A genetic perspective on the developing brain

Electrophysiological indices
of neural functioning
in five to seven year old twins

Caroline van Baal

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VRIJE UNIVERSITEIT

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Introduction

Introduction

The maturation of human brain structures forms the basis for the emergence of new brain functions, which in turn may influence more complex cognitive and behavioral development. Impressive cognitive and behavioral changes can be observed during middle childhood. Although the time course of these changes is about the same for all children, large interindividual differences can be observed in their onset and impact. These differences may be influenced by genetic and by environmental factors. A wealth of studies investigated genetic and environmental influences on human behavior (cf. Plomin et al., 1990; Plomin et al., 1994), adding to our knowledge of the nature of interindividual differences in cognitive abilities (including IQ and specific abilities (cf. Rose, 1995; Boomsma, 1993)), psychopathology (e.g., schizophrenia and affective disorders (Lander, 1988; Rutter, 1990; Vandenberg 1989)) and personality (for example neuroticism and sensation seeking (cf. Eaves et al., 1989; Koopmans et al., 1995)). In a large number of these phenotypes moderate to high genetic influences on interindividual differences were found. However, behavior itself is not inherited: what is inherited is DNA, encoding for proteins that are important for the development, maintenance, and regulation of the brain structures and functions. These ultimately produce behavior, in solid interaction with the environment. Knowledge about the etiology of individual differences in brain structure and function during development may therefore provide insight into the development of behavior and cognition.

To study the relative importance of genes and environment on interindividual differences in a trait, information from genetically related subjects is required. In the research project described in this thesis, 89 identical, monozygotic twin pairs (MZ), who are 100% genetically the same, and 120 fraternal, dizygotic twin pairs (DZ), who share an average of 50% of their genes were studied. This thesis presents results of a study on electrophysiological indices of neural functioning during childhood in these twins. Because children's brains show striking changes, especially during the period between about 5 and 7 years of age, the children were asked to participate twice, once when they were 5 years old, and a second time when they were about 7 years old. Using these longitudinal twin data, one has an opportunity to get insight into the genetics of electrophysiological brain activity at each age, and into changes in genetic influences during brain development.

In this introduction a brief overview on postnatal brain development is given. Next, the electrophysiological indices used in this study will be introduced, and the twin methodology will be explained in more detail.

Brain development

During childhood, a high degree of development is seen in a number of indices of neural functioning. In addition to the substantial prenatal development (e.g., most of the cerebral neurons evolve prenatally, cf. Nowakowski, 1987), some of the most profound changes are seen in early childhood. The largest increase in brain weight is seen before age 4 (Blinkov & Glezer, 1968). Myelination, which is responsible for faster and more efficient transport of information along the axonal cortico-cortical connections, is most important early in life, especially for sensory and motor systems, but continues until senescense for other connections (Yakovlev & Lecours, 1967). Cerebral metabolic rate (Chugani et al., 1987; Chugani, 1994) shows a rapid increase during the first postnatal years, and a decline to adult level during adolescence, indicating a higher level of glucose utilization during childhood. Synaptic density (Huttenlocher, 1979; 1990; 1994) roughly shows the same pattern as cerebral metabolic rate: a higher synaptic density is found in childhood than in adulthood. However, this does not necessarily imply that the same synapses continue to exist throughout childhood. Rather, synapses are believed to vanish and others to emerge again according to the demands of the environment (Changeux & Danchin, 1976; Changeux & Dehaene, 1989). This waxing and waning of synapses is believed to greatly improve functional connectivity in the brain, and may correlate with electrophysiological indices, as was suggested by Thatcher and colleagues (Thatcher, 1991; 1992; 1994a; 1994b; Thatcher et al., 1986; 1987). However, most of these indices of maturation cannot easily be assessed in vivo in humans, because they can only be assessed post-mortem, or are highly invasive. For example, because Positron Emission Tomography (PET) uses radio-actively labeled glucose, it is not feasible to use this technique in healthy children. Fortunately, a very extensive area of research exists on developmental aspects of electrophysiological indices of neural functioning. These indices are non-invasive, and therefore provide an important tool for studying children. To study the genetic and environmental influences on neural development during middle childhood, a number of these electrophysiological indices were assessed.

Electrophysiological indices of neural functioning

Electrical brain activity, was recorded for the first time by Berger (1929). The principle source of EEG activity is formed by the joined electrical activity of the excitatory postsynaptic potentials (EPSP) of the apical dendrites of the pyramidal cells in the cortex.

The summed activity of these EPSPs can be measured with scalp electrodes, and thus yields the electroencephalogram (EEG). In electrophysiological research, two major approaches can be distinguished: studies of ongoing, background EEG, and studies of time-locked electrophysiological responses to a stimulus, the event related potentials (ERPs). In this section the electrophysiological parameters used in this project, EEG power and EEG coherence from the background EEG, and P3 amplitude and P3 latency from the ERPs are outlined.

EEG power

EEG can be described as a summation of cyclic waves, each with its own wave-length, or frequency (in Hz). This complex, composite signal can be decomposed into different frequency components by way of a spectral analysis, or Fourier transformation. This results in a power spectrum, which is a description of the EEG signals in the frequency domain instead of the time domain. For every frequency component, the variance in the signal due to that component is obtained, and is expressed as power in the signal (in μV^2). EEG frequencies are commonly subdivided into a number of broad bands. The slowest waves are called delta waves, and range from 0.5 to 4 Hz. These waves are prominent during sleep, and are the main frequencies in infancy. Slightly faster waves, the theta waves (4-8 Hz) are important in young children, but then decrease with age. Alpha rhythm, ranging from 8 to 12 Hz and mostly found on posterior scalp locations, is the most obvious rhythm in the EEG of relaxed, awake adults. It is very high in amplitude and can easily be identified in the EEG of most individuals. In young children alpha rhythm is not very prominent, but with increasing age it becomes the leading rhythm. Beta activity, the fast activity in the EEG, ranges from 13 to 30 Hz, and is seen on frontal scalp locations in aroused, alert subjects. EEG power shows clear developmental trends (Anokhin et al., 1996) and therefore is often used as a maturational index. Matoušek and Petersen (1973) provided valuable EEG data of subjects aged 1 to 21 years. These data were later reanalyzed by others, including John (1977) and Hudspeth and Pribram (1992). The relative contribution of slow frequencies is high in infancy, and then decreases with age (e.g., Matoušek & Petersen, 1973; John et al., 1980; Benninger et al., 1984; Gasser et al., 1988). This is mainly caused by a decrease in low frequency power (delta, theta and alpha) with age. High frequency power (beta) remains the same throughout life. Although large developmental changes can be seen in human EEG activity, it appears to be a stable intraindividual characteristic (Salinski et al., 1991). Deviant patterns of EEG power have been related to neurological and behavioral problems, like dyslexia and hyperactivity (John, 1989), but also in normal children a

substantial interindividual variation is seen. Currently, no information on the etiology of these individual differences in large, normative samples is available.

EEG coherence

EEG coherence is the association between electrophysiological signals at different scalp locations and is suggested to be an index for cortico-cortical connectivity (Thatcher et al., 1986). Mathematically, it can be operationalized as the cross-correlation between two EEG-signals recorded at different scalp-locations. Changes in coherences with increasing age suggest that it may be considered as an index of functional brain maturation. In addition to continuous changes through childhood, discontinuous growth spurts were observed in EEG coherence, notably around six years of age. Thatcher and colleagues (1987) pointed out in a cross-sectional study of 577 children aged 2 months to 17 years, that periods of increase in coherence alternate with periods of decrease. They suggested that these changes can be viewed as a manifestation of changes in number and strength of axonal connections between the brain areas. Thatcher indicated that no answer can be given about the precise mechanisms which underly coherence, but that it could be caused by a change in synaptic density, by an increased myelination, by differences in neurotransmitters, or by other mechanisms (Thatcher, 1991; 1992; 1994a; 1994b). Differences in all these parameters can be the cause of interindividual differences in EEG coherence. Interindividual differences in EEG coherence have been shown to be related to mental retardation (Gasser et al., 1987) and schizophrenia (Hoffman, 1991). As for EEG power, substantial variation in EEG coherence is observed in healthy subjects.

P3 amplitude and latency

In addition to background EEG parameters we studied the P3 of the Event Related Potential (ERP). An ERP is a stimulus locked electrophysiological reaction to a certain event. Such a reaction is very small, and can not be discriminated directly from the ongoing background EEG. But since this reaction is time locked to the stimulus, we an ERP can be extracted from the background EEG by averaging the EEG time series of a large number of trials. Thus, an ERP is obtained by averaging a number of electrophysiological reactions to a stimulus. It consists of different components, which are associated with a particular stage in information processing. Early components (< 100 millisec), sometimes called exogenous, reflect structural characteristics of the processing system. Later components are sensitive to the type of processing and the way the subject is consciously processing, and are called endogenous. A prominent endogenous component is the P3, the third positive peak in the ERP. This is a positive deflection which occurs 300 ms or more after presentation of the stimulus. The P3 (or P300, or P3b) is suggested

to be an index of stimulus evaluation (Donchin et al., 1986). It was first discovered by Sutton and colleagues in 1965, and has been extensively studied since that time. P3 amplitude is influenced by the (subjective) probability of the stimulus and by task relevance, and is suggested to be a manifestation of a process related to the updating of models of environment or context in working memory (Donchin & Coles, 1988). P3 latency is an index of speed of stimulus evaluation (Donchin et al., 1986). During development, a decrease in P3 latency and P3 amplitude with age is found (Friedman, 1991; Courchesne, 1978, 1990). Therefore, these parameters have been suggested as indices of cognitive development.

Genetic perspective

Human brain development roughly follows the same pattern and time course in most individuals. However, substantial interindividual differences can be observed. It is highly likely that genetic influences on these interindividual differences in brain functioning will turn out to be important: approximately 30% of the entire genome is expressed in the brain (Sutcliffe and Milner, 1984; Sutcliffe, 1988; Adams et al., 1995). Behavior genetics investigates the nature of these interindividual differences. A major source is found in variation of the genetic make-up of the subject. When genes influence a trait, variation in these genes will consequently show up as variation in the observed phenotype. The expression of these genes can vary with age. Several types of control mechanisms can regulate human gene expression at specific developmental stages (cf. Strachan & Read, 1996). Another source of influences on interindividual differences is provided by the environment. In some periods of development, this may be the major source of influences, whereas in other periods genetic influences may be relatively more important. In the following paragraph the methodology for the decomposition of genetic and environmental variances is explained.

Quantitative genetics: the twin methodology

The decomposition of observed, phenotypic variance into genetic and environmental variances can be accomplished by using genetically related subjects, such as siblings or cousins, or using subjects that share the same environment, but not the same genes, such as adoptees. A widely used design is the twin design, in which the resemblance between identical, monozygotic twins (MZ) is compared to the resemblance between fraternal,

dizygotic twins (DZ). Studying twins has two major advantages: first, twins are siblings of the same age. Therefore possible age differences in gene expression will not confound the results. Second, MZ twins are subjects who at birth received the same genetic material and for whom the most important source of differences occur as a consequence of environmental influences.

A first way to get an impression of the importance of genetic or common environmental influences is to compare the correlations between MZ and DZ twins. A more sophisticated way is structural equation modeling. The last method has the advantage of being able to test for significance of factors that influence the trait, and of establishing genetic and environmental correlations between different traits. In the next sections, first some formulas are explained that describe the variance and covariance components for traits in MZ twin pairs and in DZ twin pairs. Next, the comparisons between MZ and DZ twin correlations are described. Univariate model fitting is explained, and in the last sections an outline of modeling reliability and development is given.

A phenotype (the observed trait) can partly be a result of genetic factors, and partly of environmental factors: $P = G + E$, where P is the phenotype (the observed trait), G is the genotype, and E is the environment. Sometimes the genetic influences are a resultant of 1 gene and are called monogenic traits. Gregor Mendel postulated a theory of the way genes segregate and are expressed in the phenotype. Examples of monogenic traits are color blindness, PKU or hemophilia. Some rare EEG variants seem to show monogenic inheritance: e.g., autosomal dominance for low-voltage EEG (Anokhin et al., 1992). More EEG variants are reviewed by Vogel (1970). Genetic influences on most kinds of behavior cannot be explained by a monogenic model, but are polygenic in nature. Fisher (1918) first suggested that the mechanism of monogenic inheritance could be extended to polygenic inheritance. He indicated that each gene in the polygenic model segregates according to Mendelian rules, and that the small effects of these polygenes could be summed to form the total genetic effect. When a gene only has one allele, then all humans have the same genotype on that locus, and genetic variability in the population due to that gene will be zero. However, when more alleles exist, genetic variability due to that gene emerges. For behavior probably a large number of polymorphic genes exist. These would all express a small effect, which results in a continuous trait that is normally distributed in a population. Environmental influences can also be different for individuals, and this causes environmental variability. Therefore, phenotypic variance can be decomposed into variance due to genetic factors and to environmental factors. Three sorts of genetic influences can be distinguished: additive, dominant and epistatic genetic variances. Additive genetic effects sum the effects of all alleles at all loci that influence a trait, whereas dominant genetic effects stem from the summation of the interactions between

two alleles at the same locus. Epistatic variance reflects the interactions between alleles at different loci. Epistatic variance will usually be confounded with dominance (Neale & Cardon, 1992), and it will not be considered here. Two sources of environmental variance are usually distinguished: common environmental variance and unique environmental variance. Common environmental variance is due to a shared environment within a family, like the same food habits or social economic status (SES). Unique environmental variance results from influences that are unique to a person, and often also includes measurement error (Plomin et al., 1990; Neale & Cardon, 1992). The total variance of a trait thus may be decomposed as follows:

$$Vp = Va + Vd + Vc + Ve,$$

where Vp = phenotypic variance,
Va = additive genetic variance,
Vd = dominant genetic variance,
Vc = common environmental variance,
Ve = unique environmental variance.

To decompose the observed, phenotypic variance into these components, data of genetically related subjects are needed. In this study data of monozygotic (MZ) and dizygotic (DZ) twin pairs reared together were analyzed. Because MZ twins share all their genes, and are raised in the same family, the covariance between MZ twins is composed of all genetic and common environmental variance: $Cov(MZ) = Va + Vd + Vc$. DZ twins also are raised in the same family, and therefore will share all common environmental variance. However, DZ cotwins share only half their genes on average, meaning that only half the additive genetic variance contributes to the dizygotic covariance, and a quarter of the dominant genetic variance (Neale & Cardon, 1992):
$Cov(DZ) = .5 \times Va + .25 \times Vd + Vc$.

The differences between MZ and DZ covariances (or MZ and DZ correlations) thus give information about sources of variation. Note that it is not possible to estimate both dominant genetic and common environmental variance when using a design which only includes twins reared together. Dominant genetic variance will increase differences between MZ and DZ covariances, whereas common environmental variance will decrease the difference. A third group would be necessary with a different composition of the covariance, for example monozygotic twins reared apart, whose covariance exists of additive and dominant genetic variance, but does not include common environmental variance. These data are not available in our design, and only models including Va and Vd, or Va and Vc (and Ve) were considered.

When genetic variance attributes to total variance, the standardized covariances (i.e., correlations), will also be higher in MZ twins than in DZ twins. A number of outcomes can be conceived on theoretical grounds, which will point to different factors contributing to the observed variance. If both MZ and DZ correlations are not significantly different from zero, then only unique environment influences the trait (E model). If both MZ and DZ correlations are different from zero, but not different from each other, then common environmental influences are present (CE model). If the MZ correlation is twice the DZ correlation, then additive genetic influences are of importance (AE model). If the MZ correlation is larger than twice the DZ correlation, then dominant genetic effects will also influence the trait (ADE model).

Using this logic, heritability (h^2) can be calculated as 2(rMZ - rDZ), and common environmentability (c^2) as 2rDZ - rMZ, where rMZ is monozygotic twin correlation, and rDZ is dizygotic twin correlation (cf. Falconer, 1989). This methodology is often employed in older studies. However, only examining twin correlations (or correlations between other relatives, for example fathers with sons) has several disadvantages. First, not all available information is used. For example, when heritability becomes higher with age, it would be interesting to know if genetic variance increases or environmental variance decreases. Correlations do not give this information. Second, the heritabilities calculated from the twin correlations are a descriptive statistic. Using this method we cannot test whether the genetic or environmental variances are significantly different from zero. Newly developed statistical techniques should be used to test the estimates of the genetic influences on electrophysiological indices (Boomsma & Gabrielli, 1985).

Univariate model fitting analysis

A model according to the formulas of variance and twin covariances given above can be fitted to the data using structural equation modeling. A number of models can then be tested by comparing their goodness-of-fit, namely ADE, ACE, AE, CE and E models, in which A refers to genetic variance, D to dominant genetic variance, C to common environmental variance, and E to unique environmental variance. These models can be tested with or without constraining parameters to be equal in males and females, thus testing for sex differences in the relative influences of genetic and environmental factors. Using these models, the following estimates can be calculated:

(narrow) heritability	(h^2) = Va / Vp
dominant heritability	(d^2) = Vd / Vp
common environmentability	(c^2) = Vc / Vp
unique environmentability	(e^2) = Ve / Vp

where Vp = Va + Vd + Vc + Ve

Modeling reliability

In this thesis, in addition to the univariate models described in the last paragraph, a more extended model is used. This model was developed to obtain heritabilities for the reliable part of the variance in electrophysiological indices. When measuring these indices, the possibility of measurement error has to be taken into account (particularly in children). Measurement error will show up as unique environmental variance, and as such, it will increase total variance. Since heritability expresses the proportion of genetic variance over total variance, a large proportion of measurement error will lower h^2. Preprocessing of electrophysiological data always involves some kind of averaging over trials before the observed phenotype is obtained. To decompose the observed variance into reliable variance and variance due to measurement error, we obtained two estimates of the trait were obtained by separately averaging over all odd, and over all even trials or epochs. These two phenotypes should ideally be exactly alike, but if not, reflect the measurement error of that phenotype. The covariance between these two phenotypes reflects the reliable variance of the true underlying phenotype, and the variable-specific variance reflects variance due to measurement error.

Modeling development

The children in this study were measured twice with a 1.5 years' interval. In this period, relative influences of genetic and environmental factors may stay the same, or may change. Two questions are investigated:

1. do new genetic and environmental factors emerge at age 7?
2. is the correlation across time between the (reliable) phenotypes genetic or environmental of origin?

To answer these questions, a bivariate model was used. For each source of variation (G and E) two factors were specified: one factor that influences the true phenotype at age 5 and at age 7 and a new factor that influences the true phenotype at age 7. The first factor is common to both ages and causes covariance between true phenotype measured at ages 5 and 7. In this way, the true phenotype covariance (or stability) is decomposed into a genetic and an environmental part. By constraining the factor loadings of the true phenotype at age 7 on the first set of factors to be zero, the hypothesis is tested that all genetic (or environmental) variance at age 7 is due to new genetic (or environmental) influences being expressed at that age. By constraining the factor loadings of the true phenotype at age 7 on the second set of factors to be zero, the hypothesis is tested that no new influences are expressed at age 7.

Combining the models

The various elements of data analysis that were introduced above, that is, the twin model, the bivariate model and the reliability model can be combined into one structural model. Figure 1.1 shows a path diagram of the developmental structural model that was fitted to the P3 and coherence data reported in this thesis.

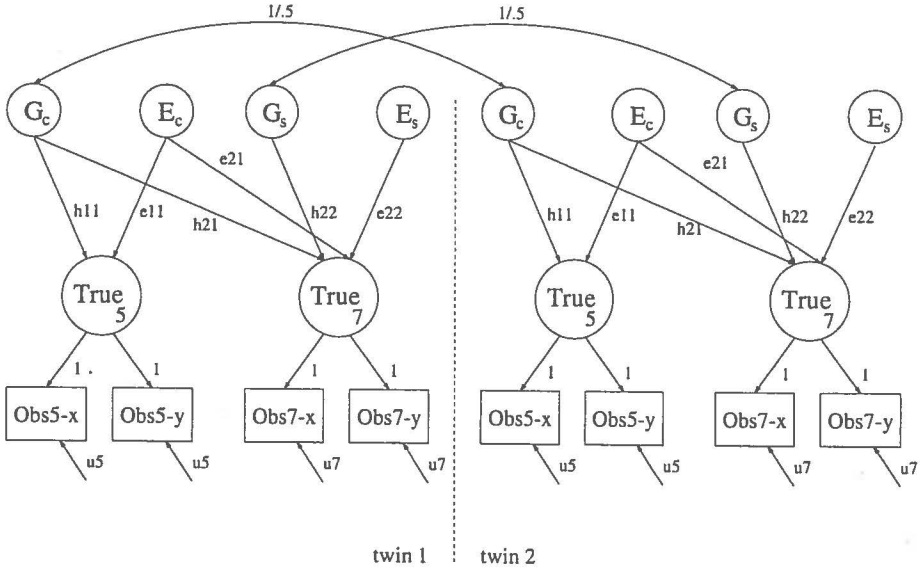

Figure 1.1 *Path model of a multivariate genetic model. For each twin, four observed phenotypes are shown (rectangulars): two phenotypes at age 5 years (Obs5-x and Obs5-y) and two phenotypes at age 7 years (Obs7-x and Obs7-y). These observed phenotypes are influenced by the true underlying phenotype at that age (True$_5$ and True$_7$) and by measurement error (u_5 and u_7). The True phenotypes are influenced by genetic and by environmental factors. One set of factors is common to both ages (G_c and E_c), accounting for the covariance between the phenotypes. The other set of factors is specific for age 7 years (G_s and E_s). Correlations between the genetic factors of twin 1 and twin 2 is 1 for monozygotic twins, and .5 for dizygotic twins.*

Figure 1.1 shows that the observed phenotype (rectangulars) can consist of measurement error and a true part. The true part is composed of a genetic part and a environmental part: P = True + Error = G + E + Error. Thus, the phenotypic variance can be written as the sum of true, reliable variance and variance due to measurement error:

$$Vp = V_{True} + V_U = V_G + V_E + V_U.$$

For this model for age 5 and age 7 heritability was calculated as the percentage of additive genetic variance of total *reliable* variance. Likewise, it was calculated which percentage of the observed covariance between ages 5 and 7 was due to genetic factors. In addition, an estimate was made of the amount of genetic variance that was shared at both measurement occasions. The genetic correlation, that is, genetic covariance divided by the square root of genetic variance at age 5 times the square root of genetic variance at age 7, provides an estimate to which extent the same or other genes influence the trait at ages 5 and 7 years. A genetic correlation of 1 indicates that no new genetic factors emerge at age 7. A genetic correlation of 0 indicates that no common genetic factor exists for the trait at ages 5 and 7. The same estimate was obtained for environmental correlations.

