and CPT type were entered into the model as covariates, after screening for homogeneity of within-group regression coefficients. A $P \leq .05$ was considered statistically significant.

Results

Characteristics of the Alcoholic and Nonalcoholic Fathers

The assignment of children to the three groups was based a priori on the father's drinking behavior. The SAA group had fathers who were nonabstaining alcoholics, the SRA group had fathers who were recovered alcoholics (4.43 ± 0.59 years of abstinence), and the SSD group had fathers who were not alcoholic but who drank alcohol socially. The alcoholic fathers of these children, besides being differentiated on their ability or inability to abstain, showed the following characteristics. Fathers of the SAA group compared with fathers of the SRA group were slightly younger (42.6 ± 1.0 vs. 44.7 ± 1.1 years of age) and had a lower annual income ($50,700 ± 9,000 vs. $58,300 ± 6,000). They also began to consume alcohol earlier in their lives (15.7 ± 0.6 vs. 16.5 ± 0.5 years), were more frequently drug dependent (40.6% vs. 25.0%), and had more first-degree alcoholic relatives (1.83 ± 0.26 vs. 1.26 ± 0.16). None of these differences achieved statistical significance. However, fathers of the SAA group compared with fathers of the SRA group had a significantly greater number of medical, legal, and social problems associated with their drinking (0.88 ± 0.15 vs. 0.47 ± 0.11, $P = .03$).

Fathers of the SSD group were 46.9 ± 1.1 years old, had an annual income of $73,600 ± 6,800 and began to consume alcohol when they were 19.1 ± 0.5 years of age. Fathers of the SSD group were significantly older than fathers of the SAA group but not older than fathers of the SRA group ($F[2,95] = 3.85$, $P = .02$). They began to consume alcohol at a significantly later age than either fathers of the SRA or SAA groups ($F[2,95] = 10.50$, $P = .0001$). However, the three groups of fathers did not significantly differ on income. None of the fathers of the SSD group had a history of drug dependence or problems associated with their drinking or had any alcoholic relatives.

DRD2 Genotypes of the Children

Table 1 presents *Taq*I A DRD2 genotypes of the children in the SAA, SRA, and SSD groups. In the SAA group, A1 allele frequency was .313 compared with .139 and .133 in the SRA and SSD groups, respectively. A significant difference in A1 allele frequency was found among these three groups ($\chi^2 = 8.56$, $P = .01$). Further, while the frequency of the A1 allele was very similar in the SRA and SSD groups, the frequency of this allele in the SAA group was significantly higher when compared with either the SRA group ($\chi^2 = 5.94$, $P = .02$) or with the SSD group ($\chi^2 = 5.69$, $P = .02$).

Age, Education Level, and IQ of the Children

The relationship of age, education level, and IQ to *Taq*I A DRD2 alleles in the three groups of boys is shown in table 2. No significant differences in age and educational level were found among boys carrying the A1 or A2 allele or among the different groups (SAA, SRA, and SSD). There were also no significant interaction effects between allele (A1 and A2) and group (SAA, SRA, and SSD) on these two variables. There was a significant difference in IQ among the three groups of boys ($F[2,92] = 3.42$, $P = .04$). However, there was no significant difference in IQ among boys of different allele types and no significant interaction between allele and group on this variable.

Table 2

Relationship of Age, Education Level, and IQ to *TaqI* A DRD2 Alleles in Sons of Active Alcoholic, Recovered Alcoholic, and Social Drinker Fathers

	SAA		SRA		SSD	
MEASURE (mean)	A1 (n = 17)	A2 (n = 15)	A1 (n = 9)	A2 (n = 27)	A1 (n = 8)	A2 (n = 22)
Age in years (12.5 ± 0.1)	12.4 ± 0.3	12.5 ± 0.3	12.9 ± 0.4	12.1 ± 0.2	12.5 ± 0.4	12.8 ± 0.2
Education level (5.5 ± 0.1)	5.3 ± 0.3	5.6 ± 0.4	6.1 ± 0.4	5.0 ± 0.3	6.1 ± 0.6	5.8 ± 0.2
IQ (112.5 ± 1.3)	105.8 ± 2.7	111.7 ± 3.2	119.2 ± 5.6	111.6 ± 2.3	119.0 ± 4.8	114.3 ± 2.3

NOTE.—A1 allele includes A1/A2 and A1/A1 genotypes. A2 allele includes A2/A2 genotype only. Values represent mean ± SEM.

ERP Profiles of the Children

Figure 1 shows the grand mean ERP responses of the total number of boys with A1 and A2 alleles, recorded from Fz, Cz, Pz, 01, and 02 electrode sites. As may be evident from an inspection of this figure, P3 was most prominent at Pz; subsequently for this initial study, analyses were confined to this site.

