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Stability of Genetic and Environmental Influences on P300 Amplitude: A Longitudinal Study in Adolescent Twins

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This study examined the stability of genetic and environmental influences on individual differences in P300 amplitude during adolescence. The P300 component is an event-related brain potential (ERP) that has attracted much attention as a biological marker for disturbed cognitive processing in psychopathology. Understanding the genetics of this biological marker may contribute to understanding the genetics of the associated psychopathologies. In a group of 213 adolescent twin pairs, the P300 component was measured twice, the first time at age 16 and the second time 18 months later. A large part of the variance of the P300 amplitude could be explained by familial factors, with estimates ranging from 30% to 81%. Whether the familial resemblance was due to genetic or shared environmental factors depended on sex. For males, genetic factors explained familial resemblance in P300 amplitude, but for females such resemblance was likely due to shared environmental factors. The phenotypic stability of the P300 amplitude from 16 to 18 years was high in both sexes, and stability could be attributed largely to the same familial factors. There was no evidence that new familial influences emerged at age 18.

KEY WORDS: P300; twins; genetics; development; event-related potentials.

INTRODUCTION

Genetic influences contribute to most psychopathologies, including depression, schizophrenia, and addiction (McGuffin *et al.*, 2001; Plomin *et al.*, 1994; Rose, 1995). Because disturbed cognition is part of many, if not all, forms of psychopathology (Carlson *et al.*, 1999; Kuperberg and Stephan, 2000; Pierson *et al.*, 2000; Polich *et al.*, 1994), a reasonable hypothesis states that genetic liability for disturbed cognitive processing partly overlaps with susceptibility for psychopathology. Genetic influences on cognition, which are expressed via the brain, are likely to be determined by a complex interaction of multiple subcortical and cortical structures, each influenced by its own set of genes. By studying confined aspects of human brain functioning it may be easier to isolate and identify the effects of each of these subsets of genes (Boomsma *et al.*, 1997).

Recording brain activity at the scalp is one of the techniques used to assess human brain functioning. One of the most investigated brain potentials is the P300. This brain potential is a large positive waveform that peaks between the 300 to 600 ms after detection of an attended and task relevant stimulus. In most studies, the P300 is elicited in an "oddball" task, in which a participant is asked to count or respond to a infrequent relevant (target) stimulus randomly altered with frequently irrelevant stimuli (nontarget). The target stimuli produce a large P300 component in normal individuals. The P300 amplitude provides an index of allocation of attentional resources during working memory operations (Kramer and Strayer, 1988) or, more generally, cognitive processing needed for encoding new stimuli or updating of representations in working memory (Donchin and Coles, 1988).

The P300 is the ERP most widely used in research with an individual differences perspective; for instance, in studies on the developmental change in cognitive pro-

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cessing (Friedman *et al.,* 1993). More important, it can provide information about the underlying nature of cognitive impairments usually observed in persons with mental disorders. For example, reduced amplitude of the P300 component is a robust clinical finding in patients with schizophrenia (Turetsky *et al.,* 1998; Weisbrod *et al.,* 1999). P300 is also increasingly considered to be a biological indicator of a variety of other psychiatric diseases, including alcohol addiction (Hansenne *et al.,* 2000; Polich *et al.,* 1994; Porjesz and Begleiter, 1998). Because biological markers are proposed to be closer to actual gene action than the psychiatric disease itself, the P300 is thought to provide greater power for gene identification (Almasy and Blangero, 2001; Cornblatt and Malhotra, 2001). This approach has been successfully applied in the Collaborative Study on the Genetics of Alcoholism (Williams *et al.,* 1999), where the P300 amplitude was used as a biological marker that mediates genetic liability to alcoholism. In a bivariate genome scan, evidence was found that the chromosome 4 region near the locus ADH3 may influence both the P300 amplitude and the risk for alcoholism.