Genetics of electrophysiological indices

The genetic and environmental influences on development of electrophysiological indices of brain functioning in middle childhood have hardly ever been studied. On the whole, most research on genetic and environmental influences on electrophysiological indices of brain functioning used rather simple ways of establishing heritabilities. In addition, very few studies concentrated on children, and on changes in genetic and environmental influences. An excellent review on the genetics of the EEG and ERPs is given by Van Beijsterveldt and Boomsma (1994). In this section, the most important conclusions for the indices that are analyzed in this thesis, are discussed. A number of studies investigated the origin of interindividual differences in EEG power in adults (e.g., Christian et al., 1996; cf. Van Beijsterveldt & Boomsma, 1994). The overall consensus, both from older studies which used visual inspection, and from later spectral analysis studies, was that EEG broad bands, especially alpha parameters, are to a large degree influenced by genetic factors. In a number of studies children were included, but only a few twin pairs were studied (e.g., Whitton et al., 1984; Zung & Wilson, 1984), or conclusions were based on visual inspection of the EEG (e.g., Lennox et al., 1945). Vogel (1958) included young twins (from age 6 onwards) in his study of 110 MZ and 98 DZ twin pairs (although only 10 twin pairs younger than 9 years). He found that for every age, MZ twins were completely concordant.

To our knowledge, only two studies about genetic and environmental influences on coherence have been conducted. The first, done in our own laboratory (Van Beijsterveldt, 1996), is identical to the study reported in this thesis, with 213 adolescent twin pairs measured at ages 15 and 17, instead of young children. Results of this study showed no

sex differences in genetic and environmental influences on coherence. Relative influences of genetic factors on coherence were about 60%. Adolescence is a period in which maturation of the brain seems to be finished for the largest part, except within the frontal area. Heritabilities of young children may be different, because maturation is not yet complete. The second study reported in the literature (Ibatoullina et al., 1994) investigated genetic and environmental influences on EEG coherence in twins aged 5 years. Coherence in 20 MZ and 17 DZ twin pairs was studied. Familial resemblance was found, which could either be attributed to genetic or to common environmental influences. However, the assessment of coherence in this study may have been unreliable, therefore these results should be viewed with some caution.

Genetic studies of P3 mostly applied an auditory oddball paradigm. The oddball task is a task especially suited to evoke a P3 reaction, and consists of frequent background stimuli (non-targets) and infrequent important stimuli (targets) for which some sort of reaction is required (i.e., counting, pressing a button). Surwillo (1980) analyzed data from 6 MZ twin pairs and 6 unrelated children, aged 9 to 13 years. He found higher resemblance for P3 latency in MZ than in unrelated children. This could imply that for that age speed of stimulus evaluation is influenced by genetic factors. Three other studies (Polich & Burns, 1987; Rogers & Deary, 1991; O'Connor et al., 1994) studied P3 amplitudes and latencies in adult subjects. All three studies found evidence for genetic influences on amplitude, but O'Connor and colleagues did not find evidence for genetic influences on P3 latency, in contrast to the two other studies. P3 amplitude and latency was also assessed in a visual oddball task in the Van Beijsterveldt study in adolescent twins (1996). This study found familial influences on amplitudes, but not on latencies. Surwillo studied P3 latency only in a very small number of children, whose age was slightly higher than in our study (9 to 13 years). In conclusion, no reliable estimation has been made of the genetic and environmental influences on P3 amplitude and latency in children.

Focus of research and outline

Studies in adolescents and adults thus have convincingly demonstrated the importance of genetic influences on individual differences in background EEG and to a lesser extent in ERPs. In this era of molecular genetics this result does not any longer come as a surprise: the list of candidate genes that can in any way alter brain function is formidable (Goldman, 1996). In adults the first associations between behavior and genetic polymorphisms that influence neurotransmitter systems have already been reported.

The first theme to be addressed in this thesis is whether genetic influences on brain function in young children are as high as in adolescents and adults, or whether in children environment plays a more important role during brain maturation. Plomin and DeFries (1985) have suggested that slight differences in neural function early in life can become amplified later on, and lead to increasing genetic influences on behavior. An important problem however, that may seriously bias conclusions on the influence of heritability in children is the reliability of measures, a problem that was already noticed also by Van Beijsterveldt and Boomsma (1994), particularly with respect to ERP in adults. Therefore, in this thesis a model is developed to analyze the reliable EEG coherence and ERP. The second theme in this thesis is whether the relative importance of genetic and environmental influences change from ages 5 to 7 years, when children start attending formal education, and large qualitative cognitive changes can be observed.

To study the genetic architecture of electrophysiological indices in children, electrophysiological data were collected in 209 twin pairs, recruited from the Netherlands Twin Register (NTR). Children visited the laboratory twice with a 1.5 years' interval, at about ages 5 and 7 years. The children's EEG was recorded on 14 scalp locations during a visual oddball task and during a rest condition (first part with eyes open, last part with eyes closed). Heart rate and respiration rate were measured during the whole EEG session. Height, weight, head circumference, IQ and conservation ability (measured with a liquid quantity task) were also assessed. Zygosity was determined by analysis of DNA or blood, and in a few cases by physical resemblance questionnaires. The EEG was used to assess a number of electrophysiological indices: EEG power, EEG coherence and Event Related Potentials (ERPs). This study has produced a vast amount of data. A selection of the results is presented in the following chapters. Chapter 2 concerns univariate genetic analyses of 6 EEG power broad bands on 14 electrode positions during the first measurement occasion. Chapter 3 continues with a multivariate analysis of P3 amplitude and latency. In the model used in this chapter, the observed variance is decomposed into a reliable part and a part due to measurement error. Genetic stability in the period of 5 to 7 years of age is tested. Chapter 4 presents univariate analyses of EEG coherences in the same twins at age 5 years. Chapter 5 then analyses EEG coherences for first and second measurement occasion, using the multivariate model which controls for measurement error. Chapter 6 provides a general discussion and conclusions.

2

Genetic architecture of EEG power spectra in early life

G.C.M. van Baal, E.J.C. de Geus and D.I. Boomsma

We measured the electroencephalogram (EEG) in 209 five year old monozygotic (MZ) and dizygotic (DZ) twin pairs to estimate the relative contribution of genetic and environmental factors to EEG power spectra in early life. Data from same-sex and from opposite-sex twin pairs were used to test for sex differences in genetic influences. Results showed high concordance for EEGs of MZ twins for absolute and relative power in delta, theta, alpha1, alpha2, beta1 and beta2 bands. A model with additive genetic and unique environmental influences explained individual differences in both absolute and relative power in almost all bands and all electrode positions. Heritability of EEG power spectra was high. For absolute power the highest heritabilities were observed in theta, alpha1, alpha2 and beta1 power bands (mean heritability = 81, 81, 78, and 73%). Somewhat lower heritabilities were found in delta and beta2 bands (mean heritability = 55 and 64%). For relative power heritabilities were 63, 76, 71, 72, 68, and 65 for delta, theta, alpha1, alpha2, beta1, and beta2 respectively. Virtually no sex differences in heritability were found. These findings indicate that the background EEG is one of the most heritable characteristics in early life.

Introduction

Most of the salient changes in brain anatomy take place in the first four years of life, for example growth in brain weight (Blinkov & Glezer, 1968), increased myelination (Yakovlev & Lecours, 1967), glucose utilization (Chugani et al., 1987), and synaptic growth (Huttenlocher, 1979). Many major developmental changes in behavior occur in the period of 4 to 6 years (Thatcher et al., 1987). It has been suggested that these changes coincide with anatomical changes that are less "massive" than those during the first years, but greatly improve functional connectivity of the various parts of the brain. Among these are selective death of irrelevant synapses and strengthening of the relevant ones (Goldman-Rakic, 1987; Nowakowski, 1987) and myelination of fibers in integrative cortical interhemispheric and intrahemispheric connections (Courchesne, 1990). EEG activity, has been proposed as a non-invasive index of these developmental changes. This is a study of the relative importance of genetic and environmental influences on the electrical brain activity in a group of five year old children.

Spectral analysis (Fourier analysis) can be used to determine the amount of variance in the EEG signal explained by cyclic components with a certain frequency ("power",

in Hertz (Hz)). Several studies have shown strong developmental changes in EEG power (Matoušek & Petersén, 1973; Chavance & Samson-Dollfus, 1978; Samson-Dollfus & Goldberg, 1979; John et al., 1980; Matthis et al., 1980; Katada et al., 1981; Benninger et al., 1984; Gasser et al., 1988). These studies all show the same basic tenet: slow activity is dominant in early life, but is substituted by faster activity with increasing age. As the child gets older, total power in the low frequency bands (delta, theta and alpha) decreases, whereas total power in the higher (beta) bands stays constant. Various studies suggest that, in addition to gradual changes, discrete growth spurts in EEG spectra may be found (Thatcher et al., 1987) that may be linked to stage transitions in cognitive development (Hudspeth & Pribram, 1990; Thatcher, 1994a; 1994b). In spite of these overall trends, individual differences in EEG power in children of the same age are striking (Benninger et al., 1984). These individual differences are increasingly being used to diagnose deviant brain development, such as that related to neurological and behavioral problems like dyslexia and hyperactivity (John, 1989). The determinants of such individual differences in children's EEG are largely unknown. It is likely that genetic factors play a major role. Evidence suggests that approximately 30% of the entire genome is expressed only in the brain (Sutcliffe & Milner, 1984), which is much more than in any other tissue.

In adults, a number of studies of genetic and environmental influences on EEG parameters have been conducted (for a review, see Van Beijsterveldt & Boomsma, 1994). Most of these studies used the twin method to estimate the relative influences of genes (heritability) and environment on individual differences in the EEG. The twin method is based on the fact that identical or monozygotic (MZ) twins share all their genes, whereas fraternal or dizygotic (DZ) twins only share 50% of their genetic material on average. Heritability (h^2) can be estimated as twice the difference between the MZ correlation and the DZ correlation: $h^2 = 2(rMZ - rDZ)$, or by model fitting techniques (Boomsma & Gabrielli, 1985). In most earlier studies only small samples were available, and no formal model fitting methods were used to estimate genetic and environmental influences. Sex differences in genetic and environmental influences on EEG were ignored, and although EEG changes significantly with age, most studies used wide age ranges with virtually no children. In spite of the methodological pitfalls, all adult studies have reported the robust finding of high to very high genetic contribution to individual differences in the EEG power spectra of adults.

We cannot assume that the genetic architecture of CNS functioning is the same in infants, older children and adults. Particularly in children, it is possible that there may be periods during development in which brain functioning is more sensitive to environmental influences than to genetic factors and vice versa. An example of changing

heritability with age is provided by intelligence: heritability of IQ increases from about 15% at infancy to about 50% at older ages (Boomsma, 1993). A few of the EEG studies in twins included in addition to adults some children (Vogel, 1958; Dumermuth, 1968; Whitton et al., 1985). However, the age ranges in these studies were too large, or the number of subjects too small to draw conclusions about the differential heritability of children and adult EEGs.

In this study we specifically address the genetic and environmental sources of individual differences in the EEG power spectrum of 5 year old children, in a period of increased cortico-cortical connectivity. The sample included male and female same-sex MZ and DZ twins to test for sex differences in heritabilities, as well as twins of opposite-sex to look at the question whether the same genetic factor is expressed in males and females.

Methods

Subjects

This study is part of a larger project in which genetic and environmental influences on neural development in early life are studied longitudinally. Two hundred and nine healthy Dutch 5 year old twin pairs (mean age = 5.26 years, sd = .19 years) participated. All subjects had normal or corrected to normal vision. Addresses of the twin pairs were obtained from the Netherlands Twin Register, which registers between 45 and 50% of all Dutch twins born after 1986 (Boomsma et al., 1992). For 103 same-sex twins zygosity was determined by blood typing (ABO, MNS, Rhesus, Kell, Duffy, Kidd, Lutheran). Parents of all same-sex twin pairs (N = 170) completed a zygosity questionnaire (Goldsmith, 1991), and discriminant analysis was used to determine zygosity of the twins for whom no blood typing was available. All multiple choice questions (19) entered the analysis, but only 4 questions (hair color, hair structure, confusion by acquaintances, confusion by close friends of the family) remained which discriminated best between MZ and DZ twins. In the group of 103 same-sex twins already classified by blood typing, 95% was classified correctly; no actual MZ twins were classified DZ, and 5 DZ twins were classified as MZ.

Eighteen twin pairs had incomplete EEG data because of difficulties during the experiment. Children who fell asleep during the experiment (11), and children who showed high level of arousal or cried (13) were also excluded from further analyses. This left 167 twin pairs (34 monozygotic males (MZM), 33 dizygotic males (DZM), 37 monozygotic females (MZF), 32 dizygotic females (DZF), 31 dizygotic opposite-sex twins (DOS)) with complete data.

Procedure

Twins were measured on the same time of day (morning or afternoon). After arrival in the laboratory the protocol was explained to the twins and their parents. Height, weight and head circumference were measured. One of the twins started with the EEG/ERP experiment, the other twin was given an IQ test (Boomsma and Van Baal, in preparation). While the child watched a video, an electrocap with electrodes in the 10-20 system of Jasper (1958) was attached. Electrode impedance was kept below 10 Kohm. Four tin electrodes for eye movement recordings, two ear electrodes as references and one tin electrode for an electrocardiogram were also attached. Testing took place in a dimly lit, electrically shielded, sound attenuated cabin with intercom facilities. Subjects lay on a bed with a black and white 25 × 30 cm monitor about 50 cm above their heads. One parent was allowed to stay with the child. The experimental conditions consisted of an auditory habituation task, a visual oddball task and six minutes of quiet rest (3 minutes eyes open, 3 minutes eyes closed). In this paper data of the background EEG measured during quiet rest with eyes closed are presented.

Apparatus

EEG was recorded continuously on an 18 channel Nihon Kohden PV-441A polygraph. Time constants were set to 5 s, and high frequency cut-off was 35 Hz and sample frequency was 250 Hz. Signals were converted with a 12 bits AD converter, and sent to an Olivetti M28 PC for offline processing.

Data quantification and data reduction

EEG was measured at the following scalp locations: prefrontal (Fp1, Fp2), frontal (F7, F3, F4, F8), central (C3, C4), parietal (P3, P4), occipital (O1, O2) and temporal (T5, T6). Linked earlobes were used as references according to the method described by Pivik and colleagues (1993). Briefly, we used two separate preamplifiers with high input impedance for each of the reference electrodes and linked the output electrically. Calibration showed highly comparable gain and accuracy range of the two preamplifiers. With the ears linked this way the effects of possible imbalances in electrode impedance introduced by the electrical double layers were prevented. Vertical eye movements were measured at infra and supra orbital sites in line with the pupil of the left eye (VEOG), and horizontal eye movements at the outer canthuses (HEOG).

Single trial EOG artifacts were removed using dynamic regression in the frequency domain (Brillinger, 1975), and the EEG signal was divided into 90 2-second epochs. Epochs with clippings were automatically excluded from further analysis. During visual inspection, epochs with abnormal EEG patterns (like ECG artifacts, movement artifacts)

were noted in the protocol, and removed from the analyses. For every epoch and for every scalp location the raw EEG was then converted from the time domain into the frequency domain using Fast Fourier Transformation (FFT). This procedure yields a power spectrum for every individual, which indicates for every cyclic component how much variance in the raw signal is accounted for by this component. The power spectra, ranging from .5 to 30 Hz, with a resolution of .5 Hz, were averaged over all epochs without artifacts (never less than 20) to obtain the average power spectrum for all 14 electrode positions. From these power spectra both absolute and relative power of more traditional broad bands per scalp location were calculated: absolute power is the sum of power in a certain frequency range, relative power is the absolute power in that band divided by the sum of all power bands in the EEG. We defined six different bands: delta is the sum of power in the frequency bands ranging from 1.5 to 3.5 Hz, theta ranged from 4.0 to 7.5, alpha1 from 8.0 to 9.5, alpha2 from 10.0 to 12.5, beta1 from 13.0 to 17.5 and beta2 from 18.0 to 25.0. To obtain a normal distribution of the data 10-log transformations were conducted on absolute values, and logit transformation (i.e., $^{10}\log(x/1-x)$ for relative powers.

Statistical analysis

In a simple oneway analysis of variance we first tested for birth order effects, that is, differences between first born and second born twins, in mean values of absolute and relative delta, theta, alpha1, alpha2, beta1 and beta2 power. We then tested for differences in EEG powers between zygosities (MZ and DZ), sexes (male and female), and electrode positions (Fp1, Fp2, F7, F3, F4, F8, C3, C4, T5, P3, P4, T6, O1 and O2) using multivariate analyses of variance (Manova, SPSSPC). Analyses were conducted separately for twin A (first born child) and twin B (second born child) of the twin pair, because due to their genetic relatedness their data are not independent. Testing for differences between zygosities is essential because an assumption of the twin design is that monozygotic and dizygotic twins all stem from the same population, and therefore will show no differences in means and variances.

Secondly, to obtain an indication of measurement error, we computed split-half reliabilities using power spectra averaged over all odd, and over all even 2-sec epochs from the total EEG registrations, for all leads and frequency bands separately.

The final class of analyses decomposed the observed variance in the EEG power into genetic and environmental factors, using structural equation modeling with the computer program Mx (Neale, 1994). With structural equation modeling, a model is constructed which specifies causal relationships between the observed phenotype, in our case the EEG variables, and the unobserved genetic and environmental factors influencing the

phenotype. Genetic factors are the sum of small effects of many genes. Two sort of effects are possible for each of these genes, additive genetic effects in which the effects of the paternal and maternal alleles are added up, and dominance effects, in which the paternal or maternal allele is dominant for some of the genes. In the environmental factors, a distinction can be made between common environmental influences and unique environmental influences. Common environmental influences are shared by relatives growing up in the same family, for instance food habits, or going to the same school. Non-shared, or unique environmental influences cause differences between cotwins even if they have the same genotype and live in the same family. These include measurement errors (Plomin et al., 1990; Neale & Cardon, 1992).

The basis for structural modeling in twin studies is the different level of genetic relatedness in monozygotic (MZ) and dizygotic twins (DZ) in combination with a similar common environment. Figure 2.1 shows a path diagram of a structural model in which the observed phenotype (P: measured in twin 1 and twin 2) is influenced by an additive genetic factor (A), a shared common environmental factor (C) and a non shared unique environmental factor (E): $P = A + C + E$.

Since MZ twins share all their genes and DZ twins share 50% on average, the correlation between additive genetic factors (A) is set to 1 for MZ twins, and to .5 for DZ twins. The common environmental correlation is 1 in both MZ and DZ twins. Correlations for the non-shared, unique environmental influences (E) are set to 0 for both types of twins. Dominance genetic effects, were not included in the model, because twin correlations did not give an indication for such effects. It is only of importance if DZ correlations are close to 25% of the MZ correlations. Furthermore, dominance for poly-genetic traits is difficult to estimate in a twin model.

The model outlined in Figure 2.1 will be tested against the observed EEG data. The aim is to find the most parsimonious model which still adequately describes the data. Estimates of the genetic, common environmental and unique environmental factors are obtained from the observed variance. These are the parameters a, c and e respectively.

In Figure 2.1, we have not accounted for possible sex differences in the relative influence of genetic and environmental effects. It is possible to account for such differences, particularly when DZ opposite sex twins are available. Sex differences can be estimated in three different models (Neale & Cardon, 1992):

1. Scalar effects sex-limitation models, in which a difference in total variance between males and females is allowed, but in which the relative contributions of A, C and E are the same for males and females;

2. Common effects sex-limitation models. In these models the relative magnitude of genetic and environmental factors can differ between the sexes, but the same genes and/or common environmental influences are expressed;
3. General sex-limitation models. These test the possibility that sex specific genes exist, which influence the trait in one sex but not in the other. For these models the DOS twins are essential.

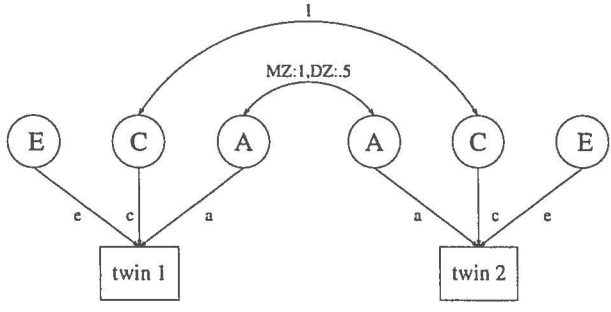

Figure 2.1 *Path diagram of a univariate twin model. Phenotypes of both twins (twin 1 and twin 2) are influenced by additive genetic factors (A), common environmental factors (C), and unique environmental factors (E). Correlation between* C_{twin1} *and* C_{twin2} *is 1, correlation between* A_{twin1} *and* A_{twin2} *is 1 for MZ twins and .5 for DZ twins.*

When the same genes explain part of the variance in both males and females, but their relative contributions differ, then the additive genetic correlation in DOS pairs is .5. When different genes account for the phenotypic variance the additive genetic correlation in DOS pairs will be smaller than .5, or even zero, and the observed correlation in DOS pairs will be smaller than in same-sex pairs.

Data on male and female same-sex MZ and DZ twins and on DZ opposite-sex twins were summarized into five 2 by 2 variance-covariance matrices. The diagonal elements of the matrices give the variances of the phenotypes for first and second born twins, the covariance between twins is given in the off-diagonal elements. The models outlined above were fitted to these matrices, such that the most parsimonious model remained. The most parsimonious model is the model with most degrees of freedom (i.e., least parameters necessary to describe the data), which is not significantly worse in describing observed variance-covariance matrices than a model with more parameters. The values of the parameters a, c and e were estimated by maximum likelihood. When the observed variances and covariances deviate only slightly from the variances and covariances as predicted by the model, the model will show a good fit. The fit between the observed

data and the model was assessed by χ^2 tests. A low χ^2 and a high p-value indicate a good fit of the model to the observed data. To compare the fit of two different models, hierarchic χ^2 tests are used. The hierarchic χ^2 is the difference between the χ^2 of a model and the χ^2 of a reduced form of that model (e.g., from a full scalar ACE model to a full scalar AE model). Heritability (h^2) is the proportion of observed, phenotypic variance that can be explained by the genetic factor, and equals to $a^2 / (a^2 + c^2 + e^2)$. Likewise, common environmentability (c^2) is the proportion of observed, phenotypic variance that can be explained by the common environment factor, and equals to $c^2 / (a^2 + c^2 + e^2)$, and unique environmentability (e^2) is the proportion of observed, phenotypic variance that can be explained by the unique environment factor ($e^2 / (a^2 + c^2 + e^2)$).

Results

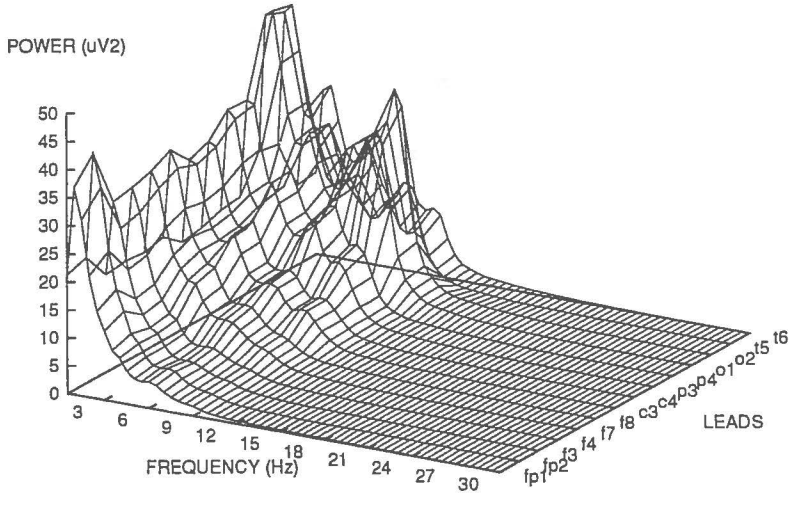

Figure 2.2 *Power spectra averaged over all subjects during rest with eyes closed for 14 electrode positions, from .5 to 30 Hz with a resolution of .5 Hz. Plotted are absolute, non-transformed values. Most power is found in the delta band. On occipital leads a clear peak in the alpha1 band at 8.5 Hz is found.*

Figure 2.2 shows the power spectra for each electrode position. In 5 year old children slow waves dominate. The sum of delta, theta and alpha1 activity explains 84 to 92% of total variance in the signal, whereas faster waves (i.e., alpha2, beta1 and beta2) explain only 8 to 16% of the variance in the EEG during rest.