Figure 2 superimposes the grand mean target waveforms at Pz of A1 and A2 allele subjects for each of the three groups of boys. Because close examination of figure 1 suggests the possibility that the CPT may generate more than one late positivity at posterior electrode sites, the waveforms in figure 2 were low-pass filtered at 6 Hz. Since most P3 power comes from frequencies below 2 Hz, this will have little effect on P3 wave shape but will greatly reduce alpha contamination. Contamination of ERPs recorded from posterior scalp sites by large-amplitude alpha waves is often a problem when young boys perform tasks with a large vigilance component, such as the CPT. Because alpha activity produces peaks about every 100 ms, it can cause the several hundred-millisecond P3 deflection to appear bifurcated. However, after removal of alpha, there is little remaining evidence for bifurcated P3 peaks (fig. 2).

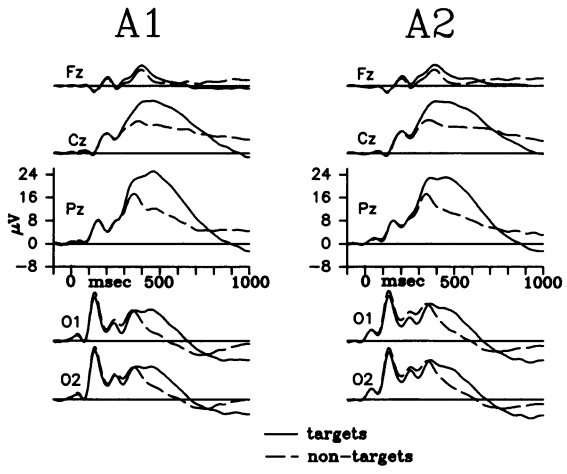

Figure 1 Grand mean ERPs of boys with the A1 (A1/A2 and A1/A1 geneotypes) and A2 (A2/A2 genotype only) alleles recorded from Fz, Cz, Pz, 01, and 02 electrode sites in response to targets and nontargets.

Table 3 presents the adjusted means for target P3 amplitudes and latencies at Pz of A1 and A2 allele subjects for each of the three groups of boys. An allele × group (2 × 3) ANCOVA was conducted on P3 amplitude. Age, IQ, and CPT type were treated as covariates because (*a*) age has been previously shown to affect P3 characteristics (Courchesne 1984), (*b*) there were significant differences in IQ among the three groups, and (*c*) two types of CPT were used. ANCOVA results showed that there were no significant main effects of allele (A1 = 29.8 ± 1.2 μV, A2 = 28.2 ± 0.9 μV; P = .28) or group (SAA = 27.1 ± 1.2 μV, SRA = 29.3 ± 1.3 μV, SSD = 30.6 ± 1.4 μV; P = .18) and no interaction between allele and group (P = .13). None of the three covariates were significantly related to P3 amplitude.

In contrast, allele × group (2 × 3) ANCOVA results of P3 latency showed a large main effect of allele (A1 = 455 ± 12 ms, A2 = 412 ± 8 ms; P = .004) and a suggestive effect of group (SAA = 409 ± 11 ms, SRA = 445 ± 12, SSD = 446 ± 13; P = .059). Whereas P3 latency difference between A1 and A2 allele subjects was greater in the SAA (55 ms) and SRA (46 ms) groups than in the SSD (28 ms) groups, there was no significant interaction between allele and group (P = .74). The covariate IQ, but not age or CPT type, was significantly and positively related to P3 latency (P = .01).

In sum, after correcting for variations associated

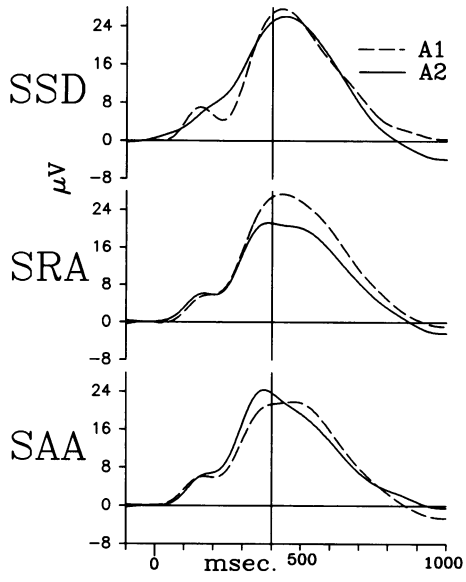

Figure 2 Grand mean ERPs at Pz of A1 and A2 allele SSD, SRA, and SAA groups in response to targets. Vertical lines demarcate 400-ms latency.

with age, IQ, and CPT type, neither allele nor group had a significant effect on P3 amplitude. However, the presence of the A1 allele was significantly associated with prolonged P3 latency across the three groups, and sons of active alcoholics tended to have reduced P3 latency, regardless of genotype.