To use the P300 amplitude as a biological marker for a genetic susceptibility, it has been suggested that (1) it should be a stable characteristic, and (2) it must be partly under genetic control (Almasy and Blangero, 2001; Cornblatt and Malhotra, 2001). This last criterion has been examined in various family (Almasy *et al.,* 1999; Eischen *et al.,* 1995) and twin studies (Katsanis *et al.,* 1997; O'Connor *et al.,* 1994; van Baal *et al.,* 1998; van Beijsterveldt *et al.,* 1998b). In 164 young twin pairs (van Baal *et al.,* 1998), visual P300 amplitude was moderately heritable at age 5 (mean heritability over various leads 34%) and highly heritable at age 7 (heritability around 70%). In 58 MZ and 39 same-sex adult DZ twin pairs, O'Connor *et al.* (1994) reported a substantial genetic influence on the auditory P300 amplitude in the posterior scalp locations (heritability around 60%). Somewhat higher heritabilities (80%) for visual P300 amplitude were reported in a sample of 30 MZ and 34 DZ 17- and 18-year-old male twin pairs (Katsanis *et al.,* 1997). The O'Connor *et al.* study (1994) did not find sex-specific differences in the variance of the P300 component, and Katsanis *et al.* (1997) measured only males. Results from the study of van Beijsterveldt *et al.* (1998b), on a sample of 213 adolescent twin pairs, confirmed that a substantial proportion of the variance of the visual P300 amplitude in adolescents is indeed familial. However, results indicated that the nature of this familial resemblance were sex-specific. For males, genetic influences on the P300 amplitude were suggested but for females, shared environmental influences were suggested. A consequence of this finding, in view of the criteria formulated above, is that the P300 may be a valid biological marker in males only. As an alternative explanation, we offered the possibility that genetic factors may be expressed only periodically during development and that sex differences in maturational rate explained the unexpected sex differences in genetic architecture (van Beijsterveldt *et al.,* 1998b).

The present study reports the results of a longitudinal follow-up in this same sample of twins. At the first assessment the twins were 16 years of age. The second assessment was done 1.5 to 2 years later. Using this follow-up, we were able to examine whether the sex-specific contribution of genetic and shared environmental factors to P300 at age 16 could be replicated at age 18. The longitudinal genetic design used in the present study simultaneously allowed us to examine the stability of familial influences of the P300 amplitude. Magnetic resonance imaging scans (MRI) show that there are still changes in brain structure and functioning until the 20s (Pfefferbaum *et al.,* 1994; Posner and Raichle, 1994), although changes occur mainly in the frontal lobe. Therefore, it is possible that different genes are expressed at ages 16 and 18 or that different environmental factors contribute to the P300 at each age.

The genetic analyses used a model in which genetic and environmental influences were estimated from the reliable part of the phenotypic variance using the odd/even trial correlation to estimate error variance (Van Baal *et al.,* 1998). A Cholesky decomposition was employed to address the following questions: (1) What is the relative contribution of genetic and environmental influences to P300 amplitude, and are these influences the same for males and females? (2) Do the same genetic and environmental influences underlie the individual differences in P300 amplitude at ages 16 and 18 years? and (3) Do new genetic and environmental influences emerge at age 18? Confidence intervals were calculated in order to test quantitative changes in genetic and environmental influences over an interval of 1.5 to 2 years.

METHODS

Participants

A sample of 213 adolescents twin pairs participated twice in the study, with a mean interval of 1.5 year between sessions. The mean age of the twins at the first test session was 16.2 years ($SD = 0.55$) and at the sec-

ond session 17.6 years (SD = 0.54). Addresses of twin pairs were obtained from participants in a large questionnaire study on health-related behavior (Boomsma *et al.*, 1994). Participants were invited by letter to participate in the experiment. They received a present as a reward for their participation. Costs they incurred when traveling to the laboratory were reimbursed.

The participants were divided into five groups by sex and zygosity: 38 monozygotic males (MZM), 37 dizygotic males (DZM), 52 monozygotic females (MZF), 38 dizygotic females (DZF), and 48 twins of opposite sex (DOS). For 114 same-sex twin pairs zygosity was determined by blood or DNA typing. For the other same-sex twins the zygosity was determined based on a questionnaire that was completed by the mother. The questionnaire contains items relating to physical similarity (similarity of face, eye color, hair color, and skin color) and the frequency with which family members and strangers confused the twins. In 17 twin pairs zygosity was determined based on the questionnaire that they completed themselves. Agreement between zygosity based on this questionnaire and zygosity based on blood group polymorphism was 95%.

No complete data were available for 19 twin pairs, because they did not return for the second test session. In addition, data were discarded for further analysis when a person did not perform the counting task well and/or had bad electroencephalogram (EEG) signals (10 twin pairs in the first session and 7 twin pairs in the second session). This left complete data for 203 and 187 twin pairs for the first and second test session, respectively.

Procedure and Task

The procedure and task were identical at test sessions 1 and 2. A detailed description of the procedure is found in van Beijsterveldt *et al.* (1998b). An oddball task was administered which consisted of two types of visual stimuli, infrequent stimuli (targets, n = 25) and frequent stimuli (non-targets, n = 100). The stimuli were line drawings of cats and dogs (Snodgrass and Vanderwart, 1980), which were pseudorandomly distributed and presented on a black and white monitor. Build-up time of the pictures on the screen was less than 20 ms. The duration of a stimulus was 100 ms and the time between them varied quasi-randomly (was the same for all participants) from 1.5 to 2 sec (mean = 1.75 sec). During the interstimulus interval (ISI), a central square was shown on the video monitor. To reduce eye movements, participants were instructed to fixate on this central square. The participants were instructed to silently count the infrequent stimuli. After the task they were asked how many infrequent stimuli they had counted.