Table 2.1 *F-values of multivariate analyses of variance with independent variables sex (male or female), zygosity (MZ or DZ), electrode position (Fp1, Fp2, F7, F3, F4, F8, C3, C4, T5, P3, P4, T6, O1 or O2) and interaction effects of sex × zygosity (s × z), zygosity × electrode position (z × e), sex × electrode position (s × e), and sex × zygosity × electrode position (s × z × e). Table shows results of twin A only.*

Absolute power							
	df	delta	theta	alpha1	alpha2	beta1	beta2
sex	1,163	< 1	< 1	3.57	5.04*	11.47*	6.36*
zygosity	1,163	< 1	< 1	< 1	1.22	1.81	< 1
electrode	13,151	63.09*	213.06*	308.06*	187.92*	102.59*	32.68*
s × z	13,151	1.23	< 1	< 1	< 1	< 1	< 1
s × e	13,151	1.46	1.71	< 1	< 1	1.76	1.44
z × e	13,151	1.11	1.25	< 1	< 1	< 1	1.02
s × z × e	13,151	< 1	< 1	< 1	< 1	< 1	< 1
Relative power							
	df	delta	theta	alpha1	alpha2	beta1	beta2
sex	1,163	2.92	< 1	3.82	4.36*	11.70*	3.06
zygosity	1,163	< 1	< 1	< 1	< 1	< 1	< 1
electrode	13,151	89.01*	69.48*	74.73*	46.82*	40.48*	108.52*
s × z	13,151	< 1	< 1	< 1	< 1	< 1	< 1
s × e	13,151	1.81*	1.62	2.25*	1.13	< 1	< 1
z × e	13,151	< 1	< 1	< 1	1.77	1.33	1.54
s × z × e	13,151	< 1	< 1	< 1	< 1	< 1	< 1

* $p < .05$
Significant F-values (df = 1,163): 3.90 for $\alpha = .05$ and 6.79 for $\alpha = .01$,
significant F-values (df = 13,151): 1.79 for $\alpha = .05$ and 2.25 for $\alpha = .01$.

Most power was found in the lowest frequency band (delta). On posterior scalp locations, power in theta, alpha1 and alpha2 bands was larger than on anterior scalp locations and a peak was found at frequency 8.5 Hz on occipital scalp locations. Powers in the beta1 and beta2 bands were small but significantly different from zero. Mean EEG powers of first born twins were not significantly different from those of the second born twins at any scalp location in any band. Multivariate analysis of variance of both absolute and

relative power showed no differences between zygosities for power in all frequency bands. No sex differences were found for mean absolute delta, theta and alpha1 power, but females had slightly higher absolute powers in alpha2, beta1 and beta2 bands. For relative power, sex differences were significant in alpha2 and beta1 band: relative power was larger in females. Mean differences for electrode positions were highly significant. Absolute power was highest at posterior scalp locations, except beta2 power, which was highest at frontal leads. Relative power was higher on anterior leads than on posterior leads for delta, beta1 and beta2 band. For theta, alpha1 and alpha2 band relative power was higher on posterior than on anterior leads. A summary of multivariate analyses of variance with sex, zygosity and electrode position as independent variables is shown in Table 2.1.

Split-half reliabilities for absolute and relative power in the 6 broadbands were uniformly high. No differences between electrode locations were found, and average reliabilities were computed across all electrodes for each of the 6 frequency bands. Split-half reliabilities for absolute power were .89, .96, .96, .96, .95 and .93 for delta, theta, alpha1, alpha2, beta1 and beta2 respectively. For relative power the reliabilities were .88, .91, .92, .92, .90 and .92 respectively.

Genetic analyses

As a first step, MZ and DZ correlations were computed on absolute and relative powers of all bands and all leads (appendix 2.1). These correlations give an indication of which factors influence the trait: When MZ correlations are twice the DZ correlations, genetic factors are important. When MZ and DZ correlations are equal (but not zero), common environment is of importance. There is a large concordance between children from the same family for both absolute and relative power. For all electrode positions and all bands correlations of MZ twins with their cotwins are very high, and correlations of same-sex DZ twins with their cotwins are about half the MZ correlations. Correlations of the male-female DOS twins with their cotwins are about the same as the DZ same-sex correlations, indicating that the same genetic and/or environmental influences are expressed in boys and girls.

Whether the influences of genetic and common environmental factors on the EEG powers are significant can be tested using structural equation modeling. Model fitting showed that the variance in the data was best explained by models containing additive genetic and unique environmental factors. Large heritabilities were found for absolute and relative power in almost all bands and electrode positions. We further tested for

influences of common environment, which would cause both MZ and DZ twins to be more alike, but this factor hardly ever was significant for any band or electrode position. The most parsimonious models were, therefore, models with additive genetic and unique environmental factors only (AE models), as is shown in Table 2.2.

Table 2.2 *Best fitting models and their χ^2. Models containing additive genetic and unique environmental factors without sex differences (AE) have 13 degrees of freedom (df), scalar models (AEsc) have 12 df, and models with non-scalar sex differences (AEsx) have 11 df.*

Absolute power

Electrode	delta		theta		alpha1		alpha2		beta1		beta2	
Fp1	AE	*** 8.96	AE	*** 8.36	AEsx	** 12.84	AEsx	** 11.30	AE	23.94	AE	* 20.73
Fp2	AE	*** 6.58	AE	** 9.86	AEsx	*** 5.97	AEsc	*** 7.92	AEsc	** 13.95	AE	** 14.37
F7	AEsx	* 16.61	AE	** 15.46	AE	* 16.32	AE	** 9.54	AE	** 13.09	AEsc	** 14.08
F3	AE	*** 7.13	AE	** 14.72	AE	** 13.75	AE	** 9.72	AE	* 18.29	AE	** 12.60
F4	AE	** 13.72	AE	* 18.45	AE	*** 9.12	AEsc	*** 7.13	AEsc	** 14.22	AE	** 11.10
F8	AE	* 21.39	AE	*** 8.88	AE	*** 5.52	AE	** 11.81	AE	** 9.90	AE	** 10.10
C3	AE	* 21.62	AE	** 15.46	AE	** 12.67	AEsx	** 9.05	AE	* 19.91	AE	** 15.19
C4	AE	* 20.56	AE	* 19.08	AE	** 10.84	AEsx	*** 5.34	AE	26.78	AE	* 17.82
T5	AE	* 18.97	AE	** 12.07	AE	* 18.87	AE	*** 7.75	AE	** 15.81	AE	** 15.30
P3	AE	33.63	AE	** 9.39	AE	*** 8.71	AE	*** 6.33	AE	** 13.52	AE	** 13.89
P4	AE	24.20	AE	** 10.98	AE	*** 6.17	AE	*** 5.64	AE	* 18.40	AE	** 10.59
T6	AE	* 19.62	AE	*** 6.17	AE	* 18.82	AE	** 14.04	AE	** 11.12	AE	** 10.50
O1	AE	* 18.74	AE	*** 8.53	AE	* 18.14	AE	** 9.36	AE	** 11.78	AE	** 11.84
O2	AE	24.31	AE	*** 6.80	AE	** 12.92	AE[1]	** 15.09	AE	*** 9.28	AE	** 11.75

Relative power

Electrode	delta		theta		alpha1		alpha2		beta1		beta2	
Fp1	AE	*** 6.33	AE	** 10.72	AE	** 12.92	AE	* 18.82	AE	** 12.19	AE	** 12.28
Fp2	AE	*** 6.13	AE	** 14.99	AE	** 14.67	AEsc	* 18.79	AE	** 12.64	AE	** 11.00
F7	AEsx	* 14.41	AEsx	* 15.88	AEsx	*** 6.78	AEsx	** 8.08	AEsx	*** 5.88	AEsc	*** 7.60
F3	AE	** 14.36	AE	** 14.75	AE	*** 7.51	AE	** 14.06	AEsx	*** 5.23	AE	*** 3.60
F4	AE	* 16.55	AE	* 18.07	AE	*** 7.70	AE	** 15.72	AE	* 16.67	AE	** 12.16
F8	AE	* 21.84	AE	23.11	AE	* 19.55	AE	** 15.99	AEsx	** 8.24	AE	** 11.17
C3	AE	** 13.64	AE	*** 5.19	AE	*** 8.94	AE	** 15.87	AEsx	** 15.86	AE	*** 7.11
C4	AE	* 19.70	AE	** 14.93	AE	** 10.53	AEsx	** 8.18	AEsc	** 17.54	AE	** 9.78
T5	AE	* 17.22	AE	*** 5.03	AE	** 12.27	AE	** 11.21	AE	*** 5.68	AEsc	*** 7.29
P3	AE	23.47	AE	*** 9.16	AE	*** 9.15	AEsx	** 8.55	AE	** 10.48	AE	*** 4.98
P4	AE	*** 8.62	AE	*** 7.11	AE	*** 5.51	AE	** 15.04	AE	** 12.11	AE	*** 7.77
T6	AE	** 11.62	AE	*** 7.89	AE	*** 6.57	AE	*** 6.55	AE	** 12.68	AE	*** 7.06
O1	AE	** 14.55	AE	** 11.45	AE	** 11.62	AE	** 14.28	AE	** 13.52	AE	** 9.50
O2	AEsx	** 9.17	AE	** 12.97	AE	*** 6.43	AE	* 20.69	AE	* 16.10	AE	*** 8.97

Probability of the models: * = $p > .05$; ** = $p > .25$; and *** = $p > .75$.
[1] including a common environment factor gave a slightly better fit.

For almost all EEG powers on all scalp locations heritabilities were the same for males and females. A scalar sex-limitations model, in which the total variance was allowed to differ between males and females, but the relative contributions of genes and environment is the same, gave a better fit to the data in some cases. In these models variance was always larger in males than in females. A common sex-limitations model was found a few times. Frontal lateral left electrode position (F7) showed larger heritabilities of absolute power in females for delta power at F7. As a consequence, higher heritabilities in females were found for relative powers on that same location. Heritabilities of absolute alpha power was higher in females at prefrontal scalp locations, and higher in males at central scalp locations (alpha2). These sex differences are scant and show no obvious systematic pattern. In all instances, the same genetic factor always influenced the observed trait in both sexes, since a general sex-limitations model was never the best fitting model. For the largest part of our data, therefore, the most parsimonious models were AE models without sex-differences. Figures 2.3, 2.4 and 2.5 display the heritabilities for absolute and relative power for 6 bands and 14 electrodes for the best fitting models.

With regard to the success of model fitting: for absolute power these models fitted the data well in theta, alpha1 and alpha2 bands, with 29% of the models showing a probability of more than .75 that the data were described correctly by the model. 89% of the models showed a probability of more than .25. For beta bands 66% of the models showed a probability of more than .25, but for delta band this was only 32%, indicating that the lower heritabilities of absolute power in the delta band must be interpreted with some caution. For relative power the AE models fitted the data extremely well in all bands, with a moderate exception of relative delta power. 36% of the models showed a probability of more than .75 that the data were described correctly by the model. 87% of the models showed a probability of more than .25.

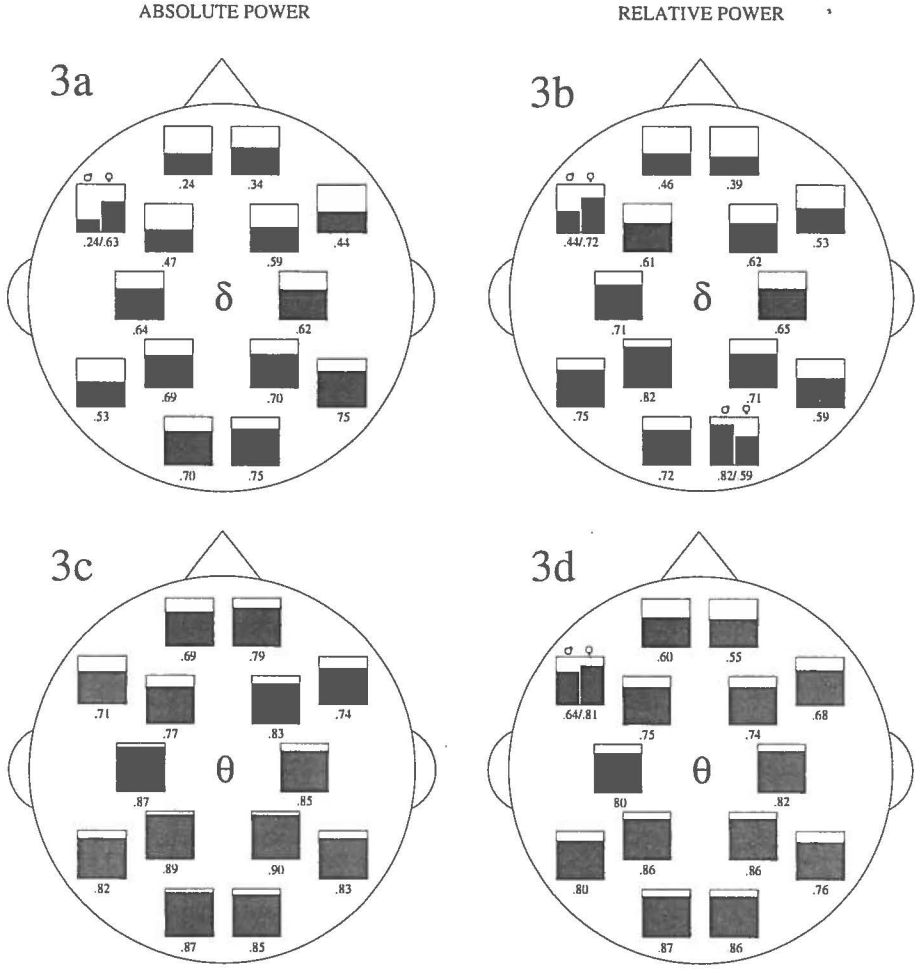

Figure 2.3 a, b, c and d *Heritabilities of absolute and relative delta (a and b) and theta (c and d) power, during rest with eyes closed.*

Heritabilities in theta, alpha1 and alpha2 bands were extremely high, with mean heritabilities of absolute power of 81%, 81% and 78% respectively. Heritability also explained a large part of the individual differences in absolute power in the delta and beta bands, particularly in the occipital and parietal leads. Mean heritabilities were 55%, 73% and 64% respectively. Heritabilities of relative power were high in all bands, with a mean heritability of 63%, 76%, 71%, 72%, 68%, and 65% for relative delta, theta, alpha1, alpha2, beta1 and beta2 power respectively.

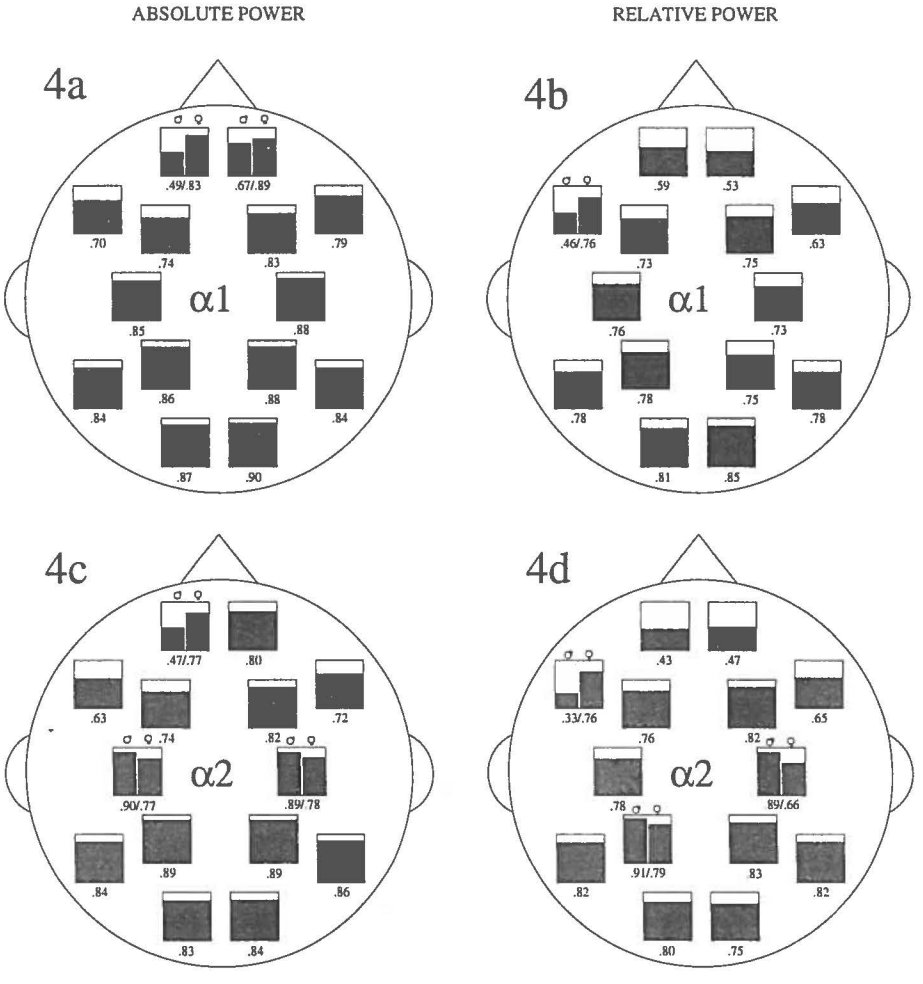

Figure 2.4 a, b, c and d *Heritabilities of absolute and relative alpha1 (a and b) and alpha2 (c and d) power, during rest with eyes closed.*

In our models no explicit multivariate testing of electrode location was performed. However, according to the 95% confidence intervals, heritabilities for both absolute and relative power in all bands decreased significantly from posterior to anterior leads. No significant differences in heritabilities were found between homologous electrodes at left and right hemispheres. 95% confidence intervals are 21 to 56% for $h^2 = 40\%$, 33 to 64%

for h^2 = 50%, 45 to 71% for h^2 = 60%, 58 to 79% for h^2 = 70%, 71 to 86% for h^2 = 80%, and 85 to 93% for h^2 = 90%. Some caution is in order in interpreting inter-regional comparisons, since they may be slightly confounded by different contributions of common activity coming from the linked ears reference.

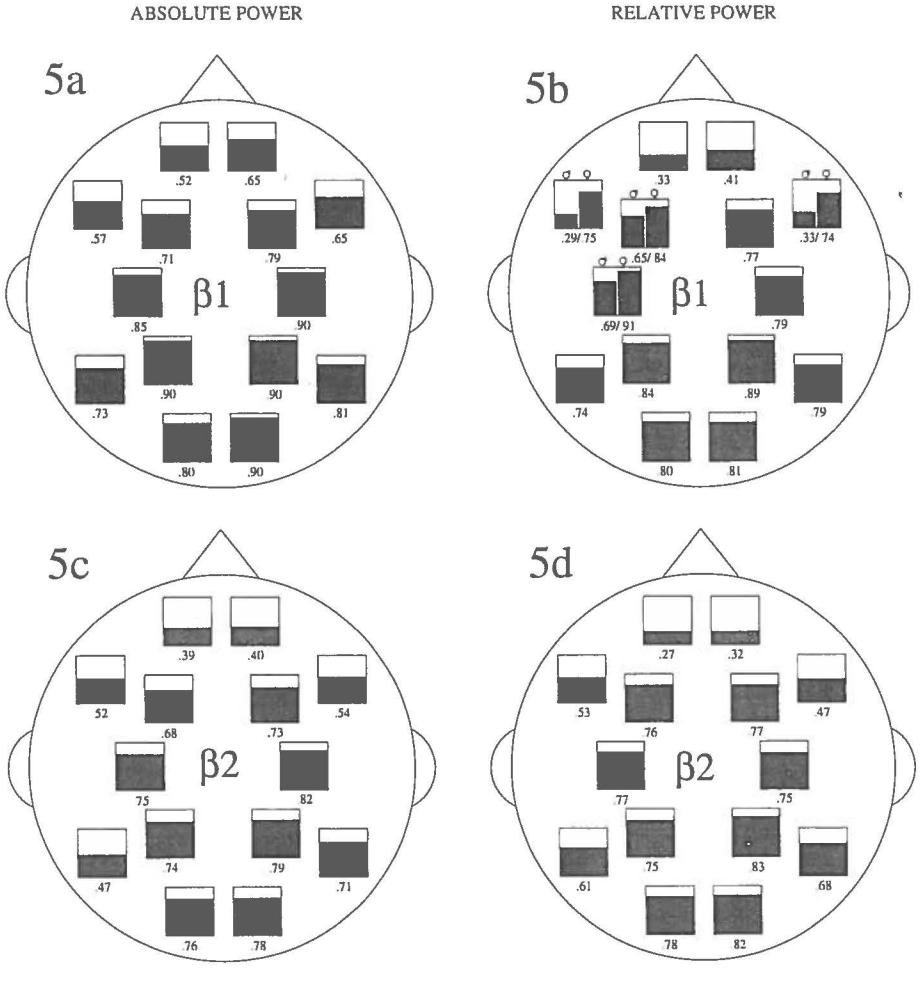

Figure 2.5 a, b, c and d *Heritabilities of absolute and relative beta1 (a and b) and beta2 (c and d) power, during rest with eyes closed.*

Discussion

The objective of the present study was to determine the genetic architecture of electrical brain activity in 5 year old children. In short, the results are that nearly all EEG power spectra measures are highly heritable, and that heritabilities were nearly always the same for boys and girls. Heritabilities of EEG power were slightly higher at posterior than at anterior scalp locations, but no differences were found between left and right hemispheres.

On visual inspection, the dominant picture found in the EEG of 5 year olds usually is that of posterior alpha, frequently interrupted by intermingled slow waves, mostly in the range of 1.5-4 Hz, extending from occipital into the posterior temporal, and less impressively, into the parietal regions (Niedermeyer & Lopes da Silva, 1993). Frequency analysis in our twins generally confirmed this pattern. Delta contributed strongly to power on all leads with a peak in the posterior leads. Power in the alpha and theta bands were also high throughout, except for the frontal leads. Although activity of the low voltage beta1 and beta2 contributed only little to the total power, it was present in virtually all children and, as in the other bands, large individual differences were found. The absolute values for EEG powers found in this study, referenced to linked ears, correspond well to power values reported previously (Gasser et al., 1988) and to mean amplitudes (i.e., square root of power) reported from visual scoring (Niedermeyer & Lopes da Silva, 1993).