Accuracy and Reaction Times of the Children to Targets

Table 3 also presents the effects of the experimental variables on the accuracy and RT associated with response to targets on the CPT. Two separate allele × group (2 × 3) ANCOVAs were conducted on these two behavioral measures. No significant effects of allele ($P = .79$), group ($P = .19$), or their interaction ($P = .88$) were found on the accuracy of target identification, although IQ was found to be significantly and positively related to accuracy ($P = .03$). Moreover, the speed of the button press was not significantly related to allele ($P = .54$), group ($P = .31$), or their interaction ($P = .13$), nor was it related to any of the three covariates.

In sum, although IQ was related to the accuracy of target identification, after correcting for variations associated with age, IQ, and CPT type, there were no significant effects of allele, group, or their interaction on the behavioral measures of accuracy and RT.

Discussion

Characteristics of the Alcoholic and Nonalcoholic Fathers and Their Children

The present sample consisted of unrelated volunteers drawn from the general population of non-Hispanic Caucasian boys attending school and included only physically and mentally healthy subjects. This sample of above-average-intelligence boys had not yet begun to consume alcohol or use other drugs, obviating the effects of these substances on brain function. The boys were divided into three groups on the basis of their fathers' drinking behavior. Two of the groups were sons of alcoholics, with the SAA group having fathers who were unable to abstain and the SRA group having

Table 3

Relationship of Target P300 Amplitude and Latency at Pz and Percent Correct Responses and RT to *TaqI* A DRD2 Alleles in Sons of Active Alcoholic, Recovered Alcoholic, and Social Drinker Fathers

	Amplitude (μV)		Latency (ms)		% Correct Responses		RT (ms)	
Group	A1	A2	A1	A2	A1	A2	A1	A2
SAA[a]	26.3 ± 1.7	27.9 ± 1.8	437 ± 15	382 ± 17	88.2 ± 2.2	86.4 ± 2.3	658 ± 26	580 ± 27
SRA[b]	32.2 ± 2.3	26.4 ± 1.3	468 ± 21	422 ± 12	90.8 ± 3.0	91.1 ± 1.6	660 ± 35	663 ± 19
SSD[c]	31.0 ± 2.5	30.2 ± 1.5	460 ± 23	432 ± 14	91.2 ± 2.8	91.2 ± 1.9	621 ± 34	653 ± 23
Mean[d]	29.8 ± 1.2	28.2 ± 0.9	455 ± 12	412 ± 8	90.1 ± 1.5	89.6 ± 1.1	646 ± 18	632 ± 13
Significance	.28		.004		.79		.54	

NOTE.—A1 allele includes A1/A2 and A1/A1 genotypes; A2 allele includes A2/A2 genotype only. Values represent ANCOVA-adjusted mean ± SEM (see text).

[a] $N = 32$; A1 = 17; A2 = 15.

[b] $N = 36$; A1 = 9; A2 = 27.

[c] $N = 30$; A1 = 8; A2 = 22.

[d] $N = 98$; A1 = 34; A2 = 64.

fathers who were able to abstain (approximately 4.5 years of sobriety). The third group, SSD, had fathers who were never alcoholic but did consume alcohol socially. Data analysis showed that fathers of the SAA group compared with the SRA group had a tendency to consume alcohol earlier on in life and had a greater familial load of alcoholism; moreover, they had a significantly greater number of alcohol-related problems. These observations, taken together with a differential ability to abstain, suggest that fathers of the SAA group compared with the SRA group had a more heritable and severe type of alcoholism, possibly akin to the type 2 (i.e., "male-limited") alcoholics described by Cloninger (1987).

DRD2 Alleles in Alcoholic and Nonalcoholic Fathers and Their Children

The A1 (less-frequent) allele of the DRD2 gene has been shown to be strongly associated with severe alcoholism (Blum et al. 1990). A further study has shown a lower frequency of the A1 allele in less-severe compared with more-severe alcoholics (Blum et al. 1991). This finding has been replicated in a number of additional studies (Bolos et al. 1990; Gelernter et al. 1991; Noble and Blum 1991; Parsian et al. 1991; Noble et al., in press; for reviews, see Noble 1993; Uhl et al. 1993). Indeed, when adult Caucasian nonalcoholics and less-severe and more-severe alcoholics were compared, A1 allele frequency was .097, .150, and .337, respectively (Blum et al. 1991). No significant difference in A1 allele frequency was found between the nonalcoholic and the less-severe alcoholic group. However, the severe alcoholics had a significantly higher frequency of the A1 allele than did each of the other two groups. While we did not ascertain DRD2 alleles in the fathers of the present sample of children, A1 allele frequency in the SSD, SRA, and SAA groups of boys was .133, .139, and .313, respectively.