Brain Activity Recording

Tin electrodes mounted in an electro-cap were used to measure EEG and electro-oculogram (EOG) activity. Recordings were made at the following scalp locations: central frontal (Fz), left and right central (C3, C4), left and right parietal (P3, P4), and left and right occipital (O1, O2). Linked earlobes were used as references according to the method described in Pivik *et al.* (1993). In order to record horizontal eye movements, tin electrodes were placed at the canthus of each eye. To detect vertical movement, EOG was recorded from intra-orbital and supra-orbital electrodes, in line with the pupil of the left eye. A ground electrode was attached to FPz. For both EEG and EOG, ECI (electrogel) EEG paste was used and the electrode impedance for EEG and EOG was less than 5 Kohm. All EEG and EOG signals were displayed and recorded by a 18-channel Nihon Kohden electroencephalograph (type EEG-4414A1K). The high-pass filter was set to .03 Hz and the low-pass filter was 35 Hz. Signals were sent to a 12-bit analog-digital converter and computer-stored for off-line processing. During the ERP recording, the sampling rate of the AD-converter was set to 100 Hz.

Preprocessing of the EEG consisted of automatic removal of single trials with gross shifts in EEG amplitude and clipping. Single-trial eye movements were removed by means of a dynamic regression routine in the frequency domain (Brillinger, 1975). Remaining trials (minimum 20 trials) were averaged, resulting in averaged ERP wave form for each participant, condition (target and non-target), and electrode location (Fz, C3, C4, P3, P4, O1, and O2). Because averaged ERPs were flattened because of latency jitter, a Woody filter (window of 200 to 700 ms) was used (Woody, 1967). At each electrode location, the P300 component was automatically scored and defined as the highest positive peak within a time window from 300–600 ms after stimulus onset. The amplitude of the P300 component was measured as the difference between the highest peak and a 100 ms pre-stimulus baseline period. All averaged ERPs were checked by outlier identification followed by visual inspection of the ERP simultaneously in all leads. If necessary, peak scorings were adjusted. The study reports the results of the P300 in response to the targets (infrequent stimuli).

Statistical Analysis

Split-Half Reliability and Temporal Stability of P300 Amplitude

The reliability of the P300 amplitude within a test session was obtained by using the split-half method. Two separate averaged ERPs were formed; one ERP averaged over even trials and one ERP averaged over odd trials. For each averaged ERP (odd and even), the peak amplitude of the P300 component was scored using the same procedure as in the ERP averaged over all trials. Split-half reliabilities were calculated as Pearson correlations between the even and odd averaged ERPs. Because of the low number of target trials, the Spearman-Brown formula was applied [$r_{sb} = 2r_{xy}/(1 + r_{xy})$; where r_{sb} is the split-half reliability coefficient, and r_{xy} represents the correlation between the even and odd averaged ERP].

In order to obtain information about the temporal stability of the P300 amplitude, the test-retest correlation between the two sessions (with one and half years between) was calculated for the reliable part of the P300. To this end, the correlation was calculated between the latent factor "true P300 at age 16" and the latent factor "true P300 at age 18" (see also Fig. 7, part A).

Genetic Analyses

To estimate the relative contributions of genetic and environmental influences to individual differences in P300 amplitude at two test sessions, we employed the method of genetic model fitting (Neale and Cardon, 1992). The genetic model consisted of two parts (Fig. 1). In part A of the model, a latent factor is formed by the covariation of the P300 amplitude from the odd and even averaged ERPs. In this way, the latent factor represents the reliable part of the P300 amplitude. The remaining variance is due to measurement error. In the model the measurement error was allowed to differ between males and females, and for sessions 1 and 2. As shown in part B of Fig. 1, the remaining variance of the latent factor of the P300 amplitude is decomposed into genetic and environmental factors. To this end, a bivariate Cholesky decomposition was applied, with common factor effects on both test sessions and specific-factor effects for the second test session. The information from the Cholesky decomposition could be used to test whether the same genes (or environment) influence the P300 amplitude at ages 16 and 18 years (by constraining the factor loading from the common genetic [or environmental] factor to P300 to be zero at age 18). In addition, constraining the loading of specific genetic (or environmental) factor to be zero tests whether new genes emerged at test session 2. In the full model, the variance was decomposed into additive genetic influences (A), unique environmental influences (E), and shared environmental influences (C) and their estimates were allowed to differ between males and females.