Small but significant mean differences between boys and girls were found for absolute alpha2 and beta power, and for relative alpha2 and beta1 power. Girls had higher absolute alpha2, beta1 and beta2 power and higher relative alpha2 and beta1 power than boys in all leads. This contrasts with the results of others. Some studies did not find significant sex differences in EEG amplitudes (Matoušek & Petersén, 1973; Gasser et al., 1988). Other studies (Matthis et al., 1980; Benninger et al., 1984) showed higher relative alpha1 and alpha2 in boys and higher relative theta in girls below the age of 6. However, our results do agree with Petersén and Eeg-Olofsson (1971) who, using visual analysis, found higher alpha frequencies in girls compared with boys up to 11 years of age. Since girls are more advanced with regard to many biological events compared to boys, higher alpha might be interpreted as reflecting a faster maturation in girls than in boys. In older age groups higher beta power in girls has been found before (Matoušek & Petersén, 1973; Matsuura et al., 1985). We do not know why girls have higher beta power than boys. A possible explanation might be that girls were less relaxed, and more alert during the experimental sessions, which would yield more fast and less slow waves.

However, no sex differences were found in slow waves (i.e., delta, theta and alpha bands).

Only a few studies thus far have been conducted on genetic and environmental influences on EEG parameters in children. For slightly older twins, ranging from 9 to 22 years of age, Dumermuth (1968) found that 6 MZ pairs showed larger resemblance than 4 DZ twin pairs. We know of only one other study that has estimated genetic and environmental influences on power of frequency bands in the EEG of a group of 5 year old children (Gavrish & Malykh, 1994). In agreement with our results, their abstract indicated high heritabilities for the 7.5 to 13 Hz frequency band which was in bins of 1.5 Hz. Other studies using large age ranges but including some 5 year olds (Vogel, 1958; Whitton et al., 1985) also agree with our results in that high heritability was found for alpha power. The overall conclusion, therefore, must be that the individual differences in absolute and relative EEG power spectra seen at age 5 are largely genetically determined.

The heritabilities for absolute power of 5 year old children found in this study can be compared directly with heritabilities obtained in a sample of adolescent twin pairs, who participated in a similar study with an identical procedure, conducted at our laboratory (Van Beijsterveldt et al., 1996). Heritabilities tended to be a little bit higher in adolescence (mean heritability was about 90%, except for delta, for which heritability was about 75%). In both age cohorts it was shown that genetic factors explained the larger part of variance in EEG power spectra.

Estimating heritability by using the twin method can be confounded by a number of factors as indicated by Falconer (1989, pp. 174). Some of these factors, like the number of amnia and choria shared by MZ twins in utero, or parental treatment of the twins, will have an unpredictable effect. Other factors, like genotype-environment interaction, or the exact contemporaneity of twins as compared with singletons, would result in a larger estimate of environmental effects, and a lower estimate of genetic effects. Since heritabilities in this study are very high, these factors obviously are not very important.

Heritability estimates cannot be understood fully without a notion of the size of the measurement errors in obtaining the trait. In EEG measurements a good estimator of the measurement error is the use of split-half reliability. When MZ twins are compared with their cotwins, they can never be more alike than the split-half reliability of the trait. Therefore, split-half reliability of the trait gives an upper boundary for heritability. By the same token, a high correlation in MZ twins indicates that the split-half reliability if the trait under study must be high (Lykken, 1982). We calculated split-half reliabilities for absolute and relative power in 6 broadbands. All split-half correlations were very

high, suggesting that only a very small part of the environmental variance is due to measurement error. Apart from measurement error, specific conditions of the experiment, time-of-day, duration of signal conditioning, mood state, temperature, etc. may create additional unique environmental variance that detract from heritability. Such influences might be estimated by looking at temporal stability of the EEG parameters. Gasser and colleagues (1985) measured EEG power spectra twice in 10 to 13 year old children with a ten months' interval. The retest reliabilities of absolute power in delta, theta, alpha1, alpha2, beta1 and beta2 power were .59 .70 .80 .72 .58 and .66 respectively. Unfortunately, it is unclear to what extent the imperfect test-retest correlation is confounded by maturational effects. Measurements in Gasser's study were 10 months apart. In fact, the heritabilities found in our study (i.e., 55, 81, 81, 78, 73 and 64% respectively), compare favorable to their test-retest correlations.

The large environmental influences for absolute power in the delta band on all frontal (Fp1, Fp2, F7, F3, F4, F8) leads may have resulted from true environmental influences, for instance related to drowsiness. Although children who were visibly drowsy were always removed from the analyses, at this age, drowsiness is not easily detected in the EEG, so it may have confounded our assessment of the causes of individual differences in delta. On the other hand, it is unclear why this did not affect h^2 in the theta-band. An alternative explanation for the large environmental contribution to delta may be found in eye-movements. Because eye-movements are in the delta frequency range and because the frontal leads are most sensitive to admixture of EEG with EOG, it is tempting to suggest that the low h^2 in delta activity resulted from measurement error. In fact, the slight negative occipital-frontal gradient in h^2 in the other bands may also be related to admixture of the EEG with EOG. Finally, it was pointed out by Dumermuth and Molinari (1987) that EEG power may be decomposed into white, pink (amorphous or arrhythmic EEG) and colored ("true" EEG peak powers) components. The power in the delta bands is particularly affected by pink noise. Heritability may thus suffer from the influences of pink noise, instead of reflecting genetics of true delta activity.

Several authors have suggested that the developmental changes in the EEG power spectra can be seen as an index of CNS maturation (Matoušek & Petersén, 1973; Gasser et al., 1988). This age-EEG relationship is not necessarily linear and shows different patterns with topography such that posterior regions are seen to mature earlier than anterior ones (Matoušek & Petersén, 1973; Katada et al., 1981; Gasser et al., 1988). Our results could be interpreted as pointing to a strong genetic determination of CNS maturation around the 5th year of life. Also, since for both sexes the same genetic factors underly power, maturation of the CNS seems controlled by the same set of genes in boys and girls.

If the development of rhythmical activity in the brain is strongly under genetic control, as we have shown in our study, one could be tempted to suggest that the development in cognitive functioning is under genetic control too. In a series of articles Thatcher and coworkers have suggested that developmental changes in the EEG may closely reflect the stages proposed by Jean Piaget (Thatcher, 1991; 1992). The finding of high heritabilities for power in the current study appears to lend support to their idea that "human cognitive development occurs ontogenetically, i.e., by the genetically programmed unfolding of specific brain functions and specific brain functions and specific brain connections" (Thatcher et al., 1987).

Appendix 2.1: *Twin correlations for log transformed absolute power and logit transformed relative power for 5 groups in 6 bands, 14 electrode positions.*

delta: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.21	.36	.23	.48	.68	.41	.67	.69	.61	.69	.71	.80	.65	.79
DZM	-.21	.01	.05	.48	.47	.22	.59	.51	.50	.45	.35	.31	.34	.41
MZF	.42	.39	.70	.38	.57	.64	.50	.47	.32	.52	.56	.65	.63	.58
DZF	.01	.04	-.01	.15	.05	.10	-.12	.07	-.04	-.04	.07	.04	.09	.19
DOS	.22	.21	.43	.28	.19	.24	.34	.52	.25	.58	.66	.63	.55	.62

theta: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.59	.75	.60	.76	.85	.67	.84	.83	.84	.89	.88	.80	.87	.85
DZM	.32	.44	.63	.47	.49	.53	.48	.39	.53	.48	.43	.43	.54	.53
MZF	.71	.78	.73	.71	.78	.73	.82	.80	.80	.84	.85	.83	.86	.83
DZF	.47	.46	.42	.49	.47	.38	.33	.37	.26	.29	.32	.27	.24	.28
DOS	.36	.28	.45	.33	.35	.43	.45	.52	.38	.52	.51	.44	.55	.47

alpha1: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.49	.70	.66	.71	.81	.73	.86	.84	.83	.86	.86	.80	.87	.89
DZM	.36	.44	.50	.55	.53	.43	.63	.57	.64	.63	.56	.64	.69	.67
MZF	.84	.90	.79	.81	.88	.86	.86	.89	.91	.89	.90	.91	.90	.92
DZF	.66	.54	.55	.59	.55	.36	.57	.50	.44	.50	.50	.53	.47	.54
DOS	.24	.22	.25	.20	.31	.39	.46	.42	.23	.43	.41	.31	.38	.35

alpha2: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.52	.83	.62	.69	.82	.69	.89	.89	.86	.90	.87	.84	.80	.84
DZM	.17	.40	.39	.56	.54	.14	.45	.48	.55	.59	.53	.55	.63	.70
MZF	.79	.81	.67	.75	.82	.80	.77	.77	.83	.88	.88	.87	.83	.83
DZF	.46	.42	.32	.46	.53	.31	.43	.46	.29	.40	.48	.46	.44	.52
DOS	.17	.09	.26	.23	.30	.27	.35	.47	.40	.44	.35	.41	.53	.53

beta1: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.52	.72	.64	.76	.76	.59	.85	.86	.78	.88	.87	.76	.71	.76
DZM	.04	.58	.37	.60	.64	.29	.55	.53	.40	.52	.38	.38	.58	.56
MZF	.60	.59	.55	.55	.73	.74	.77	.86	.65	.85	.87	.81	.83	.79
DZF	.15	.05	.16	.32	.41	.32	.29	.28	.31	.34	.33	.23	.35	.36
DOS	.05	.08	.21	.14	.19	.22	.24	.28	.16	.27	.30	.35	.46	.43

beta2: absolute power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.44	.58	.55	.79	.75	.52	.82	.85	.58	.82	.82	.76	.74	.75
DZM	.14	.34	.30	.61	.62	.35	.48	.64	.28	.51	.45	.37	.62	.65
MZF	.45	.27	.52	.55	.69	.62	.69	.77	.43	.67	.76	.68	.69	.72
DZF	-.04	-.16	.17	.37	.41	.23	.37	.35	.24	.32	.38	.32	.33	.43
DOS	.27	.41	.45	.45	.40	.22	.56	.56	.09	.47	.54	.56	.53	.48

delta: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.51	.43	.54	.66	.75	.61	.75	.71	.71	.86	.74	.68	.81	.83
DZM	.29	.16	.18	.32	.14	.23	.11	.01	.17	.02	.25	.12	.34	.34
MZF	.45	.29	.78	.68	.63	.64	.71	.66	.81	.77	.68	.57	.72	.66
DZF	.12	.17	.20	-.11	.03	.11	.03	.17	.00	.14	.16	.18	.05	.18
DOS	.35	.43	.10	.25	.20	.08	.31	.36	.48	.45	.33	.37	.26	.21

theta: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.63	.62	.72	.77	.84	.76	.80	.82	.79	.86	.88	.77	.89	.88
DZM	.39	.35	.27	.21	.25	.34	.18	.13	.30	.11	.40	.33	.43	.47
MZF	.59	.42	.83	.76	.69	.71	.80	.82	.81	.84	.80	.75	.79	.76
DZF	.20	.14	.47	.32	.37	.23	.42	.42	.38	.48	.49	.56	.38	.45
DOS	.42	.52	.23	.40	.41	.30	.46	.63	.45	.47	.48	.36	.38	.42

alpha1: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.62	.48	.48	.74	.74	.52	.74	.74	.85	.83	.77	.78	.88	.90
DZM	.18	.13	.39	.46	.46	.59	.53	.54	.39	.50	.49	.40	.45	.53
MZF	.63	.56	.80	.76	.78	.79	.79	.71	.78	.75	.72	.81	.76	.79
DZF	.43	.44	.37	.43	.49	.42	.47	.52	.50	.57	.52	.58	.51	.48
DOS	.36	.50	.18	.31	.33	.11	.49	.51	.20	.37	.36	.22	.13	.26

alpha2: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.27	.29	.25	.70	.82	.55	.83	.89	.88	.89	.89	.85	.87	.82
DZM	-.05	-.03	.23	.35	.20	.10	.32	.39	.56	.45	.36	.48	.43	.49
MZF	.48	.45	.77	.79	.81	.74	.74	.64	.76	.79	.75	.72	.67	.56
DZF	.40	.45	.31	.45	.54	.55	.43	.57	.47	.53	.53	.42	.49	.43
DOS	.33	.36	.18	.09	.18	-.01	.21	.41	.44	.50	.50	.44	.56	.63

beta1: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.19	.24	.26	.68	.72	.40	.72	.74	.72	.83	.86	.68	.77	.82
DZM	-.16	-.03	.18	.26	.14	.02	.25	.19	.16	.26	.27	.20	.34	.43
MZF	.41	.40	.73	.81	.79	.70	.88	.81	.72	.82	.87	.83	.81	.79
DZF	.24	.41	.24	.37	.39	.42	.35	.33	.40	.46	.48	.43	.44	.48
DOS	.41	.30	.26	.36	.39	.24	.34	.35	.36	.43	.42	.48	.50	.27

beta2: relative power

	Fp1	Fp2	F7	F3	F4	F8	C3	C4	T5	P3	P4	T6	O1	O2
MZM	.13	.33	.42	.74	.80	.43	.74	.75	.66	.80	.79	.61	.80	.82
DZM	.00	.01	.25	.32	.22	.27	.28	.35	.25	.29	.33	.22	.44	.31
MZF	.38	.24	.60	.78	.75	.48	.80	.73	.58	.70	.83	.76	.78	.82
DZF	.09	.16	.28	.44	.36	.18	.42	.34	.41	.43	.40	.43	.26	.40
DOS	.48	.40	.30	.40	.41	.17	.36	.37	.20	.42	.44	.30	.32	.29

3

Longitudinal study of genetic influences on ERP-P3 during childhood

G.C.M. van Baal, E.J.C. de Geus and D.I. Boomsma

The P3, a component of the Event Related Potential (ERP), is an electrophysiological reaction of the brain to a stimulus. It has been extensively studied as an index of attentional and memory processes in humans and the substantial individual variation in its amplitude and latency have been related to individual differences in cognitive function and ability. Little is known about the relative contributions of genetic and environmental influences to the individual differences in this ERP component. Furthermore, it is unclear whether and how these influences vary during maturation in childhood. In the present study, P3 was measured twice in 164 young twin pairs, once at age five, and once at age seven. Subjects performed a visual oddball task with 100 nontarget and 25 target stimuli. P3 amplitudes and latencies were obtained at 6 scalp locations (C3, Cz, C4, P3, Pz and P4). Results show an effect of age (smaller amplitudes and shorter latencies at age seven than at age five), stimulus type (larger amplitudes and longer latencies for targets than for nontargets), and electrode location (largest P3 amplitude at Pz, longest P3 latencies at central electrodes). No sex differences were found for mean amplitude or latency. A genetic model was fitted to the data that decomposed the reliable variances and covariances of P3 at ages five and seven years into genetic and environmental parts. A significant part of the true variance in P3 latency was genetic. Heritabilities were 13 - 78% at age five, and 36% - 99% at age seven. Heritabilities for P3 amplitude in response to targets were low (0 - 19%), but high in response to non-targets (36 - 86%) at both ages. At most scalp locations the same set of genes influenced latency and amplitude from age five to age seven. Only for Cz and P3 scalp locations was an additional genetic factor common to the latency of targets and nontargets found at age seven. We conclude that genetic influences are responsible for the stable interindividual differences in P3 latency and non-target P3 amplitude and that these influences are largely established at age five.

Introduction

Converging evidence from animal studies (Zecevic et al., 1989) and morphometric, PET and MRI studies in humans (Huttenlocher, 1979; Huttenlocher et al., 1982; Chugani et al., 1987; Jernigan et al., 1991) suggests that early brain development from childhood to adolescence is characterized by a gradual decrease in grey matter and an increase in white matter. The decrease in grey matter, starting at about 4 years (Pfefferbaum et al.,

1994), is thought to reflect a pruning of synaptic contacts, such that only connections incorporated into functional networks survive, whereas random connections are eliminated. The increase in white matter may reflect the ongoing myelination of the many cortico-cortical connections. Although this general pattern of brain development occurs in all children, the extent and the time-course of myelination as well as synaptic pruning show clear differences between individuals (Huttenlocher et al., 1982; Benes, 1994). It is likely that these individual differences in the maturation of the brain affect the development of interindividual differences in cognitive functions as well as overall behavior. In an attempt to trace these effects, previous studies have used the electroencephalogram (EEG) as a non-invasive index of brain development (Thatcher et al., 1987; Friedman, 1991; Stauder et al., 1993; 1995). Generally, their results support the existence of both continuous maturation and discrete growth spurts in EEG activity.

At present, the nature of individual differences in these maturational changes in brain function is unclear. To which extent the same or different genetic factors are expressed with the passing of time is unknown, as is the extent to which the timing of their expression is modified by environmental effects, for instance those related to family or school. Using genetically related subjects, such as twins, it is possible to distinguish between genetic and environmental contributions to interindividual differences. Monozygotic (MZ) twins share all their genetic material, while dizygotic twins share on average 50% of their genes. If differences in the EEG are larger in DZ twins than in MZ twins, then the greater resemblance in the EEG phenotype of MZ twins is caused by their greater genetic resemblance. The present study is part of an ongoing research project in which 209 twin pairs were tested when they were 5 years old and when they were 7 years old. Collecting data repeatedly in time on the same twins makes it possible to address the question whether heritability changes as children grow older and enter a different maturational stage. More interestingly, longitudinal data from twins can distinguish between genetic and non-genetic causes of phenotypic stability and estimate the extent to which the covariance across time is caused by the same genes operating at different time periods (Eaves et al., 1986). This means that changes in the genetic and environmental effects on maturation across time can be studied directly. By including opposite sex twins in the sample, possible sex differences in the stability of genetic and environmental factors can be tested as well.

In a previous part of this research project we showed high heritabilities of absolute and relative EEG alpha and theta power during quiet rest in a sample of 167 five year old twin pairs (Van Baal et al., 1996). Since the ratio between EEG alpha and EEG theta is generally seen as an index of brain maturation (Petersén & Eeg-Olofson, 1971), our findings suggested that the individual differences in brain maturation are largely under

genetic control. However, the exact relationship between EEG power and cognition and behavior is unclear. EEG rhythms are driven mainly from subcortical areas and may bear little relationship to the functional development of the cortical cell layers. In contrast, development of stimulus evoked changes in EEG, the so-called event related potentials (ERPs), appears to closely mirror the time-course of development of grey and white matter (Courchesne, 1977; Courchesne et al., 1987). The P3 (also known as P300) shows particular promise. It can be reliably evoked, even in young children, by a simple oddball paradigm. The P3 is a positive going wave in the ERP which occurs 300 ms or more after stimulus presentation. It is a late, endogenous component in the ERP, and is associated with the information processing demands of the task rather than the obligatory activation of neuroanatomical structures in the stimulated primary pathways, which is indexed by the earlier, exogenous components. The latency of the P3 (i.e., the timing of its peak) provides a measure of mental processing speed that is independent of behavioral responding (Donchin et al., 1986). Latency gradually decreases with age until young adulthood (Courchesne, 1978; 1979; 1990; Polich et al., 1990; Friedman, 1992). Individual differences in P3 latency have been suggested to be related to faster processing speed in various tests of cognitive function (Ladish & Polich, 1990; Emmerson et al., 1990). P3 amplitude is sensitive to task relevance and (subjective) probability of the stimulus and is suggested to be proportional to attentional resources invested in the maintenance and updating of working memory (Polich, 1996). Indeed, larger P3 amplitude has been associated with superior memory performance (Fabiani et al., 1990; Noldy et al., 1990). Taken together, these findings suggest that knowledge about the genetic architecture of P3 will contribute to the theory of individual differences in cognitive maturation.

Genetic and environmental influences on individual differences in P3 amplitude and latency have been investigated in a small number of studies only (for a review see Van Beijsterveldt & Boomsma, 1994). Most studies used a twin design to estimate heritability. Surwillo (1980) studied P3 latency in an auditory oddball task in 6 MZ twin pairs and 6 unrelated pairs of children, aged 9 to 13 years, and found evidence for genetic influences. O'Connor and colleagues (1994) studied P3 amplitude and latency in an auditory oddball task in a group of adult twins (59 MZ and 39 DZ). They found significant genetic influences on P3 amplitude, but not on P3 latencies. In two smaller studies with adult subjects, (Polich & Burns, 1987; Rogers & Deary, 1991) genetic influences on both amplitude and latency of an auditory P3 were suggested. A family study also provided evidence of familial resemblance in P3 latencies and amplitudes in both auditory and visual tasks (Eischen & Polich, 1994). However, the latter study included only 10 families, and no distinction could be made between common environmental and genetic influences. A study of P3 amplitudes and latencies in adolescent twins

using a visual oddball task was conducted in our laboratory (Van Beijsterveldt et al., submitted). In this study, 213 adolescent twin pairs participated. Strong genetic influences were shown on amplitudes for nontarget stimuli. For target amplitudes results were less clear, but familial resemblances were found. No genetic influences on P3 latencies were observed.

Except for Surwillo's study, none of the previously mentioned studies included children. Because P3 changes in amplitude and latency from childhood to adolescence, results from adult genetic studies cannot be extrapolated to children. More importantly, no study has attempted to assess the changes in genetic contribution to P3 across childhood. This is surprising because cognitive abilities show remarkable changes in genetic architecture as children grow up. At a young age, individual differences in intelligence and verbal and non-verbal abilities are more determined by shared environment than by the genotype. When children get older, the influence of heritability increases. Between ages 6 and 12 heritability reaches its adult values of 50%-60% (cf. Plomin & Rende, 1991; Boomsma, 1993; McGue et al., 1993; Thompson, 1993). If P3 reflects the brain maturation necessary for cognitive development, as has been suggested (Courchesne, 1990; Stauder et al., 1993; 1995), than we would expect the changes in genetic contribution to cognitive abilities to coincide with changes in genetic contribution to the neural P3 generators. The age range chosen in this study, from 5 to 7, seemed optimal to detect a sudden shift in genetic contribution. In the longitudinal data from the Colorado Adoption Project (Cherny & Cardon, 1994) heritability of childhood IQ increased after 4 years of age with new genetic factors emerging somewhere between age 4 to 7. In addition, in middle childhood most children show a qualitative change in cognition referred to within a Piagetian framework as a shift from the pre-operational to the operational phase (Piaget & Inhelder, 1969).

Although the longitudinal design is an essential and valuable aspect of this study, it also induces specific methodological problems. In children, the P3 amplitude and latency are more difficult to detect than in adult data, since the P3 wave is much broader. This may result in less reliable P3 measures, which strongly affects estimates of heritability. Also, because the reliability of the P3 wave improves with age, comparing data from age 5 to 7 may yield spurious increases in heritability that are due solely to a decrease in measurement error, since in the usual genetic model measurement error is included in the environmental component. Thus a large error component will lead to a low heritability (h^2), because h^2 is the ratio of genetic variance over total variance. In this paper a model is used in which genetic and environmental influences and measurement error can be distinguished, and h^2 can be estimated for the reliable part of the phenotypic variance.