The similarity in the high frequency of the A1 allele in the SAA group and adults who are severe alcoholics suggests the involvement of the DRD2 gene in the possible development of severe alcoholism in these children. By the same token, the low frequency of the A1 allele in the SRA group and in adults with a less-severe form of alcoholism suggests a lack of involvement of the DRD2 gene in the future development of alcoholism in the SRA group. It is hypothesized that environmental factors and/or genes other than the DRD2 may be implicated in the possible future development of alcoholism in the SRA group. Long-term follow-up studies are underway to determine the validity of this notion.

P300 Characteristics as a Function of Group and DRD2 Alleles

The relationship of P3 characteristics to group and allele affiliation showed an interesting pattern. While no significant difference was found in P3 amplitude among the present three groups of boys, P3 amplitude was largest in the SSD group, followed in descending order by the SRA and SAA groups. This is consistent with the many studies that have shown reduced P3 amplitude in children of alcoholics compared with children of nonalcoholics (for a recent review, see Polich et al., in press). Moreover, a separate analysis indicated that our present data replicated the significantly reduced P3 amplitude in sons of alcoholics, which we have previously reported, with the CPT version featuring all 10 digits (Whipple et al. 1991). To make the comparison as similar as possible to our earlier report, (1) only data from the CPT_{10} were analyzed, (2) sons of alcoholic (SAA combined with SRA) fathers were compared with SSD fathers, and the effect of allele was not considered. We retained the use of a mixed linear model ANCOVA, with IQ and age as covariates. The ANCOVA adjusted mean (±SEM) P3 amplitudes were 28.3 (±0.8) μV for sons of alcoholics and 32.6 (±1.2) μV for sons of social drinkers, a difference that was statistically significant ($P < .006$).

In the main analysis, however, there was no significant effect of either an allele or a group × allele interaction on P3 amplitude. In contrast, there was a large main effect of allele on P3 latency, with boys carrying the A1 allele having an average of 43 ms (10%) prolongation of the P3 latency compared with those carrying the A2 allele. There was no significant interaction of allele × group on P3 latency. However, a strong trend of group effect was found on P3 latency, with boys with the A1 or A2 allele in the SAA group having shorter latencies than the comparable boys with the A1 or A2 allele in the other two groups. The reasons for the shortened latencies in the SAA group are unclear and merit further investigation.

A Possible Neurochemical Substrate of P3 Latency

The neurochemical basis for the prolonged P3 latency observed in the present sample of boys with the A1 allele is not known. However, as indicated earlier, evidence from a variety of sources suggests the involvement of the dopaminergic system, particularly the D_2 dopamine receptors, in P3 latency. It is proposed that the prolonged P3 latency of boys with the A1 allele may be due to an inherent deficit in the number of their brain D_2 dopamine receptors. Support for this notion comes from a previous study of postmortem brain sam-

ples where D_2 dopamine receptor binding characteristics were determined in subjects with the A1 or A2 allele (Noble et al. 1991). In subjects with the A1 allele, the number of D_2 dopamine receptor binding sites (B_{max}) was significantly reduced (about 30%) compared with the B_{max} of subjects with the A2 allele. Moreover, a progressively reduced B_{max} was found in subjects with A2/A2, A1/A2, and A1/A1 genotypes, in that order. It has been suggested (Comings et al. 1991) that a mutation causing *Taq*I A polymorphism or a mutation in linkage disequilibrium with *Taq*I A (*Taq*I B polymorphism [Hauge et al. 1991]) is associated with some functional decrease of the DRD2 gene. To test the possibility of a differential expression of D_2 dopamine receptors in brains of children with the A1 or A2 allele, additional studies are needed, e.g., position-emission-tomography imaging techniques using radiolabeled D_2 dopamine receptor ligands.

In conclusion, the present study presents the first evidence for a molecular genetic determinant of P3 latency. While the sample studied was not small, it was nevertheless restricted to Caucasian boys with a limited age range, of whom a majority were children of alcoholics. Further studies are needed to determine the generality of our findings in other populations, including females and other age and racial groups, and whether prolonged P3 latency is associated with unique cognitive and other behavioral patterns and is a predictor of future problems.

Acknowledgments

The authors acknowledge the support of R. Brinkley Smithers and the Christopher D. Smithers Foundation, Inc. This study was also supported by USPHS grant AA-08020. We thank David K. Grandy for providing the primer sequences used in DRD2 genotyping, Karl Syndulko and David Friedman for critically reading the manuscript, and Donald Guthrie and Stan Bentow for statistical consultation. We appreciate the dedicated efforts of Shawn Prokopec in recruiting the subjects in this study and thank the families for their participation.

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