Model fitting was performed on the raw data in five zygosity groups. Using raw data allowed us to include the data of twins, who participated at test session 1 but did not return for the second test session. Parameters estimates were obtained using maximum

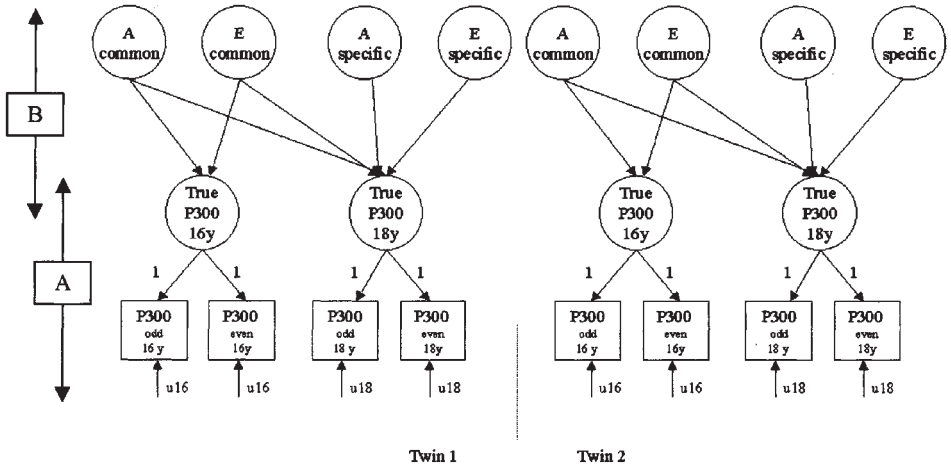

Fig. 1. Path model of the multivariate genetic model. Part A represents the decomposition of the observed variance into true variance and measurement error. Part B represents the genetic Cholesky decomposition. A (E) common refers to a genetic (environmental) factor common to ages 16 and 18. A (E) specific refers to a genetic (environmental) factor specific for age 18.

likelihood methods (Mx, Neale, 1997). Goodness-of-fit of submodels was assessed by likelihood-ratio χ^2-tests. Submodels were compared by subtracting -2*log likelihood for the full model from that for a reduced model. This difference is distributed as a χ^2. The degrees of freedom (*df*) for this test are equal to the difference between the number of estimated parameters in the full model and that in the submodel.

RESULTS

Means Differences

Table I depicts the means of the P300 amplitude for males and females, aged 16 and 18 years. A MANOVA was performed, with "test session" and "scalp location" as within-subject factors and "sex" and "zygosity" as between-subjects factors. The analysis was performed separately for the oldest and youngest of the twin pair in four zygosity groups (MZM, DZM, MZF, and DZF). A significant main effect appeared for scalp locations. The largest amplitudes were observed for the parietal scalp locations and the smallest for the frontal locations, replicating the well-known parietal maximum of the P300 (Polich and Kok, 1995). In addition, a significant interaction effect between test session and sex was found (targets: $F(1,131) = 13.326$; $p < 0.01$)). *Post hoc* analysis showed that the target P300 amplitude of males reduced significantly, whereas for females no significant changes occurred. There were no main effects of zygosity or significant interactions between zygosity and sex, scalp locations, and test session.

Split Half Reliability and Temporal Stability

The second column of Table II reports the split-half reliabilities for the P300 amplitude measured at both test sessions (16y and 18y). The reliability of the P300 amplitude within a session was good. Averaged over test sessions and electrode locations, the mean split-half reliability coefficient was .72. Stability over time of the true part of the P300 amplitude, corrected for measurement error using the split-half reliability, was averaged over electrode locations .78.

Twin Correlations

The MZ and DZ twin correlations were also calculated for the reliable part of the P300 component. Table II depicts these twin correlations for P300 amplitude in response to targets. For the various scalp locations, the MZ correlations for the P300 amplitude ranged from .39 to .83. In general, these MZ correlations were higher for the P300 measured at posterior scalp locations (P3, P4, O1, and O2) than at frontal and central locations (Fz, C3, and C4). On the whole, MZ correlations seems not to differ between males and females. This is not the case for the pattern of DZ correlations of males and females. With the exception of central scalp locations, male DZ twin correlations were lower than MZ twin pairs for P300 amplitude measured at test sessions 1 and 2. This pattern of correlations suggests genetic influences on the P300 amplitude in response to targets for the posterior locations (P3, P4, O1, and O2). In contrast, the female DZ correlations were identical (posterior) or even higher (frontal, central) than the female MZ correlations. This suggest that the P300 in females may be influenced by shared environmental influences. Differences in genetic versus environmental determination of the P300 in males and females would predict correlations in the opposite twin pairs that are close to zero. Indeed, at both sessions low DOS correlations were found for targets.