Methods

Subjects

Two hundred and nine healthy Dutch twin pairs participated twice, with a 1.5 years' interval ((96% of the twins were tested within 1 year and 6 months). Subjects were 5 years old at the first measurement occasion (mean age = 5 years and 3 months, sd = 0.2 years), and around 7 years old at the second measurement (mean age = 6 years and 10 months, sd = 0.2 years). All twin pairs were registered in the Netherlands Twin Register, which contains between 50 and 60% of all Dutch twins born after 1986 (Boomsma et al., 1992). Zygosity for the same-sex twin pairs was determined either by blood typing (ABO, MNS, Rhesus, Kell, Duffy, Kidd, Lutheran) or by DNA fingerprinting (N = 159 pairs). No blood typing or DNA analyses were available for 11 same-sex twin pairs, who were assigned to a zygosity group based on their physical appearance by means of a discriminant analysis.

No complete data were available for 17 twin pairs, because they did not participate the second time. Data from 26 twin pairs were discarded from further analysis because of difficulties during data collection at the first or second measurement occasion (i.e., could not perform the task, distorted ERP signals due to movement artifacts). One twin had an extremely high P3 amplitude, and another twin had an extremely long P3 latency. These two twin pairs were also discarded from the analyses. This left 164 twin pairs with complete data (33 monozygotic males (MZM), 37 dizygotic males (DZM), 33 monozygotic females (MZF), 31 dizygotic females (DZF), 31 dizygotic opposite sex twins (DOS)). No significant differences in IQ, sex or age were found between children who participated once and children who participated twice. Twins were measured on the same time of day (morning or afternoon) and had normal or corrected to normal vision. Subjects were rewarded with a small present.

Procedure

The protocol was the same on both measurement occasions. After the twins and their parents arrived in the laboratory, the protocol was explained to them and height, weight and head circumference were measured. Next, one of the twins participated in the electrophysiological experiment, while the other twin was given an IQ test in an adjacent room (Boomsma & Van Baal, in press). To measure EEG activity, an electrocap with electrodes in the 10-20 system of Jasper (1958) was attached, while the child watched a video. Four tin electrodes for eye movement recordings and two ear electrodes as references were also attached. Electrode impedance was kept below 10 Kohm. Testing took place in a dimly lit, electrically shielded, sound attenuated cabin with intercom facilities. Subjects

lay on a bed and watched a black and white 25 × 30 cm monitor about 50 cm above their heads. One parent was allowed to stay with the child. The experimental conditions consisted of an auditory habituation task, a visual oddball task and six minutes of quiet rest. This paper presents the ERP data acquired during the oddball task.

Task

Subjects performed a visual oddball task that consisted of 125 stimuli with line drawings of dogs as nontargets (n = 100) and line drawings of cats as targets (n = 25) (Snodgrass & Vanderwart, 1980). Pictures 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, and stimulus duration was 100 ms. After one or more short practice series (15 trials with 4 targets), five sets of 25 trials were presented (Number of targets per set: 6 3 8 4 4). Subjects were instructed to silently count the targets and report the result for each set. Inter-stimulus intervals varied (but were the same for all subjects) and ranged from 1.5 to 2.0 s (with a mean of 1.75 s). During the interstimulus interval (ISI) a fixation point was shown, and subjects were instructed to look at it.

Data quantification and data reduction

EEG was recorded continuously on a 18 channel Nihon Kohden PV-441A polygraph. Time constants were set to 5 s, low pass filter was 35 Hz and sample frequency was 100 Hz. Signals were converted with a 12 bits AD converter, and sent to a PC for offline processing.

EEG electrodes were placed at the following scalp locations: frontal (F7, Fz, F8), central (C3, Cz, C4), parietal (P3, Pz, P4), occipital (O1, O2) and temporal (T3, T4, T6). Linked earlobes were used as references according to the method described by Pivik and colleagues (1993). Briefly, we used two separate preamplifiers with high input impedance for each of the reference electrodes and linked the output electrically. With the ears linked this way the effects of possible imbalances in electrode impedance introduced by the electrical double layers were prevented. Vertical eye movements were measured at infra- and supra-orbital sites in line with the pupil of the left eye, and horizontal eye movements at the outer canthuses.

P3 amplitude and latency were calculated by selecting time series of 50 ms prestimulus, and 1000 ms poststimulus. Single trial EOG artifacts were removed using dynamic regression in the frequency domain (Brillinger, 1975), and trials with clippings or large shifts in voltage were excluded from further analysis. Remaining trials were then averaged, resulting in averaged ERP wave forms per subject for targets and non-targets. Since averaged ERPs were flattened because of latency jitter, a Woody filter (window

350 to 900 ms) was used (Woody, 1967). The highest point in a window 450 to 750 ms (at first measurement) or 400 to 600 ms (at second measurement) of the Woody filtered wave form was automatically scored as the peak of the P3 wave. All signals and peak scorings were then visually checked and adjusted if necessary. P3 amplitude was defined as the difference in voltage from baseline to peak, and P3 latency was defined as the time from stimulus onset to peak.

Statistical analysis

Differences in mean values

Multivariate analyses of variance (MANOVA, SPSSPC) were used to test for differences in mean values of P3 amplitude and amplitudes between sexes (male or female), zygosities (monozygotic (MZ) or dizygotic (DZ)),stimulus types (targets or nontargets), electrode locations, and ages (5 or 7 years). All analyses were conducted separately for both twins of a pair (first and second borns), because due to their genetic relatedness, the data of the 2 children of a twin pair are not independent. A oneway analysis of variance was used to test for birth order effects on P3 latency and amplitude.

Reliability measures

Reliability of the nontarget amplitude and latency was estimated using a split-half approach. This method provides a coefficient of internal consistency. In addition to calculating an ERP averaged over 100 trials, two averaged ERPs over 50 trials (even numbered and odd numbered) were calculated. In both signals the P3 peak was picked using the same procedure as in the original ERP that was averaged over all trials. The correlation between the amplitudes (or latencies) of those two signals provides a measure of reliability.

Stability measures

Test-retest correlations between the first and second measurement (1.5 years' interval) were computed to obtain information about stability in time of P3 amplitudes and P3 latencies for targets and nontargets.

Genetic analyses

Observed phenotypic variance (Vp) in P3 amplitude and P3 latency can be decomposed into genetic (Vg) and environmental variance. Two sources of environmental variance can be distinguished: common environmental variance (Vc) and unique environmental variance (Ve). Common environmental variance is due to a shared

environment within the family. Unique environmental variance results from influences that are unique to a person, and often also includes measurement error (Plomin et al., 1990; Neale & Cardon, 1992). The total variance thus equals: $Vp = Vg + Vc + Ve$.

To decompose the observed phenotypic variance into these components, data of genetically related subjects are needed. We analyzed data of monozygotic (MZ) and dizygotic (DZ) twin pairs reared together. Because MZ twins share all their genes, and are raised in the same family, the covariance between MZ twins is composed of all genetic and common environmental variance: $Cov_{(MZ)} = Vg + Vc$. All differences between the MZ cotwins are due to unique environmental influences. DZ twins also are raised in the same family, and therefore will share all common environmental variance. However, DZ cotwins share only half their genes on average, meaning that only half the genetic variance contributes to their covariance: $Cov_{(DZ)} = .5 \times Vg + Vc$. The differences between MZ en DZ covariances (or MZ and DZ correlations) thus give information about sources of variation.

An easy way to get an impression of genetic or common environmental influences is to compare the correlations between MZ and DZ twins. If both MZ and DZ correlations are not significantly different from zero, only unique environment influences the trait (E model). If both MZ and DZ correlations are different from zero, but not different from each other, then common environmental influences are present (CE model). If the MZ correlation is twice the DZ correlation, then genetic influences are of importance (GE model).

A more sophisticated way of estimating and testing the relative influences of genes and environment is by way of structural equation modeling. A model according to the formulas of variance and twin covariances given above can be fitted to the data. A number of models can then be tested by comparing their goodness-of-fit, namely GCE, GE, CE and E models, in which G refers to genetic variance, C to common environmental variance, and E to unique environmental variance. G refers to additive genetic variance only. Dominant genetic variance, that is variance due to interaction between genes at the same locus, was not reported, because twin correlations did not give an indication for such effects. Models containing additive and dominant genetic and unique environmental factors were tested, but never gave a significantly better fit to the data. The models can be tested with or without constraining parameters to be equal in males and females, thus testing for sex differences in the relative influences of genetic and environmental factors.

Multivariate genetic analyses

A univariate analysis that addresses the issue of heritability of a certain phenotype, uses information on the resemblance between relatives, such as was outlined above for

twins. A multivariate genetic analysis also uses the additional information in the cross-correlations (e.g., correlation between P3 elicited by targets at age 5 in twin 1 with P3 elicited by targets at age 7 in twin 2) to determine the extent to which genetic influences are shared by several phenotypes or are phenotype specific. Using this additional information, a multivariate design can improve the power to detect genetic or shared environmental influences. In addition, the multivariate approach provides information about the extent to which different P3 measures (e.g., in response to target and non-target stimuli) are influenced by the same genetic and environmental influences. Finally, the approach used allows us to correct the estimates of genetic and environmental contribution to P3 for measurement error. For the latter purpose we calculated two averaged ERPs for nontarget trials. These two variables were used as observed phenotypes in our multivariate genetic model. This allows us to distinguish true variance (the covariance between these variables) from variance due to measurement error (the variable-specific variance) in P3 elicited by nontargets. Assuming that the measurement errors in both stimulus types were the same, the multivariate solution also improves the estimation of genetic and common environment effects on P3 from targets.

A path diagram of this multivariate genetic model used is given in Figure 3.1. In this figure, the observed variables are indicated with rectangulars. For both twins and both measurement occasions, three phenotypes (one target P3 and two non-target P3s) are analyzed simultaneously. Variance of each observed phenotype for each twin at each time point consists of two parts: a true part (true P3) and a part that is due to measurement error (U). The variance of the true part can be influenced by genotype (G), by common family environment (C) and by unique environmental influences (E). For each twin four true phenotypes are defined: P3 responses to targets and nontargets at age 5, and P3 responses to targets and nontargets at age 7. A simultaneous analysis of these four phenotypes will allow insight about the extent to which P3 responses to targets and nontargets are influenced by the same genetic and/or environmental factors. Likewise, a multivariate genetic analysis will provide information about the extent to which P3 responses at ages 5 and 7 years are influenced by the same genes or environmental factors. Stated otherwise, a multivariate genetic analysis will also show whether new genetic factors are expressed at age 7. We used this general multivariate genetic model to obtain estimates of heritabilities and genetic and environmental correlations between the true part of phenotypic measures. As is shown in Figure 3.1, the true P3 phenotype can be influenced by G, C or E. The first genetic factor (G1) is common to all four traits, the second factor (G2) has loadings on all traits except the first one, and so on (same for C and E). When the full four factor model is fitted to the data, the ordering of the phenotypes is arbitrary, as long as there are no sex differences in the genetic and

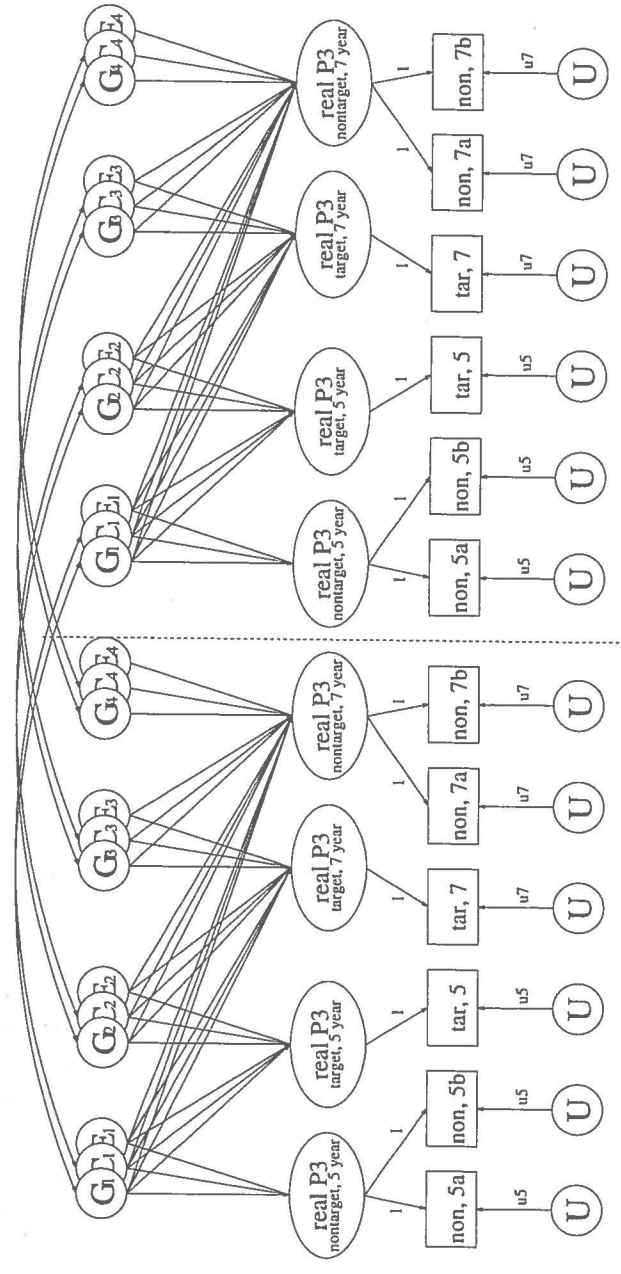

Figure 3.1 *Path diagram of multivariate model. Rectangles are observed variables. For each age, three variables are available, one P3 elicited by targets and two P3s elicited by nontargets (series a and b, averaged over all odd and all even trials respectively). These are influenced by two latent factors: the true P3, and a measurement error factor (U). The true P3 is influenced by genetic (G), common environmental (C) and unique environmental (E) factors. Correlations between G's of twin 1 and twin 2 are 1 for MZ twins, and .5 for DZ twins. Correlations between C's of twin 1 and twin 2 are 1 for all twins.*

environmental factor loadings (Heath et al., 1994). Multiplication of the triangular matrices of genetic and environmental factor loadings gives maximum likelihood estimates of genetic and environmental covariance matrices. Standardization of these matrices gives the genetic and environmental correlations between measures.

First, this model was tested allowing for differences in G, C and E parameter estimates between males and females. Second, estimates were constrained to be equal for males and females. Third, the common environmental factors were left out to test whether they are necessary to describe the model. Fourth, an E model was tested by constraining the genetic factors to be zero. Based on the results, two other models were indicated and tested: a model with 1 or 2 genetic factors instead of a triangular decomposition was fitted to the data. The second factor could indicate age- or stimulus specific genetic effects. For the best fitting, most parsimonious model, heritabilities and proportion of measurement error were calculated according to the following formulas:

- heritability (h^2) = Vg / $V_{True\text{-}P3}$ = Vg / (Vg + Vc + Ve)
- measurement error (u^2) = Vu / $V_{observed}$ = Vu / (Vu + $V_{True\text{-}P3}$)

Genetic and environmental variances were estimated by Maximum Likelihood, using the computer program Mx (Neale, 1994). Data on male and female MZ and same sex DZ twins and on DZ opposite sex twins were summarized into 12 by 12 variancecovariance matrices. The diagonal elements of the matrices give the observed variances of the phenotype for first born (boy in DOS) and second born (girl in DOS), the covariance between twins is given in the off-diagonal element. The goodness-of-fit was assessed by χ^2 tests. A low χ^2 and a high p-value indicate a good fit of the model to the observed data.

Nested models were compared by hierarchic χ^2 tests. The hierarchic χ^2 is the difference between the χ^2 of a full model and the χ^2 of a reduced form of that model (e.g., an GCE model and an GE model). The degrees of freedom (df) of the hierarchic test is the difference between degrees of freedom of the nested models. The best fitting model is the model with most degrees of freedom (i.e., least parameters necessary to describe the data), without being significantly worse than a model with more parameters. For h^2, 80% maximum likelihood based confidence intervals (Neale & Miller, in press) are provided.

Results

Visual inspection of the P3 wave forms showed that in a large part of the subjects frontal, temporal and occipital scalp locations did not yield signals in which a P3 peak could be reliably detected in both target and nontarget ERPs. Since availability of adequate numbers of complete twin pairs is essential for genetic analyses, we decided to analyze central (C3, Cz and C4) and parietal (P3, Pz and P4) electrodes only. ERPs elicited by targets and non-targets, as a function of age and electrode location are depicted in Figure 3.2. Differences between stimulus types, measurement occasions, scalp locations, sexes and zygosities were tested. All results and analyses are reported for first-born twins, because results for second-born twins showed the same effects, and oneway analyses of variance indicated no effect of birth order. MANOVA's showed that amplitudes elicited by targets were significantly higher than P3 elicited by nontargets ($F(1,160) = 67.21$, $p < .001$), and slightly higher at the first measurement occasion than at the second ($F(1,160) = 7.49$, $p = .007$), although the latter effect was very small (about 1 μV). Topographical differences were significant, with the Pz electrode showing the highest amplitude ($F(5,156) = 89.43$, $p < .001$). Only one interaction effect was significant: the differences between targets and nontargets were larger on parietal scalp locations than on central locations ($F(5,156) = 10.79$, $p < .001$). Analysis of P3 amplitude showed no effect of sex and zygosity.

Latencies for targets were longer than for nontargets ($F(1,160) = 17.77$, $p < .001$), and shortest at parietal electrodes ($F(5,156) = 17.63$, $p < .001$). Latencies were longer at the first measurement occasion than at the second ($F(1,160) = 462.56$, $p < .001$). The interaction between stimulus type and measurement occasion was significant: the difference in P3 latency between targets and nontargets was larger for the first measurement than for the second measurement occasion ($F(1,160) = 9.93$, $p = .002$). Analysis of P3 latency showed no effects of sex and zygosity.

Reliability

Split-half correlations for P3 amplitudes and P3 latencies of nontarget ERPs (i.e., the correlation between two ERPs averaged over 50 trials within one measurement session) are presented in Table 3.1. For P3 amplitudes the correlations are around .5 to .6, with a mean of .54 on central scalp locations and a mean of .62 on parietal locations. For P3 latencies correlations were on average .57 on central and .48 on parietal locations. Thus, a large part of the variance in P3 latency and amplitude in these children can be ascribed to measurement error. This is probably due to the broad P3 wave at these ages which makes it difficult to detect its exact highest point.

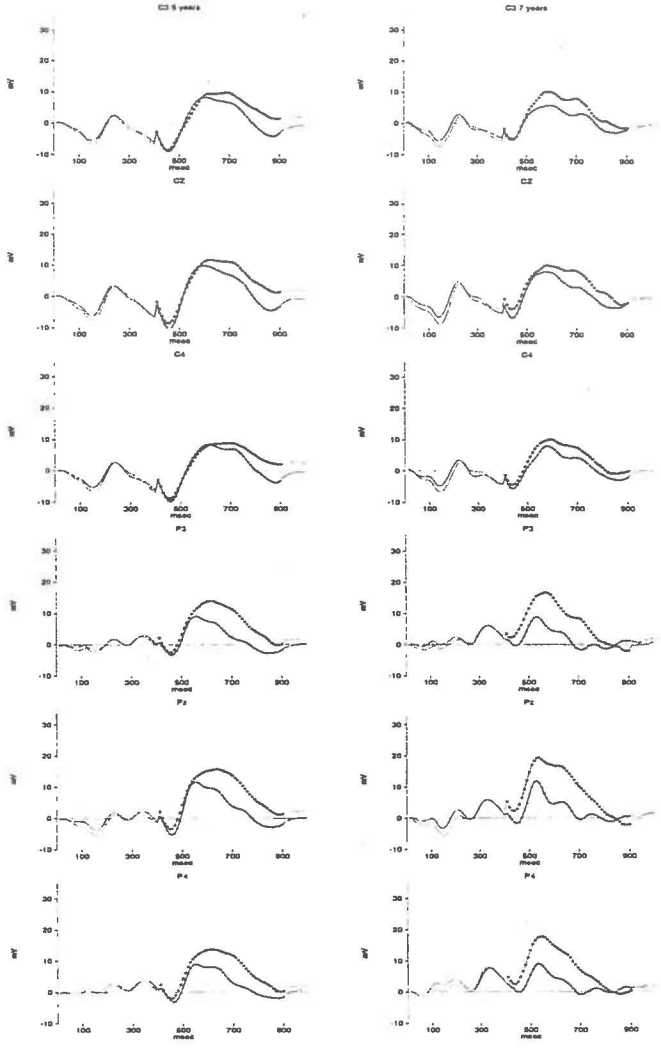

Figure 3.2 *Event Related Potentials for 6 scalp locations (C3, Cz, C4, P3, Pz, P4), for two stimulus types (targets and non-targets) and 2 measurement occasions (age 5 and age 7). The thin lines from 0 to 400 ms represent the non-Woody-filtered ERP, whereas the thicker lines from 400 to 900 ms represent the Woody-filtered ERPs, in which the P3 peak is identified. The Woody filter is applied to correct for latency jitter. The peak at the border is a result of applying the Woody filter. Lines with dots are ERPs elicited by target stimuli, lines with crosses are ERPs elicited by nontarget stimuli. Although amplitude at age 5 seems to be smaller than at age 7 (notably at Pz and P4 scalp locations for targets), this is a result of the averaging across subjects. Variability between subjects in latency is larger at age 5 than at age 7, and a grand average ERP then results in a broad wave with a lower amplitude. When amplitudes and latencies are obtained in each individual, and then averaged, amplitude is found to be higher at age 5 compared with age 7.*

Stability

Test-retest correlations between the first and second measurement occasion (1.5-years' interval) were computed to obtain information about stability of P3 amplitudes and P3 latencies (see Table 3.1). For nontarget P3 amplitudes correlations varied from .28 (C4) to .38 (Pz). Test-retest correlations for target P3 amplitudes were slightly lower. Likewise, P3 latencies showed low test-retest correlations for both targets and nontargets. Overall, midline electrodes (Cz and Pz) showed higher test-retest correlations than lateral electrodes for amplitude and latency.

Table 3.1 *Split-half correlations for nontargets at age 5 years and at age 7 years and test-retest correlations (1.5 years' interval) for targets and nontargets are depicted for amplitudes and latencies. Since the calculated split-half correlation (r^2) is the reliability of half the test, a correction was applied to estimate the reliability of the whole test (Drenth, 1975) using the Spearman-Brown formula: Split Half Reliability = $2 \times r^2 / (1 + r^2)$.*

Amplitudes		C3	Cz	C4	P3	Pz	P4
Split-half	nontarget, 5 year	.53	.56	.50	.62	.66	.55
Split-half	nontarget, 7 year	.54	.55	.58	.62	.66	.63
Test-retest	target	.25	.30	.17	.25	.36	.29
Test-retest	nontarget	.28	.35	.28	.35	.38	.35
Latencies							
Split-half	nontarget, 5 year	.48	.52	.56	.52	.37	.40
Split-half	nontarget, 7 year	.58	.59	.68	.46	.64	.49
Test-retest	target	.18	.12	.18	.27	.17	.09
Test-retest	nontarget	.24	.29	.17	.11	.24	.11

Univariate genetic analyses

Observed twin correlations for P3 amplitude and P3 latency, for targets and nontargets, for ages 5 and 7 are given in the appendix 3.1. For nontarget amplitudes MZ correlations were generally higher than DZ correlations at parietal electrode locations, but this pattern was less pronounced at central electrode locations. For target amplitudes most correlations were low, and no consistent pattern of familial resemblance could be distinguished. Twin correlations for nontarget and target latencies were low at age 5. At age 7 moderate MZ correlations were found for nontarget latencies which were higher than DZ correlations. For targets correlations point to some form of familial resemblance, but the pattern is not consistent.

To make our study comparable to previous analyses in twins (e.g., Van Beijsterveldt et al., submitted; O'Connor et al., 1994) a series of univariate models were fitted to these

data. For P3 amplitudes a model containing genetic and common environmental factors (GCE model) was never significantly better than a GE or CE model. However, most of the time it was difficult to make a distinction between the latter two models. In general, for nontarget amplitudes a GE model fitted the data best, although a CE model also described the data better than an E model. Heritabilities for nontarget amplitudes were about 40% at age 5, and slightly higher at age 7. For target amplitudes, heritability was zero and E models were sufficient to describe the data on both measurement occasions, and for all electrodes.

For nontarget latencies at age 5 an E model was found at central scalp locations, and a CE or GE model at parietal electrode locations. At age 7, nontarget latencies showed GE models for all scalp locations. Individual variability in target latencies could best be explained with a CE or GE model for the first, and with a GE model for the second measurement occasion. At age 5 h^2 in nontarget latencies was zero on central electrode locations, and about 30% at parietal electrode locations. At age 7 genetic contribution was about 40% on all electrode locations. For target latencies, heritabilities were about 20 to 35%.

Multivariate genetic analyses

The univariate results suggest a large contribution of unique environmental variance to individual differences in P3. The reliability data clearly suggest that this is due to high measurement error. To obtain a better estimate of the heritability of the true phenotype, the data were analyzed multivariately using a target P3 and two nontarget P3s. This allows us to distinguish variances due to the true phenotype and to measurement error.

P3 amplitude

First, an GCE model with sex differences was fitted to the data. In this model estimates for genetic, common environmental and unique environmental factor loadings were allowed to differ between males and females. Factor loadings of error variance were allowed to differ between sexes and between ages. Next, we tried to simplify the pattern of genetic and environmental influences on true-P3s. Testing influences on the reliable part of the variance of P3 amplitude showed that sex differences in parameter estimates were not significant (χ^2s for the comparison of nested models are given in Table 3.2). Common environmental influences could be omitted without significantly reducing the fit of the model. In addition, one common genetic factor was enough to explain the genetic variance in all observed variables. Leaving this genetic factor out (E model) significantly reduced the fit of the model, indicating the importance of genetic influences.

Table 3.2 *χ^2s for 6 electrodes, for 6 models, for amplitudes. Nested models are compared using the difference between χ^2 of the model with its more parsimonious model, indicated in the column "compare". the difference in degrees of freedom is given in the column "Δdf". Critical values at alpha = .05 for 3, 10 or 30 degrees of freedom is 7.82, 18.31 and 43.77 respectively. χ^2 of the best fitting model is printed bold. G refers to a model containing 4 genetic factors, C to a model containing 4 common environmental factors, E to a model containing 4 unique environmental factors. 1G and 2G refers to models containing 1 or 2 genetic factors respectively. "sex" indicates a model in which parameter estimates are allowed to be different for males and females, "no" indicates a model in which parameter estimates are constrained to be equal for males and females.*

Amplitudes	df	Δdf	compare	C3	Cz	C4*	P3	Pz	P4
1. ACEsex	326	-	-	493.45	465.38	469.32	501.68	410.59	433.63
2. GCEno	356	30	1	515.49	478.65	487.46	521.25	438.11	458.12
3. GEno	366	10	2	515.87	481.15	490.94	524.39	438.35	461.19
4. Eno	376	10	3	544.63	521.52	533.00	570.61	493.23	510.23
5. 2G,Eno	369	3	3	516.18	483.93	499.22	525.44	439.12	462.23
6. 1G,Eno	372	3	5	**518.70**	**486.46**	**502.91**	**528.33**	**439.36**	**466.04**

* C4: The difference between χ^2s of the GEno model and the 1G,Eno model is 11.97, critical value = 12.59, with 6 degrees of freedom.

Environmental triangular decomposition showed no clear pattern, and no attempt was made to reduce it. The last model constrained error variances to be the same in males and females, and at ages 5 and 7, except for C3 and C4 scalp location, where error variance was slightly higher at age 7. Table 3.3 shows heritabilities with confidence intervals of the true P3 amplitude, proportion measurement error of total variance, and factor loadings of the common genetic factor and the environmental triangular decomposition. For nontargets the heritabilities ranged from 36% at C4 electrode to 86% at P3 electrode. Heritabilities were low for P3 amplitudes elicited by targets, ranging from 0% to 19%, and true unique environmental influences (i.e., unique environmental influences without measurement error), were high. Measurement error itself explained 39 to 66% of the total variance in P3 amplitude. The confidence intervals indicate that for targets genetic influences were not significantly different from zero at C3, C4 and P3 electrodes. At Cz, Pz and P4 electrodes heritabilities on amplitudes were larger than zero, but significantly smaller than heritabilities for nontarget amplitudes. The lower heritabilities for targets can be a result of lower genetic variance or higher environmental variance in targets than in nontargets. The factor loadings in Table 3.3 show that both these effects are present. Squared genetic factor loadings were smaller for target amplitudes than for nontarget amplitudes, whereas squared (and summed) environmental factor loadings were larger.

Since only one genetic factor accounted for all genetic variances for targets and nontargets at both ages, the genetic correlations between these variables were one: the same genes influence P3 amplitudes for targets and for nontargets, at ages 5 and 7.

Table 3.3 *Results of the best fitting multivariate model of P3 amplitude in response to target and nontarget stimuli at ages 5 and 7 years: factor loadings of genetic factors common to both ages and both stimulus types (Gc), factor loadings of environmental factors (triangular decomposition, E1 to E4) and factor loadings of measurement error factor (U). Estimated heritabilities with their 80% confidence intervals, and percentage of total observed variance explained by measurement error are given in the last two columns.*

		Gc	E1	E2	E3	E4	U	h^2	conf.int.	u^2
	C3									
nontargets	5 yr	2.59	2.24	-	-	-	4.59	.57	(.40-.75)	.64
targets	5 yr	0.91	2.84	3.99	-	-	4.59	.03	(.00-.17)	.46
targets	7 yr	1.17	1.58	1.82	4.05	-	5.21	.06	(.00-.17)	.54
nontargets	7 yr	3.01	0.00	1.59	0.00	2.16	5.21	.56	(.38-.74)	.63
	Cz									
nontargets	5 yr	3.56	2.35	-	-	-	5.86	.70	(.49-.92)	.65
targets	5 yr	2.31	3.13	4.51	-	-	5.86	.15	(.05-.29)	.49
targets	7 yr	2.14	2.55	2.24	5.57	-	5.86	.10	(.01-.26)	.42
nontargets	7 yr	4.03	0.00	0.19	0.98	2.80	5.86	.65	(.45-.85)	.58
	C4									
nontargets	5 yr	2.70	1.79	-	-	-	4.49	.69	(.28-.94)	.66
targets	5 yr	0.67	3.16	4.58	-	-	4.49	.01	(.00-.11)	.39
targets	7 yr	0.31	2.87	0.00	4.17	-	5.20	.00	(.00-.11)	.51
nontargets	7 yr	2.64	0.00	0.50	1.90	2.90	5.20	.36	(.19-.78)	.58
	P3									
nontargets	5 yr	4.21	3.53	-	-	-	5.76	.59	(.41-.75)	.52
targets	5 yr	0.89	2.99	4.11	-	-	5.76	.03	(.00-.12)	.55
targets	7 yr	1.11	1.84	1.95	4.32	-	5.76	.05	(.00-.13)	.55
nontargets	7 yr	4.32	0.32	1.75	0.00	0.00	5.76	.86	(.68-.95)	.60
	Pz									
nontargets	5 yr	5.27	2.91	-	-	-	6.00	.77	(.63-.90)	.50
targets	5 yr	2.55	3.59	4.97	-	-	6.00	.15	(.06-.25)	.45
targets	7 yr	2.46	3.60	2.11	4.59	-	6.00	.14	(.06-.24)	.45
nontargets	7 yr	5.20	0.00	0.00	0.00	3.03	6.00	.75	(.61-.88)	.50
	P4									
nontargets	5 yr	3.77	3.02	-	-	-	5.94	.61	(.44-.79)	.60
targets	5 yr	2.01	2.03	5.30	-	-	5.94	.11	(.04-.21)	.49
targets	7 yr	2.27	0.42	2.60	3.93	-	5.94	.19	(.06-.35)	.56
nontargets	7 yr	4.70	0.00	0.00	0.82	2.37	5.94	.78	(.61-.93)	.55

P3 latency

Table 3.4 shows χ^2s for the nested models. For P3 latency sex differences and common environmental influences were not significant in the reliable part of the model. A genetic one factor model was sufficient to describe the data for C3, C4, Pz and P4 location, whereas a second genetic factor was necessary for Cz and P3 electrode location. For the Cz electrode, the correlations between targets and nontargets are almost 1 (respectively 1 and .91 for ages 5 and 7), indicating that the same genes influence variability in both stimulus types. However, genetic correlations between ages 5 and 7 were .29 for nontargets and .65 for targets, indicating new genetic influences at both targets and nontargets at age 7. For P3 electrode position the same pattern was seen: genetic correlations between stimulus types were .97 and .98 at age 5 and age 7 respectively, but genetic correlations between ages 5 and 7 were .39 and .74 for nontargets and targets respectively.

Table 3.4 *χ^2s for 6 electrodes, for 6 models, for latencies. For additional information, see table 3.2.*

Latencies	df	Δdf	compare	C3	Cz	C4	P3	Pz	P4
1. GCEsex	326	-	-	460.51	450.18	490.30	405.41	462.46	484.03
2. GCEno	356	30	1	474.11	467.27	505.67	433.25	476.18	492.39
3. GEno	366	10	2	476.32	471.83	511.80	436.01	477.61	500.41
4. Eno	376	10	3	508.19	503.97	539.93	482.30	532.79	439.85
5. 2G,Eno	369	3	3	476.40	**472.51**	513.87	**440.88**	480.66	500.99
6. 1G,Eno	372	3	5	**479.45**	482.09	**521.22**	451.30	**485.99**	**506.19**

Table 3.5 depicts heritabilities with confidence intervals of the true P3 latency, proportion measurement error of total variance, and factor loadings of the common genetic factor and the environmental triangular decomposition. Based on the confidence intervals heritabilities were always significantly larger than zero. On the whole, estimates of latency heritabilities were higher at age 7 than at age 5. For age 5 h^2 ranged from 13% to 78%, for age 7 h^2 ranged from 36% to 99%. The confidence intervals of these estimates mostly overlapped, so that there do not seem to be reliable differences between latency heritabilities at ages 5 and 7. Measurement error explained 49 to 83% of the total variance in P3 latency at both ages. Although the differences between heritabilities at ages 5 and 7 are not significant, a trend for an increase with age in heritability seems to stem from an increasing genetic variance and a decreasing environmental variance with age. Measurement errors were the same for males and females (except for Cz electrode

location), but they were significantly larger at age 5 than at age 7, as is shown by the factor loadings of U.

Table 3.5 *Results of the best fitting multivariate model of P3 latency in response to target and nontarget stimuli at ages 5 and 7 years: factor loadings of genetic factors common to both ages and both stimulus types (G1) and an additional genetic factor mainly loading on latencies at age 7 (G2), factor loadings of environmental factors (triangular decomposition, E1 to E4) and factor loadings of measurement error factor (U). Estimated heritabilities with their 80% confidence intervals, and percentage of total observed variance explained by measurement error are given in the last two columns.*

Latency

		G1	G2	E1	E2	E3	E4	U	h^2	conf.int.	u^2
	C3										
nontargets	5 yr	15.63	-	27.5	-	-	-	45.79	.24	(.10-.40)	.68
targets	5 yr	19.95	-	20.8	27.75	-	-	45.79	.25	(.12-.42)	.57
targets	7 yr	25.70	-	0.29	0.00	6.62	-	37.42	.94	(.78-1.00)	.67
nontargets	7 yr	24.71	-	0.00	0.00	24.14	0.00	37.42	.51	(.36-.66)	.54
	Cz										
nontargets	5 yr	23.48	-	26.4	-	-	-	46.18	.44	(.23-.66)	.63
targets	5 yr	27.78	0.00	8.26	26.97	-	-	46.18	.49	(.22-.81)	.58
targets	7 yr	14.94	17.25	8.18	0.00	17.08	-	38.35	.59	(.31-.95)	.63
nontargets	7 yr	7.28	24.10	10.7	0.00	5.77	16.22	38.35	.61	(.39-.80)	.58
	C4										
nontargets	5 yr	15.47	-	31.6	-	-	-	44.68	.19	(.07-.39)	.62
targets	5 yr	15.53	-	20.0	34.97	-	-	44.68	.13	(.03-.42)	.52
targets	7 yr	22.08	-	0.00	6.15	18.54	-	34.97	.56	(.26-.91)	.58
nontargets	7 yr	21.48	-	0.58	0.00	11.26	26.06	34.97	.36	(.10-.55)	.49
	P3										
nontargets	5 yr	25.69	-	28.67	-	-	-	51.88	.45	(.19-.78)	.64
targets	5 yr	32.85	8.38	0.00	18.09	-	-	51.88	.78	(.40-1.00)	.65
targets	7 yr	13.70	21.03	0.00	5.77	1.80	-	43.04	.95	(.40-1.00)	.74
nontargets	7 yr	8.96	22.00	9.57	0.00	18.99	0.00	43.04	.54	(.31-.77)	.66
	Pz										
nontargets	5 yr	19.36	-	21.12	-	-	-	52.30	.46	(.29-.68)	.77
targets	5 yr	17.87	-	19.54	21.88	-	-	52.30	.27	(.12-.51)	.70
targets	7 yr	23.50	-	0.00	2.05	17.65	-	35.63	.64	(.43-.89)	.59
nontargets	7 yr	30.37	-	0.00	0.00	0.00	15.80	35.63	.79	(.63-.94)	.52
	P4										
nontargets	5 yr	15.02	-	26.49	-	-	-	55.38	.24	(.11-.41)	.77
targets	5 yr	13.94	-	23.33	17.45	-	-	55.38	.19	(.03-.43)	.75
targets	7 yr	19.99	-	0.00	0.19	1.38	-	44.65	.99	(.71-1.00)	.83
nontargets	7 yr	32.80	-	0.00	0.00	8.73	0.00	44.65	.93	(.73-1.00)	.63

For Cz measurement errors were not the same for males and females at age 7. u^2 was .57 (targets) and .54 (nontargets) for males, and .66 and .63 respectively for females. For purposes of uniformity the estimates based on the model without sex differences are given.

Discussion

This paper examined the genetic and environmental contribution to individual differences in children's P3 latency and amplitude and the changes in that contribution from age 5 to 7. The first part of this section will briefly discuss the main findings on heritability at both ages. The second part will discuss the stability of genetic factors across time.

To evoke a P3, the present study used the same simple visual oddball task that was used in two previous studies of Dutch children of similar age (Wijker, 1991; Stauder, 1992). Mean values of P3 amplitude and P3 latency of the twins agreed well with those found in the non-twin children from these studies. There were no sex differences for mean values of amplitude or latency of the P3.

Multivariate genetic analyses showed that heritability for the P3 amplitude to targets was low. In contrast, high heritability of non-target P3 was found at age 5 as well as age 7. Two previous studies showed higher heritability for target P3 amplitude. An auditory oddball task in adult twins estimated heritability at 41 to 60% (O'Connor et al., 1994). No data were reported on non-target amplitudes. Using the same visual oddball as the present study, Van Beijsterveldt (1996) found heritability to range from 42% to 60% in a large group of adolescent twins. In agreement with our finding in children the individual variation in P3 amplitude to non-targets was mainly genetic in the adolescents and, there too, heritability of non-target amplitude was higher than that of the targets. The difference in heritability of targets and non-targets is intriguing. Although its impact on individual phenotypic variance was less strong in targets, the genetic factor influencing targets was the same as that influencing non-targets. This implies that the same set of neural generators are responsible for (automatic) generation of the P3 wave to target and non-target stimuli. However, the substantial difference in heritability suggests that during (attentive) processing of the relevant target the influence of unique environmental sources increases strongly. Based on current interpretations of the P3 amplitude (Polich, 1996) these environmental influences may be related to general arousal or motivational effort to allocate attentional resources to the target stimuli. Although we deliberately chose a very simple task, the latter is far more difficult to standardize in young children than in adults.

The striking differences in heritability between P3 amplitude to targets and non-targets has repercussion for the use of P3 amplitude as a marker for pathophysiological states. Past characterization of P3s normative values has yielded baseline measures against which deviant behaviors can be evaluated. For instance, individual differences in P3 amplitude have been used as an indicator of clinical disorders, in autism (Kemner et al., 1992) and alcoholism (Begleiter et al., 1988; Polich et al., 1994). Polich and

colleagues (1994) conducted a meta-analysis on 22 studies which showed that relatives of alcoholics demonstrated smaller P3 amplitudes than controls. The strongest effects were found for young boys (younger than 18). This may indicate that P3 amplitude has a predictive value as an index of susceptibility for alcoholism. In as far as this susceptibility has a genetic nature, our present results suggest that the P3 to non-targets may be a better marker than that to targets, at least in young children. Stated otherwise, linkage studies aimed at finding the locations for genes influencing P3 generators might best use the P3 amplitude to non-targets as the quantitative trait rather than the P3 to targets.

There were no differences in heritability for the P3 latency. Speed of target and non-target processing appeared to be influenced strongly by genetic effects. This contrasts with the results of two other large twin studies on P3 that did not find evidence for heritability of P3 latency in adults or adolescents (O'Connor et al., 1994; Van Beijsterveldt et al., 1996). However, measurement error may have seriously affected their model fitting. In the present study, split-half reliabilities of P3 amplitude and latency were unacceptably low, particularly at age 5. A large measurement error can have serious implications for detecting genetic or common environmental influences on individual differences. This is reflected clearly in the differences between heritabilities from the univariate and the multivariate model fitting analyses. In the univariate models low estimates of either h^2 or c^2 were obtained and it was difficult to decide if the familial resemblance was of genetic or common environmental origin. In the multivariate model, however, using odd and even trials of P3 latency as two separate variables, the contribution of unique environment clearly decreased, yielding true estimates of heritability for the various locations of 34% on average at age 5 and 70% at age 7. The importance of genetic influences on P3 latency has in fact already been demonstrated in candidate gene research (Noble et al., 1994). They showed that P3 latency was significantly longer in 10 to 14 year old boys with the D2 dopamine receptor A1 allelle than with the A2 allelle.

The heritability of latency implies that speed of information processing in young children is to a large extent influenced by genetic factors. As with amplitude, the same genetic factor influenced targets and non-targets. In contrast to amplitude, however, no differences were seen in heritability of target and non-target latency. Apparently, the speed of target and non-target processing depends on the same individual characteristic. Although many genes may contribute to this processing speed, it has been argued convincingly that the A1/A2 polymorphism for the D2 dopamine receptor plays a significant role, probably by affecting the total number of D2 binding sites in the mesolimbic system (Noble et al., 1994).

Longitudinal analyses

Apart from establishing heritability of P3 in childhood, this study aimed to detect the emergence of new environmental or genetic factors during maturation. Children from 5 to 7 years of age were used, because this is a period in which large cognitive changes occur. Across the repeated measurements, the P3 amplitude was seen to decrease slightly at central and parietal locations. Previous studies employing simple oddball or novelty tasks in this age range have yielded mixed results. Mostly P3 amplitude is seen to decrease in childhood (Courchesne, 1977; 1978; 1983; 1990; Friedman, 1991; Stauder, 1992) although some have reported no change (Wijker, 1991) or even increases (Mullis et al., 1985; Taylor, 1988; Polich et al., 1990). However, an apparent increase in P3 amplitude with age may be caused by the age-related decrease in variability in the latency of single trials. Correcting latency jitter by means of a Woody filter, as in the current paper, allows for a better comparison across time. In agreement with virtually all published reports (e.g., Johnson, 1988; Friedman, 1992), P3 latency decreased from age 5 to age 7. This decrease reflects an increase in information processing speed. Several studies show that the decrease will continue until adolescence when it reaches its final value of around 300 ms. Imposed on this general developmental trend, large individual differences in P3 amplitude and latency decreases were seen in the children in the present study. As a consequence, phenotypic correlation between first and second measurements was rather low. The low stability of the P3 parameters is mainly due to measurement error but could also be affected by session-specific effects, like seasonal effects or effects due to errors in electrode placement (Polich & Kok, 1995). In addition, differences in maturation are likely to be a relevant major source. Our results suggest that the maturation of P3 is a continuous process. Exactly the same genetic factors appeared to influence P3 amplitude at ages 5 and 7. An additional genetic factor emerged only for P3 latency at Cz and P3. Generally the genetic factor found at age 5 still had large effects on P3 latency at age 7, including locations Cz and P3.

The stability of genetic contribution to the P3 amplitude and latency was an unexpected finding. Previous research on IQ has suggested that new genetic factors emerge between the ages 4 and 7 (Cherny & Cardon, 1994). Because latency has been associated with neural speed and IQ (Chalke & Ertl, 1965, Barrett & Eysenck, 1992), we expected new genetic factors to emerge for P3 latency as well. Instead, we only found an increase in heritability from age 5 to age 7. However, in the same sample of twins we found an exactly similar pattern for IQ data (Boomsma & van Baal, in press): an increase in genetic variance coinciding with a decrease in environmental variance. Preliminary results of a combined analysis further indicate that correlations between latency at P3 electrode and IQ are around -.19 for both targets and nontargets. This

correlation emerged in spite of the large measurement error in P3 latencies. It is possible that amplification of genetic effects on neural speed, as indexed by P3 latency, precede the increased heritability of IQ. To test this hypothesis, we need to use an extended model, which includes IQ measures and P3 measures and which accounts for measurement errors. This next step will be pursued in the future.