Table I. Means of the P300 Amplitude at Test Session 1 (16 y) and Test Session 2 (18 y)

	Males				Females			
	16 y		18 y		16 y		18 y	
	M	SD	M	SD	M	SD	M	SD
Fz	11.04	4.92	10.70	4.91	10.98	5.15	11.71	4.89
C3	14.28	4.88	13.43	4.53	14.33	5.04	15.21	5.01
C4	14.06	4.96	13.72	4.39	14.81	5.24	15.23	5.04
P3	17.18	6.09	16.29	4.92	17.84	5.62	18.31	5.61
P4	17.57	5.97	16.04	5.09	18.40	5.83	18.52	5.48
O1	16.55	6.80	15.39	5.50	16.53	5.75	16.95	5.56
O2	16.07	6.59	14.91	5.49	16.35	5.71	16.88	5.52

M = mean; SD = Standard deviations.

Table II. Split-half Correlations, Test-Retest Correlations, and Twin Correlations for P300 Amplitude*

	r^2	rMZM	rDZM	rMZF	rDZF	rDOS
		n = 37/37	n = 35/29	n = 48/45	n = 36/32	n = 47/44
FZ						
16y	0.61	0.59	0.08	0.53	0.77	0.15
18y	0.70	0.56	0.53	0.54	0.39	0.01
16–18y	0.71	0.42	0.05	0.38	0.54	0.11
C3						
16y	0.67	0.46	0.50	0.39	0.60	0.21
18y	0.76	0.50	0.54	0.56	0.57	0.30
16–18y	0.77	0.36	0.39	0.30	0.46	0.16
C4						
16y	0.71	0.69	0.46	0.44	0.60	0.05
18y	0.72	0.69	0.51	0.67	0.62	0.13
16–18y	0.77	0.53	0.36	0.34	0.47	0.04
P3						
16y	0.71	0.71	0.23	0.50	0.61	−0.06
18y	0.75	0.45	0.32	0.67	065	0.02
16–18y	0.81	0.58	0.19	0.41	0.50	−0.05
P4						
16y	0.73	0.72	0.30	0.73	0.67	−0.13
18y	0.70	0.54	0.36	0.83	0.59	0.03
16–18y	0.80	0.58	0.24	0.59	0.54	−0.10
O1						
16y	0.78	0.70	0.25	0.76	0.66	−0.19
18y	0.76	0.44	0.25	0.67	0.57	0.06
16–18y	0.81	0.57	0.20	0.61	0.53	−0.15
O2						
16y	0.77	0.72	0.37	0.79	0.66	0.09
18y	0.74	0.50	0.08	0.73	0.66	0.14
16–18y	0.79	0.57	0.30	0.62	0.52	0.07

* The second column (r^2) reports the split-half correlation at ages 16 and 18 and the test-retest correlation (16–18 y). In the last 5 columns, the twin correlation and the cross-correlation (correlation between P300 amplitude of the eldest twin at age 16 with the youngest twin at age 18) are given. Above each column the number of twins at age 16 and 18 are given.

For each scalp location, the cross-correlations (i.e., the correlation between the oldest of the twin pair at test session 1 and youngest at session 2, or vice versa) are also given. The cross-correlation provides information about the nature of the stability of the P300 amplitude between test sessions 1 and 2. A higher MZ cross-correlation than DZ cross-correlation indicates that the stability is influenced by genetic factors, similar but nonzero cross-correlation indicates shared environment. Looking at the observed pattern of DZ and MZ correlations, the stability of the P300 amplitude for males seems to be determined by genetic factors, whereas for females shared environmental factors seem to be important.

Model-Fitting Results

Table III gives the log-likelihood results for the models that tested the significance of A and C for males and females. In order to get some indication of the fit of the models, we first tested a saturated model. In this model, all variances, covariances, and means in each zygosity group were estimated. The full ACE model (with sex differences) was compared with the saturated model. For half of the variables, the fit of the models was slightly worsened.

The significance of the A and C parameters was tested by constraining these parameters one for one and separately across sexes. For none of the scalp locations were the tests for A or C significant. However, there were clear differences between males and females. When A was dropped, the deterioration of the fit was larger for males than for females. When C was dropped, the opposite occurred—the fit worsened more for females than for males. When both A and C were dropped, the fit significantly worsened for both males and females. Thus, for most scalp locations, there is fa-

Table III. Results of Fitting Bivariate Genetic and Environmental Models to the Raw Data of P300 Amplitude at Ages 16 and 18. (The models tested the significance of genetic infuence [by dropping A], common environmental influence [by dropping C], and for the familiarity [by dropping A and C]. Marked values indicated significant deterioration of the submodel.)