Appendix 3.1: *Observed twin correlations for amplitudes and latencies elicited by target and nontarget stimuli at ages 5 and 7 years are given for 6 electrode locations and for 5 sex by zygosity groups.*

Amplitude, targets, 5 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.10	-.18	-.04	-.17	-.30	-.02
DZM	.09	.14	.16	-.03	.04	-.13
MZF	.51	.02	-.02	.32	.28	.25
DZF	-.16	.07	.13	.08	.06	.12
DOS	-.11	-.27	-.18	.00	.05	.04

Amplitude, targets, 7 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.16	.27	-.05	-.02	-.12	.05
DZM	.11	.26	.21	.26	.39	.32
MZF	.14	.21	.13	.17	.23	-.04
DZF	-.24	-.33	.05	-.11	-.40	.39
DOS	.02	.25	.16	.31	.11	.19

Amplitude, nontargets, 5 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.08	.34	.27	.45	.34	.49
DZM	.13	.36	.15	.37	.24	.20
MZF	.37	.37	.42	.44	.48	.32
DZF	.18	.08	.27	.37	.17	.54
DOS	.39	.71	.36	-.30	.16	-.13

Amplitude, nontargets, 7 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.13	.52	.45	.26	.65	.62
DZM	.23	-.31	.37	.02	.27	.16
MZF	.39	.44	.27	.50	.58	.59
DZF	.15	-.27	.16	.37	.28	.39
DOS	.38	.39	.19	.38	.28	.14

Latency, targets, 5 years						
	C3	Cz	C4	P3	Pz	P4
MZM	-.10	.14	.02	.21	.03	.23
DZM	.25	.11	.23	.25	-.19	.32
MZF	.26	.28	.16	.33	.36	.16
DZF	.29	.17	.07	.32	-.01	.28
DOS	.06	.30	.38	.17	.26	.21

Latency, targets, 7 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.31	.36	.34	.19	.48	.45
DZM	.31	-.04	.27	.37	.19	-.08
MZF	.55	.24	.19	.48	.34	.38
DZF	.08	.21	.25	.14	.16	-.29
DOS	.42	.23	.12	.20	.33	.40

Latency, nontargets, 5 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.08	.10	.01	.29	-.23	-.08
DZM	.19	.35	.34	.33	.24	.23
MZF	.18	-.03	.15	.39	.50	.41
DZF	.02	-.13	.09	.41	.26	.26
DOS	.03	.15	.11	.03	.13	.43

Latency, nontargets, 7 years						
	C3	Cz	C4	P3	Pz	P4
MZM	.21	.44	.44	.51	.41	.28
DZM	.29	.40	.19	.39	.04	.27
MZF	.62	.51	.42	.44	.59	.66
DZF	.20	.14	.33	.14	.34	.30
DOS	.08	.00	.01	.04	.11	-.08

4

Genetic influences on EEG coherence in five year old twins

G.C.M. van Baal, E.J.C. de Geus and D.I. Boomsma

Electroencephalographic (EEG) coherence has been suggested to be an index of the connectivity of the brain. It represents the coupling between two EEG signals from different brain areas and is mathematically analogous to a cross-correlation in the frequency domain. We obtained data from 167 pairs of five year old twins to study genetic and environmental influences on individual differences in intrahemispheric coherences. Coherence was computed in the theta band (4.0 to 7.5 cycles per second) between prefrontal, frontal, central, parietal and occipital regions during quiet rest. Univariate genetic analyses of the data showed moderate to strong genetic influences for all coherences. Broad heritabilities ranged from 30 to 71% with a mean heritability of 49%. With one exception, no sex differences were found. Split-half reliability varied with inter-electrode distances, ranging from .91 for the shortest distance to .62 for the longest distance. When split-half reliabilities are compared with heritabilities, the data suggest that for cortico-cortical connections between adjacent brain areas a large part of the variance is explained by "true" environmental influences, whereas for longer connections, that is, sensory to frontal areas, the variance is mostly genetic of origin.

Introduction

Numerous studies have demonstrated that a wide range of human behaviors are influenced by genetic factors (for reviews see Rose, 1995; Boomsma, 1993; Plomin et al., 1994). Such genetically mediated individual differences in behavior may be associated with individual differences in brain functioning. Genetic influences on brain functioning form a good starting point to study genetic influences on complex behavior (Lander, 1988). Brain functioning can be indexed by the electroencephalogram (EEG), which measures electrical activity of the brain. The EEG is composed of many cyclic signals of different frequencies, and spectral analysis is often used to quantify the contribution of these signals. With spectral, or Fourier analysis, the signal is transformed from the time domain to the frequency domain. A widely used spectral analysis EEG index is the power spectrum (i.e., the amount of variance explained by each frequency component in the spectrum). In a previous study we showed that EEG spectral powers show remarkably high heritability (Van Baal et al., 1996) in children. At first sight, this finding indeed seems to bridge the gap between genes and behavior, because EEG power in the broad bands has been associated with a number of temperamental characteristics, for example,

ratio of alpha/beta power with extraversion (Baker, 1978) and intelligence (Gasser et al., 1983). However, the association of EEG power with either behavior or cognition has not been unequivocal (Gale & Edwards, 1986; Anohkin, submitted). In addition, the neural mechanisms generating the surface EEG remain enigmatic. It would be desirable to use EEG parameters that more closely reflect anatomical and neurophysiological parameters, like axonal sprouting, synaptogenesis, myelination, and pruning of synaptic connections. Recent evidence suggests that EEG coherence may be used to index such processes (Kaiser & Gruzelier, 1996).

Coherence is the squared cross-correlation between two signals from scalp locations for each component in the frequency domain. It has been suggested to measure the number of cortico-cortical connections and synaptic strength of connections between 2 different brain areas (Thatcher et al., 1986; Thatcher et al., 1987; Thatcher, 1991; 1994a; 1994b). Based on the structural properties of the human cortex Thatcher and colleagues (1986) proposed a 'two-compartmental' model of EEG coherence. EEG generating cells in the neocortex are either (1) pyramidal cells with long distance loop connections (e.g., fronto-occipital) of an excitatory nature, or (2) highly branched stellate cells with only short distance connections (e.g., intercolumnar) of both excitatory and inhibitory nature (Braitenberg, 1978; Szentagothai, 1978). The pyramidal cells act in two different compartments: compartment A is composed of the basal dendrites that receive input primarily from the axon collaterals from neighboring or short-distance pyramidal cells, while compartment B is composed of the apical dendrites of cortical pyramidal cells that receive input primarily from long distance intracortical connections. Short distance coherence between electrodes for as far as 14 cm apart can be influenced by the short fiber system, while longer distance coherence is influenced only by the long distance fiber system, which represent the majority of white matter fibers. In children, short distance coherence has been found to be higher in cognitive dysfunctions. Gasser and colleagues (1987) showed that 10 to 13 year old mildly retarded children had higher coherences than controls. Higher short distance coherences were also found in dyslectics (Leisman & Ashkenazi, 1980) and in Down's syndrome (Schmid et al., 1992). In a population of normal children, Thatcher et al. (1983) showed that a negative correlation exists between full-scale IQ and short-distance coherences. A possible explanation for these findings is that in a normal brain selective synaptic pruning leads to less dispersion of neural signals, and thus lowers short distance coherences. Intelligence may be reflected in a greater specificity of short distance cortico-cortical connections, thus further lowering coherence.

The main question to be addressed in this paper is the extent to which the inter-individual variance in short distance coherences in 5 year old children is influenced by genetic or by environmental factors. In addition, we wanted to examine the genetic

architecture of the second compartment, which is reflected in the long distance coherences. Seventy monozygotic and 97 dizygotic twin pairs were used to estimate contribution of genetic and common and unique environmental influences on right and left intrahemispheric coherence of resting background EEG. Since large sex differences in interconnectivity of brain areas are suggested in 5 year olds, as reflected in mean differences in coherences (Marosi et al., 1993), the group of DZ twins included 31 opposite sex twin pairs to test for sex limitation. Because measurement error may be a concern when studying young children, split-half reliabilities were calculated to get more insight in the actual reliability of our data.

Methods

Subjects

The data presented in this paper were collected in a longitudinal study of genetic and environmental factors that influence neural development in early life. Initially, 209 5 year old twin pairs (mean age = 5.26 years, sd = .19 years) participated. All subjects were healthy, had a normal IQ (Boomsma & Van Baal, in press), and had normal or corrected to normal vision. The twin pairs were registered in the Netherlands Twin Register, which contains between 45 and 50% of all Dutch twins born after 1986 (Boomsma et al., 1992). Zygosity determination for same-sex twin pairs was done either by blood typing (ABO, MNS, Rhesus, Kell, Duffy, Kidd, Lutheran) or by DNA fingerprinting (N = 159). For 11 same-sex twin pairs these data were not available. These twins were assigned to a zygosity group using a discriminant analysis based on their physical appearances (hair color, hair structure, confusion by acquaintances, confusion by close friends of the family). Eighteen twin pairs had incomplete EEG data because of difficulties during the experiment. Children who fell asleep during the experiment (11), and children who showed high levels of arousal or cried (13) were excluded from further analyses. This left 167 twin pairs (33 monozygotic males (MZM), 34 dizygotic males (DZM), 37 monozygotic females (MZF), 32 dizygotic females (DZF), 31 dizygotic opposite-sex twins (DOS)) with complete data.

Procedure

Detailed procedures of data collection are described elsewhere (Van Baal et al., 1996). Briefly, an electrocap with electrodes in the 10-20 system of Jasper (1958) was used to measure brain activity on 14 scalp locations during a visual oddball task, and during three minutes of quiet rest with eyes open and 3 minutes of quiet rest with eyes

closed. Vertical and horizontal eye movements were recorded for correction of the EEG signal for eye-movement artifacts. EEG was recorded unipolarly with linked ears reference according to the method described by Pivik et al. (1993). All electrode impedances were kept below 10 Kohm. EEG was recorded continuously on a 18 channel Nihon Kohden PV-441A polygraph. Time constants were set to 5 s, high frequency cut-off was 35 Hz and sample frequency was 250 Hz. Signals were converted with a 12 bits AD converter. This paper reports on coherence measured during quiet rest with eyes closed.

Data quantification and data reduction

After removal of EOG artifacts using dynamic regression in the frequency domain (Brillinger, 1975), the EEG signal was divided into 90 two-second epochs. Epochs with clippings and with abnormal EEG patterns (detected during visual inspection) were excluded from further analysis. For every epoch and for every scalp location the raw EEG was converted from the time domain into the frequency domain using Fast Fourier Transformation (FFT), which yielded power spectra for every electrode position, and cross spectra and phase spectra for every electrode combination. The phase spectrum depicts the lead-lag relation between the signals at different scalp locations for every frequency band: Phase spectra were used to determine whether the signals of two scalp locations actually had a phase difference, because zero phase differences would point to signal transport other than via the axonal fibers. EEG coherence spectra were calculated from power- and cross-spectra. Smoothed power- and cross-spectra were obtained by calculating the mean of all spectra over all valid epochs (minimum number of epochs = 30). Power spectra ranged from .5 Hz to 30 Hz with a .5 Hz resolution. The cross spectra indicate the covariance of two signals in a certain frequency band. Coherence spectra for every frequency band were calculated using the formula:

$$\text{coherence} = \frac{(\text{cross spectrum } (1,2))^2}{\text{power spectrum } (1) \times \text{power spectrum } (2)}$$

where 1 and 2 refer to signal 1 and signal 2

As is shown in this formula, coherence measures the square of the linear association between two signals and is analogous to the square of the usual correlation coefficient. Thus, coherence ranges from 0 to 1. From the coherence spectra, mean coherence for the theta band, ranging from 4.0 to 7.5 Hz, was calculated. For children, theta is a major frequency band (Niedermeyer & Lopes da Silva, 1993). Data were transformed using the

formula transformed coherence = ^{10}log(untransformed coherence/1-untransformed coherence) to obtain a normal distribution of the data (Thatcher et al., 1983).

Since the majority of cortico-cortical connections are within the same hemisphere (Nunez, 1981), coherence was calculated intrahemispherically for the following combinations of scalp locations (depicted in Figure 4.1): *short distance coherences:* from prefrontal to frontal (Fp1-F3, Fp2-F4), from prefrontal to central (Fp1-C3, Fp2-C4), from central to occipital (C3-O1, C4-O2), from parietal to occipital (P3-O1, P4-O2), and *long distance coherences:* from prefrontal to parietal (Fp1-P3, Fp2-P4), from prefrontal to occipital (Fp1-O1, Fp2-O2), from frontal to occipital (F3-O1, F4-O2).

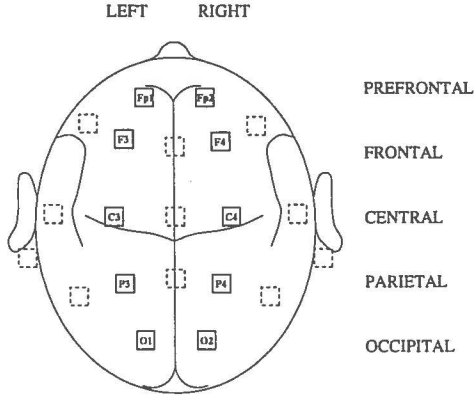

Figure 4.1 *EEG was measured at the following scalp locations: prefrontal (Fp1, Fp2), frontal (F7, F3, F4, F8), central (C3, C4), parietal (P3, P4), occipital (O1, O2) and temporal (T5 T6). This paper shows results of coherences between solid squares (i.e., Fp1 with F3, C3, P3 and O1; O1 with P3, C3, F3 and Fp1; and their right hemisphere analogues).*

Statistical analysis

Differences in mean values of coherences between males and females, and between MZ and DZ twins were tested using the ANOVA procedures from SPSSPC 5.0. Since data of twin A and twin B are not independent, cotwins of a twin pair were tested separately.

To obtain an indication of the reliability of the coherence measures, split-half correlations were calculated. Two coherence spectra per subject and per electrode combination were computed: one averaged over all odd, and one averaged over all even 2-sec epochs from the total EEG registrations (again with a minimum of 30 epochs per average). The correlation between the coherences of those two sets of signals provides a measure of reliability.

The observed variance in the EEG coherence was decomposed into genetic and environmental parts (Neale & Cardon, 1992), using structural equation modeling with the computer program Mx (Neale, 1994). Since MZ twins share all their genes and DZ twins share 50% on average, the correlation between additive (A) and between dominant (D) genetic factors equals 1 for MZ twins, and .5 and .25 respectively for DZ twins. Environmental effects were distinguished into common (C) and unique (E) environmental factors. Common environmental factors will make twins more alike, since they share the same effect from the environment. Both MZ and DZ correlations will become larger due to this effect. Unique environmental effects are responsible for differences between MZ twins. The correlation between the shared environmental factors is 1 in both MZ and DZ twins. Correlations for the non-shared, unique environmental influences (E) are 0 for both types of twins.

A number of models were fitted to the data: ADE, ACE, AE and E models, with or without sex differences. Sex differences were examined in three different models: (1) Scalar effects sex-limitation models, in which a difference in total variance between males and females was allowed, but in which the relative contributions of A, D or C, and E were the same for males and females; (2) Common effects sex-limitation models. In these models the relative magnitude of genetic and environmental factors can differ between the sexes, but the same genes and/or common environmental influences are expressed. (3) General sex-limitation models. These test the possibility that sex specific genes exist, which influence the trait in one sex but not in the other. For these models the DOS twins are essential. When the same genes explain part of the variance in both males and females, but their relative contributions differ, then the additive genetic correlation in DOS pairs is .5. When different genes account for the phenotypic variance the additive genetic correlation in DOS pairs will be smaller than .5, or even zero, and the observed correlation in DOS pairs will be smaller than in same-sex pairs.

Data on male and female same-sex MZ and DZ twins and on DZ opposite-sex twins were summarized into five 2 by 2 variance-covariance matrices. The models outlined above were fitted to these matrices. The values of the factor loadings of A, D or C, and E, expressed as parameters a, d or c, and e, and their confidence intervals were estimated by maximum likelihood. The fit between the observed data and the model was assessed by χ^2 tests. To compare the fit of two different models, hierarchic χ^2 tests were used. With these tests the χ^2 of a nested model is subtracted from the χ^2 of a more parsimonious model. This difference is again χ^2 distributed, with the difference of df's of the two models as degrees of freedom. For the best fitting models heritability (h^2) was calculated as the proportion of additive genetic variance of total variance, and d^2 as the proportion of nonadditive genetic variance of total variance.

Results

Figure 4.2 shows mean coherences in the theta band for different electrode distances, for left and right hemispheres, and for males and females. The larger the distance between the electrodes, the lower coherence becomes. The values that are presented have been transformed back after calculating means for transformed coherences. Since average coherences at longer distances become small, we may wonder whether variability will be large enough to be decomposed into different parts. Figure 4.3 demonstrates that variability of the log-transformed coherences is very much alike for short and for long distances.

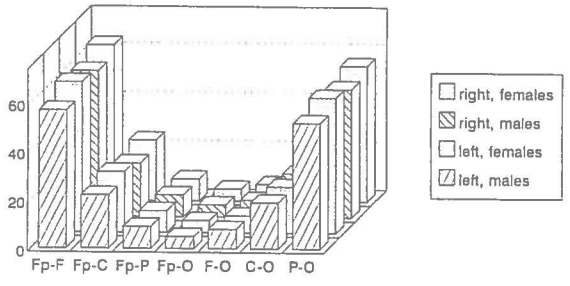

Figure 4.2 *For males and females, mean left and right theta coherences for 7 electrode combinations within each hemisphere, with different interelectrode distances are depicted. Fp is prefrontal scalp location, F is frontal, C is central, P is parietal and O is occipital. Mean distances between electrodes is 7, 14, 21, 28, 21, 14 and 7 cm respectively.*

Mean values for coherences were larger in females for a number of cortico-cortical connections. In the left hemisphere the coherences between prefrontal and frontal (twin A: $F(1,166) = 8.00$, $p = 0.005$; twin B: $F(1,166) = 7.01$, $p = 0.009$), between prefrontal and central (twin A: $F(1,166) = 6.94$, $p = 0.009$; twin B: $F(1,166) = 4.48$, $p = 0.036$), and between parietal and occipital (twin A: $F(1,166) = 5.62$, $p = 0.019$; twin B: $F(1,166) = 7.36$, $p = 0.007$) electrodes were significantly higher for females. In the right hemisphere significantly different higher coherences for females were found in twin B only, between prefrontal and frontal ($F(1,166) = 7.85$, $p = 0.006$), and between prefrontal and central ($F(1,166) = 4.18$, $p = 0.043$) electrode positions (for twin A a trend towards this effect was seen). Mean values for MZ and DZ twins were never different, and no interactions were found.

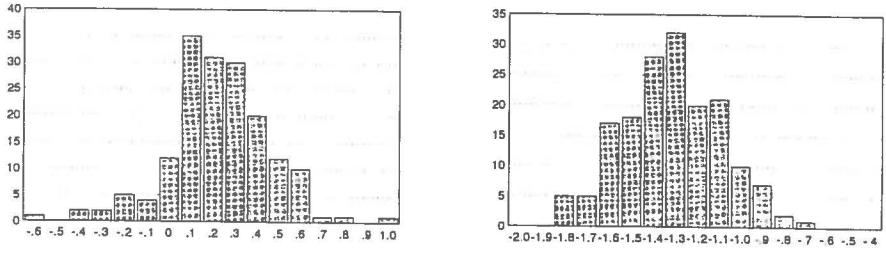

Figure 4.3 *Variability of transformed coherences (y = log(x/1-x)) for short distance (about 7 cm, Fp2-F4) and for long distance (about 28 cm, Fp2-O2). Although mean values are rather different, variabilities are comparable.*

Split-half correlations of coherences between 14 electrode combinations are shown in Table 4.1. Reliability becomes slightly lower with longer distances. Mean split-half correlations are .91, .86, .73 and .62 for 7, 14, 21 and 28 cm interelectrode distance, respectively. This would indicate that measurement error is larger for coherences at longer distances.

Twin correlations are also presented in Table 4.1. As can be seen, correlations were almost always larger for MZ twins than for DZ twins, indicating that genetic influences are of importance. Differences between MZF and MZM correlations would suggest differences in heritabilities between males and females. MZF correlations seemed to be larger than MZM correlations for coherences between left prefrontal and central scalp locations (Fp1-C3) and for frontal with occipital leads (F3-O1), suggesting sex differences in heritabilities of coherences. DOS correlations were not notably lower than same-sex DZ correlations, except for coherence between right occipital electrode (O2) with frontal or prefrontal electrodes (F4 and Fp2). All these effects were then formally tested using model fitting.

The bottom part of Table 4.1 shows model fitting statistics. Model fitting revealed that either an AE model or an ADE model fitted the data best. A common effects sex limitation model reached significance only once: females showed higher heritability than males for coherence between left prefrontal and frontal scalp locations ($\Delta\chi^2(2) = 7.86$). No indication was found for common environmental effects. In most cases a good fit was obtained with a low χ^2 and a high p-value. For coherences between near-by electrodes, an AE model was sufficient to describe the data, whereas for electrode combinations at longer distance, dominance effects were significant, except for coherence between left prefrontal and occipital electrode positions. When dominance was included in the model,

Table 4.1 *Reliabilities, twin correlations and model fitting results of intrahemispheric theta coherences. For left and right hemispheres and for 7 electrode combinations, the following information is given: Distance is the approximate distance between the electrodes; Reliability is calculated using the split-half approach; Twin correlations are given for 5 sex by zygosity groups; Model fitting results indicate best model with its χ^2, df and p value. $\Delta\chi^2$ is the difference between an AE and an ADE model without sex differences, df = 1. For the best fitting model, parameter estimates of additive heritability (h^2) and dominant heritability (d^2) are given. When dominance is not included, d^2 is indicated with -.*

Mean coherence in theta: left hemisphere, eyes closed

Distance	Fp1-F3 7 cm	Fp1-C3 14 cm	Fp1-P3 21 cm	Fp1-O1 28 cm	F3-O1 21 cm	C3-O1 14 cm	P3-O1 7 cm
Reliability:							
Split-half	.91	.86	.76	.68	.74	.86	.93
Twin correlations:							
MZM	.51	.38	.69	.48	.38	.53	.32
DZM	-.02	.09	.12	.23	-.13	-.06	-.24
MZF	.55	.70	.72	.56	.66	.64	.48
DZF	.39	.14	.07	-.04	.06	.16	.13
DOS	.28	.12	.11	.22	.02	.32	.17
Model fitting:							
Best model	AE	AEsex	ADE	AE	ADE	AE	AE
χ^2	14.96	9.02	8.60	6.51	9.18	9.63	25.77
df	13	11	12	13	12	13	13
p	.310	.620	.737	.925	.688	.724	.018
$\Delta\chi^2$	0.37	3.21*	7.00	2.29	6.08	2.45	1.78
Estimates:							
h^2	.49	.33/.70**	.00	.50	.00	.55	.30
d^2	-	-	.71	-	.56	-	-

Mean coherence in theta: right hemisphere, eyes closed

Distance	Fp2-F4 7 cm	Fp2-C4 14 cm	Fp2-P4 21 cm	Fp2-O2 28 cm	F4-O2 21 cm	C4-O2 14 cm	P4-O2 7 cm
Reliability:							
Split-half	.89	.86	.74	.57	.69	.85	.92
Twin correlations:							
MZM	.50	.34	.61	.43	.45	.44	.55
DZM	-.15	.12	.16	.05	-.18	.19	.04
MZF	.26	.34	.59	.57	.56	.65	.51
DZF	.37	.25	-.12	.06	.10	.11	.00
DOS	.26	.24	.09	-.18	-.30	.08	.05
Model fitting:							
Best model	AE	AE	ADE	ADE	ADE	AE	AE
χ^2	25.21	18.51	18.84	11.13	13.33	6.29	10.55
df	13	13	12	12	12	13	13
p	.022	.139	.092	.518	.346	.935	.648
$\Delta\chi^2$	0.00	0.00	7.40	4.67	5.25	1.59	2.10
Estimates:							
h^2	.33	.33	.00	.00	.00	.51	.43
d^2	-	-	.59	.49	.46	-	-

* = Fp1-C3 sex-difference: $\Delta\chi^2(2) = 7.86$, difference with ADE model has df = 2
** = male/female

additive genetic effects were estimated to be zero, which is biologically not very plausible.