Model	*df*	Δ*df*	Compared with model	Δ-2*log likelihood						
				FZ	**C3**	**C4**	**P3**	**P4**	**O1**	**02**
1. Saturated model	1347									
2. females $a_f c_f e_f$, males $a_m c_m e_m$	1505	158	1	211.0	160.8	181.2	180.2	216.1	248.7	216.1
Test for A										
3. females $c_f e_f$, males $a_m c_m e_m$	1508	3	2	1.37	.39	.25	.63	1.25	.46	.45
4. females $a_f c_f e_f$, males $c_m e_m$	1508	3	2	3.28	.74	4.63	7.64	5.72	.80	5.71
Tests for C										
5. females $a_f e_f$, males $a_m c_m e_m$	1508	3	2	4.61	3.46	3.36	7.11	5.65	5.51	5.42
6. females $a_f c_f e_f$, males $a_m e_m$	1508	3	2	1.95	.91	.12	.36	.00	6.40	.73
Test for familiarity										
7. females e_f, males e_m	1517	12	2	35.37	35.87	50.10	50.24	56.75	62.46	68.09
Final model										
8. females $c_f e_f$, males $a_m e_m$	1511	6	2	2.63	2.96	1.18	1.34	2.37	2.45	1.98

Note: a_f,c_f,e_f are loadings estimated for females, $a_m c_m e_e$ are loadings are estimated for males.
Critical value with 3 *df* = 7.81.
Critical value with 6 *df* = 12.59.
Critical value with 12 *df* = 21.03.
Critical value with 158 *df* = 188.33.

milial resemblance but it is not clear what causes this resemblance. Overall, the most parsimonious model, however, seems to be an AE model for males and a CE model for females. Indeed, when the ACE model was constrained to be equal across sexes, the goodness-of-fit was reduced. For this test, the critical value is 16.92 (with 9 *df*). For the various scalp locations, the χ^2 reduction ranged from 5.48 (C3) to 16.33 (P3) and most χ^2 reductions were nearly significant.

For the additional genetic analysis, an AE model for males and CE model for females was used as basis model. First, we tested whether the stability between 16 and 18 years was determined by the same genetic (for males) and shared environment (for females) factors. Therefore, the factor loading from the common genetic (shared environmental) factor to P300 at age 18 was constrained to be zero. This means that the genetic (shared environmental) variance at ages 16 and 18 would be explained each by a different genetic (or shared environmental) factor. As shown in Table IV, when this factor loading was constrained to be zero, the fit deteriorated for most of the scalp locations. The same applies to the common E factor. Thus, for both males and females the same genetic or environmental factors underlie the P300 amplitude at ages 16 and 18. Subsequently, we tested whether new genes or environmental influences emerged at age 18. This was done by constraining the specific A (C) or E factor to be zero (see also Fig. 1). New genetic influences appeared only for the right central scalp location (C4). New nonshared environmental influences appeared for the left occipital scalp location (O1). Parameter estimates and their 80% confidence intervals (CI) are provided in Table V. Although small differences existed between the various scalp locations, the overall pattern of the goodness-of-fit results suggest a "females CE, males AE" model for most scalp locations. In order to simplify the interpretation of the results, the parameter estimates were derived from the same "females CE, males AE" model. The confidence intervals were used to test whether the heritabilities/shared environmental influences differ at ages 16 and 18. In males, the contribution of genetic/environmental factors seemed to decrease at age 18 for the posterior scalp locations. In formal testing, the decrease of the heritability was significant only at O2. For females, the extent of shared environmental influences did not differ between the two test sessions. A large part of the covariance of the P300 amplitude measured at ages 16 and 18 was determined by the same genetic factors for males and by the same shared environmental factors for females.

DISCUSSION

This study examined the stability of the contribution of genetic and environmental effects to P300 amplitude in a large sample of adolescent twin pairs. In a

Table IV. Results of Fitting Bivariate Genetic and Environmental Models to the Raw Data of P300 Amplitude. (The models tested whether the same genetic and environmental factors underlie the individual differences in P300 amplitude at ages 16 and 18 years [tests for stability of A, C, and E] and whether new genetic influences emerged at age 18 [tests for new A, C, and E]. The fit of the models 2 through 9 were compared were compared with the fit of model 1 [with a difference of 1 *df*]. Marked values indicated significant deterioration of the submodel with more than 3.84, the critical value with 7 *df*.)

Model	*df*							
		FZ	**C3**	**C4**	**P3**	**P4**	**O1**	**O2**
					−2*log likelihood			
1. Females ce, males a'e'	1511	9093.03	8922.30	8937.58	9261.13	9316.60	9337.17	9455.71
					Δ −2*log likelihood			
Tests for stability A or C								
2. $C_m e_m$, $a_f e_f$, no common C females		13.04	16.21	15.31	21.75	25.90	37.38	35.34
3. $C_m e_m$, $a_f e_f$, no common A males		3.63	4.95	7.27	10.81	10.60	14.97	12.06
Tests for stability E								
4. $C_m e_m$, $a_f e_f$, no common E females		13.52	19.20	22.86	20.70	13.42	7.68	15.48
5. $C_m e_m$, $a_f e_f$, no common E males		3.37	9.04	6.74	8.34	2.65	7.71	5.23
Tests for new A or C at 18 y								
6. $C_m e_m$, $a_f e_f$, no specific C females		.98	.54	4.72	3.01	3.39	.01	2.22
7. $C_m e_m$, $a_f e_f$, no specific A males		.29	1.34	4.95	.07	.21	.01	.01
Tests for new E at 18 y								
8. $C_m e_m$, $a_f e_f$, no specific E females		.84	2.23	.00	.01	.00	6.68	.81
9. $C_m e_m$, $a_f e_f$, no specific E males		3.33	2.13	.14	1.09	2.52	2.81	1.10

Note: a_f,c_f,e_f are loadings estimated for females, $a_m c_m e_m$ are loadings estimated for males.

previous study that included the same 213 twin pairs (van Beijsterveldt *et al.*, 1998b), we found evidence for sex differences in the contribution of genetic and environmental effects to P300 amplitude. Therefore, the same twins were measured a second time and we tested whether we could replicate the sex-specific contribution to the variation of the P300 amplitude. In addition, the longitudinal design allowed us to study the stability of familial influences. MRI scans have shown that brain structure and functioning are characterized by changes that continue through the early 20s (Pfefferbaum *et al.*, 1994; Posner and Raichle, 1994). Therefore, it is possible that different genes or environmental factors influence the P300 amplitude at ages 16 and 18.

Model fitting results showed that familial resemblance was an important factor in determining the variation of the P300 amplitude at ages 16 and 18. The nature of the familial resemblance in combined data from ages 16 and 18 seems to be sex-specific. Whereas the male MZ and DZ correlations suggest genetic influence for P300 amplitude, the female MZ and DZ correlations suggest shared environment. Although the model fitting results indicate that we could not distinguish between A or C, the goodness-of-fit results are suggestive of sex differences in genetic architecture. Therefore, we decided to present the results of an AE model for males and CE model for females. In males, P300 amplitude showed average heritabilities of 62%. The heritabilities did not show large differences between ages 16 and 18. The shared environment influences for females were also substantial for each scalp location. The averaged amount of explained variance across the various scalp locations was 60%. These shared environmental effects also did not differ across ages. Thus, at both ages there appear remarkable sex-specific influences in the contribution of familial effects to P300 amplitude.

In view of previous studies, the absence of genetic influences on female P300 amplitude is a surprising finding. A number of small (Polich and Burns, 1987; Rogers and Deary, 1991) and larger (O'Connor *et al.*, 1994; van Baal *et al.*, 1998; Katsanis *et al.*, 1997) twin samples as well as the COGA family study (Almasy *et al.*, 1999; Almasy *et al.*, 2001) have yielded substantial evidence of P300 amplitude heritability (about 40%) in mixed adult samples of males and females. In principle, our sample of female DZ twins could be an odd nonrepresentative sample. This is unlikely because the many other neuroelectrical measures and IQ and behavioral data in the same sample of twin pairs did not indicate any sample abnormalities or yielded comparable sex differences (Rijsdijk and Boomsma, 1997; van Beijsterveldt *et al.*, 1996; van Beijsterveldt *et al.*, 1998a). In addition, the pattern of the female MZ and

Table V. Parameter Estimates (+80)(% CI) of the P300 Amplitude From the AE Model for Males and CE Model for Females*

		Males		Females	
		Additive genetic (80% CI)		Shared environment (80% CI)	
FZ	16	0.30	(0.03–0.61)	0.72	(0.53–0.87)
	18	0.62	(0.40–0.81)	0.41	(0.21–0.58)
	Cov	0.55	(0.20–0.86)	0.55	(0.37–0.71)
C3	16	0.42	(0.14–0.65)	0.45	(0.27–0.61)
	18	0.53	(0.31–0.71)	0.61	(0.46–0.73)
	Cov	0.48	(0.22–0.71)	0.57	(0.41–0.71)
C4	16	0.66	(0.39–0.87)	0.49	(0.33–0.63)
	18	0.72	(0.52–0.87)	0.68	(0.52–0.81)
	Cov	0.61	(0.36–0.81)	0.53	(0.38–0.66)
P3	16	0.70	(0.47–0.88)	0.51	(0.35–0.65)
	18	0.43	(0.21–0.62)	0.75	(0.61–0.85)
	Cov	0.63	(0.41–0.82)	0.59	(0.46–0.71)
P4	16	0.66	(0.42–0.85)	0.72	(0.59–0.82)
	18	0.57	(0.31–0.78)	0.70	(0.53–0.84)
	Cov	0.73	(0.48–0.95)	0.67	(0.54–0.78)
O1	16	0.79	(0.58–0.94)	0.62	(0.49–0.73)
	18	0.45	(0.21–0.66)	0.65	(0.50–0.79)
	Cov	0.68	(0.46–0.86)	0.79	(0.66–0.89)
O2	16	0.81	(0.63–0.95)	0.69	(0.57–0.79)
	18	0.36	(0.17–0.60)	0.69	(0.54–0.81)
	Cov	0.71	(0.48–0.88)	0.70	(0.58–0.80)

*Estimates are given for ages 16 and 18 years (=16 and 18) and for the covariance between 16 and 18 years (= Cov). CI = confidence interval.

DZ twin correlations for the P300 amplitude in response to non-targets suggests also genetic influences for females (van Beijsterveldt *et al.*, 1998b). Low statistical power has been cited as a possible cause of our findings (Almasy *et al.*, 1999), but our results at age 16 held up entirely at age 18. Also, the power to detect shared environment is in fact much lower than the power to detect additive genetic influences (Posthuma and Boomsma, 2000). Anokhin *et al.* (2001) evaluated these same P300 data in a bivariate genetic analysis with EEG power and also obtained significant evidence for sex differences. For males, the covariation between EEG power and P300 amplitude was mediated by common genetic factors; for females, this relationship was mediated by shared environmental influences.

A number of differences with the previous studies on P300 are evident that could explain the discrepant results. None used the specific and narrow age range of the present study. Van Baal *et al.* (1998) used very young children (ages 5 and 7 years), the mean age in the adult twins of O'Connor *et al.* (1994) was 30 years (range 22–46), and the participants in the COGA study (Almasy *et al.*, 1999; Almasy *et al.*, 2001), ascertained through alcoholic probands, had, by the nature of that study design, an even broader age range. Also, the effect of shared environment in the COGA study was tested as the correlation of the P300 amplitude of 46 spouses, which cannot detect a sex difference in environmental influences. The various studies used different P300 paradigms, e.g., we used a simple visual discrimination task but others used an auditory oddball task (O'Connor *et al.*, 1994) or a lexical decision task (Almasy *et al.*, 2001). Finally, only O'Connor *et al.* (1994) explicitly examined sex-specific influences in any detail; most other studies estimated heritability in samples pooled over males and females. When we calculated twin correlations pooled over both male and female twin pairs, we found MZ correlations that were higher than DZ correlations for P3 amplitude. This yielded estimates of additive genetic influences of about 60%. Interestingly, the heritability estimate in a sample of male twins only (Katsanis *et al.*, 1997) was 79%, i.e., almost double that of pooled samples (Almasy *et al.*, 2001; O'Connor *et al.*, 1994). Thus, pooling the data of males and females may

lower heritability estimates and blur sex differences in genetic architecture.

We have no ready explanation for the obtained shared environmental effects in adolescent females. Although difficult to interpret, they do appear to be stable determinants of the female P300, at least in the adolescent period. In the light of the present sex-specific influences, care should be taken in the use of the P300 as a biological marker of genetic susceptibility for psychopathology, at least in adolescent females. The P300 amplitude has been successful applied as a genetic marker of clinical disorders like schizophrenia and alcoholism. For instance, Williams *et al.* (1999) found evidence that the same quantitative-trait locus influences both risk of alcoholism and the P300 amplitude. Other putative loci influencing the variance in P300 amplitude have also been reported (Almasy *et al.*, 2001; Begleiter *et al.*, 1998). As long as it is unclear what causes the familial resemblance in adolescent females, these linkage results may pertain to males only. Put otherwise, linkage and association analyses of P300 amplitude may benefit from using males only.

A second question in this study concerned changes and stability of the familial influences on the P300 amplitude from age 16 to 18. Developmental decreases in the P300 amplitude during late adolescence have been interpreted as reflecting more efficiency in allocation of cognitive resources. In agreement with previous ERP studies (Johnstone *et al.*, 1996; Katsanis *et al.*, 1996), we found that the P300 amplitude diminishes with increasing age in males. No changes occurred for females, possibly implying a different rate of change in neuronal and/or cognitive processes. In both sexes, the degree to which familial factors influenced the P300 amplitude remained constant across the two ages. Only the heritability at the posterior (occipital) part of the head in response to targets decreased significantly. We also found little evidence for newly expressed familial factors at age 18.

In conclusion, the results indicated that the individual differences in P300 amplitude in adolescence are mainly influenced by familial factors. However, the causes of these familial factors seemed to be sex-specific. For males, genetic factors explained familial resemblance in P300 amplitude but for females, such resemblance was likely due to shared environmental factors. When measurement error was taken into account, the temporal stability of the P300 amplitude from age 16 to 18 was high in both sexes and could be largely attributed to the same familial factors. There was no evidence that new familial influences emerged at age 18.

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