Figures 4.4a and 4.4b show heritabilities with their 80% confidence intervals for best fitting models. A moderate to large part of the variance was explained by genetic factors for all electrode combinations. Coherences between adjacent electrodes (like Fp1 with F3, or P4 with O2) showed a relatively low heritability, in combination with a high reliability. This indicates that a large part of the variance is due to unique environmental influences, other than measurement error. Longer distances, like Fp2 with P4, or Fp2 with O2, show a relatively large heritability in combination with a somewhat lower reliability, which indicates that the reliable part of the variance is influenced for the largest part by genetic factors.

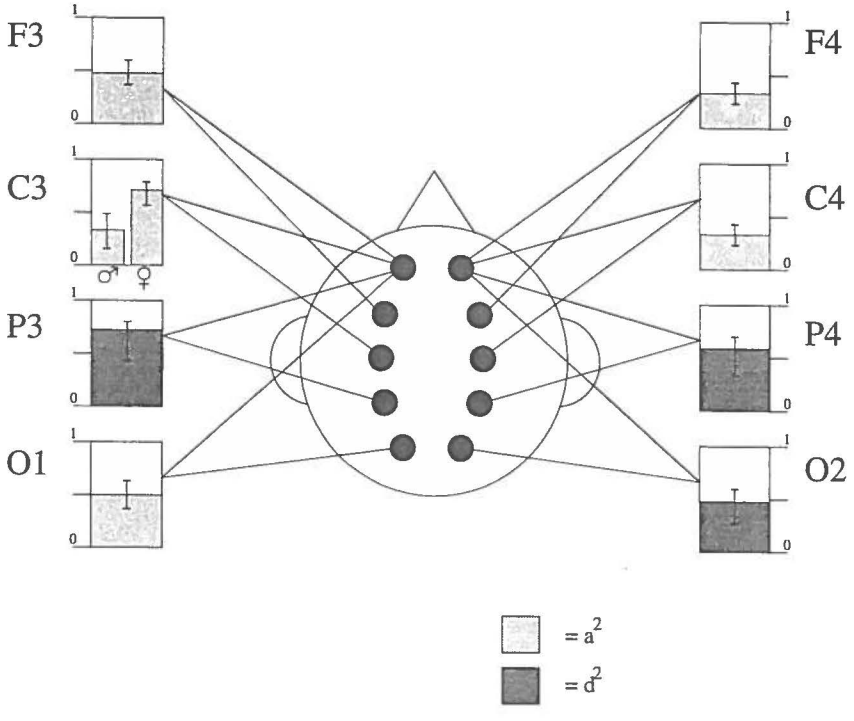

Figure 4.4a *Cortico-cortical coherences with prefrontal electrodes: heritabilities (h^2) and relative influences of dominance (d^2) are shown with their 80% confidence intervals.*

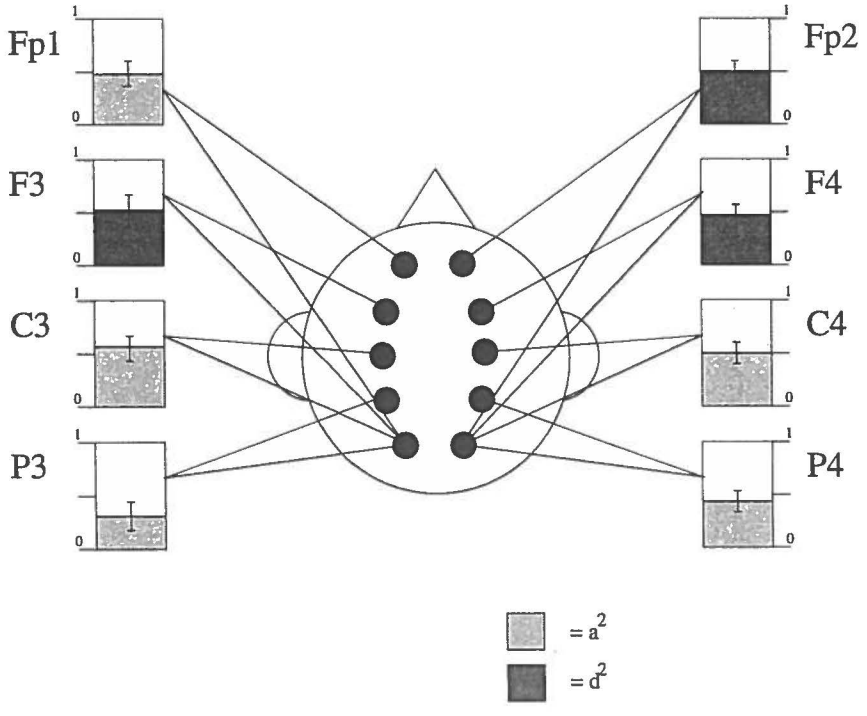

Figure 4.4b *Cortico-cortical coherences with occipital electrodes: heritabilities (h^2) and relative influences of dominance (d^2) are shown with their 80% confidence intervals.*

Discussion

This study has determined the heritability of intrahemispheric coherences in 5 year old children. Overall, genetic as well as unique environmental effects were important for coherences. Broad heritabilities ranged from 30% to 71%, with a mean of 49%. To date, information about genetic and environmental influences on coherence is restricted to a few studies. Ibatoullina and colleagues (1994) studied coherences in 5 year old twins (20 monozygotic (MZ) and 17 dizygotic (DZ)), and found evidence for familial influences. The study was too small to distinguish between genetic and shared environmental influences. Their results certainly allow for the genetic influences found in the present

study. The only other study known to us, a large twin study in 213 adolescent twin pairs (Van Beijsterveldt, 1996) also showed significant genetic influences on EEG intrahemispheric coherences. Heritabilities were slightly higher than in the present study: about 60%. This difference in heritability between children and adolescents, albeit small, agrees with the idea that, depending on age, genetic control on EEG traits may vary. The mean values of both short-range and long-range coherences in the adolescents were lower than coherences in children. The difference in coherence between adolescents and children suggests that both short- and long-range coherences decrease with increasing cognitive maturation.

We chose to study the genetic architecture of EEG coherence, because it has been empirically associated with cognitive abilities and because clear theoretical notions have been put forward to link this trait to structural aspects of the brain. The interpretation of EEG coherence in terms of cortico-cortical connectivity is largely based on a non-linear mathematical wave model by Nunez (1981) that attempts to describe the synchronicity between neural generators in terms of anatomical parameters, such as synaptic delays, conduction velocity and cortico-cortical fiber length. This model has been integrated by Thatcher et al. (1986; Thatcher, 1994a; 1994b) with specific knowledge about the structure of the human neocortex. He distinguished a short-distance fiber system, which gradually becomes less important with increasing distance, and a long-distance fiber system. Kaiser and Gruzelier (1996) hypothesized that changes in short range coherence are associated with changes in synaptic density: further differentiation of local neural circuitry through pruning leads to a smaller dispersion of neural signal and thus increased coherence. Long range coherence, on the other hand, would be lower if the number of synaptic contacts is smaller, although this may be offset by a larger degree of myelination. In spite of its theoretical elegance, the evidence for the existence of separate compartments influencing coherence is incomplete. But several aspects of our results are in good agreement with these theoretical notions about the difference in short and long range coherence. For instance, it is remarkable, that although long-distance as well as short-distance coherences are to a large degree influenced by genetic factors, the long-distance coherences seem to be controlled to a large extent by non-additive, dominant genetic factors, whereas short-distance coherences are controlled by additive genetic factors only. This was also observed by Ibatoullina et al. (1994). Some caution is in order. Using only twin data it is difficult to reliably distinguish additive and dominant genetic effects, which is also reflected in the somewhat larger confidence intervals of d^2 in the ADE models presented in this paper. Nonetheless, when computing phenotypic correlations between long and short range coherences, we found low correlations between different coherences (e.g., between coherence for Fp1-F3 and coherence for Fp1-O1 the correlation was .13).

This further testifies to the fact that different genetic factors underly short and long range coherence.

A final important difference between short and long distance coherences is the difference in the heritability, that was observed after reliability was taken into consideration. Reliability of a trait is the upper bound for heritability, so that a lower reliability would result in a low heritability. We computed split-half correlations, and found that these were high for short-distance coherences (.91), and became somewhat lower with increasing distances (.62 for longest distances). Split-half reliabilities for coherences were about the same as those measured in a group of adolescent twins in our laboratory (Van Beijsterveldt, 1996). Heritabilities roughly stayed the same or became even larger with increasing distance. This indicates that compared with longer distances, for short distances a smaller part of the reliable variance is genetic. Genuine environmental effects play a larger role for short than for long distance coherences, suggesting that, at this age, long-axonal connections between sensory and (pre)frontal areas are more genetically determined than connections between adjacent intrahemispheric cortical areas. Since large sex differences in interconnectivity of brain areas are suggested in 5 year olds (Marosi et al., 1993), we tested for sex differences in mean coherences. In our study slightly higher mean intrahemispheric coherences in females than in males were found, mainly in the left hemisphere and only on short distances. Marosi and colleagues (1993) also found higher coherences in females, but mostly in the right hemisphere. The twins used in our study (aged 5 years) were younger than the children used in their study (aged 7.6 to 13.3 years). Thatcher et al. (1987) suggest that the left hemisphere matures earlier than the right hemisphere. If short distance coherence reflects increased differentiation of the local circuitry, our data and those of Marosi may point to a maturational lead in girls.

Coherence indexes both structural and functional or task-related influences (French & Beaumont, 1984). The structural baseline depends on the anatomical features of the brain, that is, the number and synaptic strength of cortico-cortical connections. However, the actual "state" of coherence can change according to the demands of the task or the emotional state of the subject. The twins in this study were measured in the same resting state, and children with extreme behavior, like crying or sleeping, were discarded from the analyses. In that way we hoped to lower the variance due to the emotional state of the subjects. In spite of these precautions, it is still possible that, apart from estimating heritabilities of cortico-cortical connections, we are also estimating heritabilities of the emotional state of the subject. A further concern in the interpretation in coherence is the confounding by volume conduction. Coherence can in part be due to conductivity through other tissue than axonal fibers, like skull or blood. Although skull is a poor conductor,

blood may serve as a good conductor (Nunez, 1981). Coherence would thus become a function of skull size and blood supply. However, when volume conduction is responsible for coherence between two scalp locations, phase differences between these signals will be zero. When signals are transported via the much slower medium of myelinated axonal fibers, phase differences will reflect the velocity of this electric transport, and will become larger than zero. Phase differences were always non-zero for the intrahemispheric coherences reported here.

In conclusion, our data clearly showed moderate to high genetic influences on intrahemispheric coherences in 5 year old children. The genetic influences seem to be most important in long-distance coherences, whereas unique environment, other than measurement error, plays a significant role in short-distance coherences. Finding genes that influence a clearly genetic trait like intrahemispheric coherence can eventually shed some light on the proteins produced by these genes, and additionally the mechanisms through which coherence and later on behavior emerges.

5

Genetic stability of EEG coherence in young children

G.C.M. van Baal, E.J.C. de Geus and D.I. Boomsma

During middle childhood, massive changes have been observed in EEG coherence, which is an index of cortico-cortical connectivity of the brain. To examine changes in genetic and environmental influences on interindividual differences in EEG coherences from age 5 to age 7, 14 intrahemispheric coherences from the anterior to posterior and posterior to anterior axes were collected twice from 209 twin pairs. A genetic model was fitted to the data that decomposed the reliable variances and covariances of coherences at ages 5 and 7 into genetic and environmental parts, common for both measurements and genetic and environmental parts, specific for coherence at age 7. In the posterior to anterior direction, heritabilities increased with age in both hemispheres, but in the anterior to posterior direction heritabilities decreased in the left hemisphere, and remained the same in the right hemisphere. Genetic variances decreased with age in both hemispheres in the anterior to posterior direction. However, in the posterior to anterior direction genetic variances increased in the right hemisphere and remained the same in the left hemisphere. Environmental variances decreased with age. These findings suggest different rates of maturation for cortico-cortical connections. New genetic factors at age 7 were found for 10 of the 14 coherences. However, mostly the genetic factor expressed at age 5 accounted for the largest part of the genetic variance at age 7. Therefore, no clear qualitative *changes in genetic influences on interindividual differences in EEG coherence were found.*

Introduction

Cognitive development in childhood depends on the formation of neuronal networks within separate neural subsystems (e.g., basal ganglia, primary sensory cortices, motor cortex, etc.) as well as their integration through the maturation of cortico-cortical connectivity (Goldman-Rakic, 1987; Huttenlocher, 1990). Evidence for maturation of brain structures has been obtained from imaging studies (Huttenlocher, 1979; 1994; Chugani et al., 1987; Chugani, 1994; Jernigan et al., 1991; Pfefferbaum et al., 1994). Across childhood a gradual increase in white matter is found, paired to a decrease in grey matter. However, structural brain imaging does not reveal the extent to which the various brain areas become functionally connected. A non-invasive method of assessing changes in connectivity is provided by electroencephalographic (EEG) coherence, that is, the

squared cross-correlation in the frequency domain between two EEG time series measured simultaneously at different scalp locations (Nunez, 1981).

The development of EEG coherence from childhood into adolescence and young adulthood has been extensively documented by Thatcher and coworkers (Thatcher, 1991; 1992; 1994a; 1994b; Thatcher et al., 1986; 1987). Their EEG coherence data from more than 400 children aged 1 to 17 years showed that a gradual decline in coherence with age appeared to be interspersed with periods of increased coherence. A cyclic pattern of increasing coherence followed by decreasing coherence was observed with a period of 2 to 4 years (Thatcher, 1994a; 1994b). A period of major increase in coherence was seen between ages 5 and 7, and another at about ages 9 to 11 (Thatcher, 1994a). These growth spurts in coherence seem to coincide with the discontinuous, stagewise transitions between the four cognitive developmental stages of Piaget (1966). The spurt at age 6 coincides with the transition from pre-operational to operational thinking, that of age 10 with the transition of operational thinking to formal logic.

It has been assumed that these periods of large changes in cognition and EEG coherence are based on changes in the genetic architecture of brain functioning. This means that changes may occur in relative influences of genes and environment and that whole new genetic and environmental influences on the brain may emerge. A main aim of the present study was to test whether and how genetic and environmental influences on cortico-cortical connectivity as reflected in EEG coherence change in middle childhood, that is, from age 5 to age 7. Such influences on EEG coherence can be estimated with a longitudinal twin design. This design is based on the fact that identical, monozygotic (MZ) twins share all their genes, whereas fraternal, dizygotic (DZ) twins share 50% on average. A larger resemblance within MZ twin pairs than within DZ twin pairs indicates that genetic factors influence coherence. Repeated measures of coherences on the same twins make it possible to distinguish between new and persistent genetic influences.

In an earlier paper on heritability of EEG coherence in 5 year old children (Van Baal et al., in press) we used the twin method to estimate the relative importance of genetic influences on EEG coherence. The results showed that for both long and short intrahemispheric coherences a significant part of the variance was explained by genetic influences. At first, heritabilities seemed to be about the same: mean heritability over all cortico-cortical connections was 49%. However, measurement error was larger for long distance connections than for short distance connections. Measurement error will always lower heritability estimates, because it will show up as unique environmental variance, and therefore will increase total variance, whereas genetic variance stays the same. This problem may get worse in the longitudinal design of the present study, because

measurement error at age 5 may be different from that at age 7. Since a relatively large amount of EEG time series (90 epochs) was collected, it was possible to construct a model in which reliable variance can be distinguished from measurement error. Calculating two coherence measures for each child, using the split-half approach, allows a distinction between measurement error from reliable variance. The reliable variance can then be decomposed into genetic and environmental variances. The more precise estimation of heritability of different cortico-cortical coherences will allow us to better compare the results of long and short distance coherences. In addition, it will also allow us to compare differences in heritabilities of coherences along the anterior to posterior and the posterior to anterior axes and homologous coherences in left and right hemispheres. Such comparisons are of interest, since other studies indicated that anterior and posterior areas, and left and right hemispheres develop at different rates (Thatcher et al., 1987; Thatcher, 1991; 1994a; 1994b). This approach will benefit the main question of this paper, the comparison of heritabilities at age 5 and 7.

Changes in heritability as children grow older may result from both changes in genetic and environmental variance. For example, a higher heritability can be a result of an increase in genetic variance, less environmental variance, or from a combination of these two effects. Changes in genetic and environmental variances may have theoretical relevance. Changes in coherence have been suggested to be directly associated with shifts from synaptic outgrowth to synaptic "pruning" and vice versa (Thatcher et al., 1994; Kaiser & Gruzelier, 1996). In fact, the growth cycles of approximately 2 to 4 years may be explained as shifts from a phase of overproduction of synapses to a phase of pruning of the non-functional part of these new synapses. It was suggested that the increases in coherence reflect the temporary overproduction of synapses, whereas the decline in coherence is caused by selective pruning of existing synaptic connections, according to the demands of the environment (Thatcher, 1994). Synaptic growth is thought to be mainly determined by genetic effects (Changeux & Danchin, 1976; Changeux & Dehaene, 1989). During pruning however, genetic effects will interact with environmental effects that determine which synapses will be consolidated or eliminated (Greenough et al., 1987). This would suggest that, depending on the phase of brain growth, differential contribution of genetic and environmental effects on interindividual differences in coherence will be found. Thus, during development of individual differences in coherence, a phase of synaptic overproduction may coincide with increased genetic variance whereas a phase of pruning may be associated with increased environmental variance. Furthermore, we may reasonably hypothesize that different genetic factors influence pruning and growth. Thus, changes from growth to pruning from age 5 to 7 may be associated with the emergence of new genetic factors. Apart from estimating

changes in heritability, the model fitting approach in this paper allows the assessment of the changes in total genetic and total environmental variance. In addition, the emergence of new genetic and environment factors at age 7 can be tested.

Methods

Subjects

A longitudinal study on genetic and environmental influences on neural development during childhood was conducted in 209 twin pairs (mean age first measurement = 5.3 years, sd = 0.2 years, second measurement one and a half years later mean age second measurement = 6.8 years, sd = 0.2 years). The twins were all registered in the Netherlands Twin Register, which contains 50% of all Dutch twins born after 1986 (Boomsma et al., 1992). They were healthy, had a normal IQ (Boomsma & Van Baal, in press), and had normal or corrected to normal vision. Zygosity determination for same-sex twin pairs was done either by blood typing (ABO, MNS, Rhesus, Kell, Duffy, Kidd, Lutheran) or by DNA fingerprinting (N = 159). For 11 same-sex twin pairs these data were not available. These twins were assigned to a zygosity group using a discriminant analysis based on their physical appearances (hair color, hair structure, confusion by acquaintances, and confusion by close friends of the family).

At the first measurement occasion, eighteen twin pairs had incomplete EEG data because of difficulties during the experiment. Children who fell asleep during the experiment (11), and children who showed high levels of arousal or cried (13) were also excluded from further analyses. This left 167 5 year old twin pairs (34 monozygotic males (MZM), 33 dizygotic males (DZM), 37 monozygotic females (MZF), 32 dizygotic females (DZF) and 31 dizygotic opposite-sex twins (DOS)) with complete data. Eighteen twin pairs did not participate at the second measurement occasion. In addition, 9 twin pairs were excluded because of experimental difficulties, sleeping or crying during this session. This left 182 twin pairs (36 MZM, 37 DZM, 41 MZF, 33 DZF, and 35 DOS). Of these, 152 twin pairs had complete data on both occasions.

Procedure

Detailed procedures of data collection are described elsewhere (Van Baal et al., 1996). Briefly, an electrocap with electrodes in the 10-20 system of Jasper (1958) was used to measure brain activity on 14 scalp locations during a visual oddball task, and during three minutes of quiet rest with eyes open and 3 minutes of quiet rest with eyes closed. Vertical and horizontal eye movements were recorded for correction afterwards.

EEG was recorded unipolarly with linked ears reference according to the method described by Pivik and colleagues (1993). All electrode impedances were kept below 10 Kohm. EEG was recorded continuously on a 18 channel Nihon Kohden PV-441A polygraph. Time constants were set to 5 s, high frequency cut-off was 35 Hz and sample frequency was 250 Hz. Signals were converted with a 12 bits AD converter. This paper reports on coherence measured during 3 minutes of quiet rest with eyes closed.

Data quantification and data reduction

After removal of EOG artifacts using dynamic regression in the frequency domain (Brillinger, 1975), the EEG signal was divided into 90 two-second epochs. Epochs with clippings and with abnormal EEG patterns (detected during visual inspection) were excluded from further analysis. For every scalp location the raw EEG was converted from the time domain into the frequency domain using Fast Fourier Transformation (FFT), which yielded power spectra for every electrode position, and cross spectra and phase spectra for every electrode combination. The phase spectrum depicts the lead-lag relation between the signals at different scalp locations for every frequency band. Phase spectra were used to determine whether the signals of two scalp locations actually had a phase difference, because zero phase differences would point to signal transport other than via the axonal fibers. Power-, cross-, and phase-spectra were calculated over all valid epochs (minimum number of epochs = 30). Three sets of spectra per subject and per electrode combination were computed: one for all epochs, one for all odd, and one for all even 2-sec epochs from the total number of EEG registrations. Power spectra ranged from .5 Hz to 30 Hz with a .5 Hz resolution. The cross spectra indicate the covariance of two signals in a certain frequency band. Coherence spectra were calculated for every frequency band, using the formula:

$$\text{coherence} = \frac{(\text{cross spectrum } (1,2))^2}{\text{power spectrum } (1) \times \text{power spectrum } (2)}$$

As is shown in this formula, coherence measures the square of the linear association between the two signals and is analogous to the square of the correlation coefficient. Thus, coherence ranges from 0 to 1. From these coherence spectra mean coherence for the theta band, ranging from 4.0 to 7.5 Hz, was calculated. For children, theta is a major frequency band (Niedermeyer & Lopes da Silva, 1993). Data were transformed using the formula transformed-coherence = ^{10}log(untransformed-coherence/1-untransformed-coherence) to obtain a normal distribution of the data.

Coherence was calculated intrahemispherically, since the majority of connections are within the same hemisphere (Nunez, 1981). Coherences were calculated over the anterior-posterior axes from prefrontal electrodes to other electrodes, and from occipital electrodes to other electrodes: *short distance coherences:* from prefrontal to frontal (Fp1-F3, Fp2-F4), from prefrontal to central (Fp1-C3, Fp2-C4), from central to occipital (C3-O1, C4-O2), from parietal to occipital (P3-O1, P4-O2), and *long distance coherences:* from prefrontal to parietal (Fp1-P3, Fp2-P4), from prefrontal to occipital (Fp1-O1, Fp2-O2), from frontal to occipital (F3-O1, F4-O2). The scalp locations are shown in Figure 5.1.

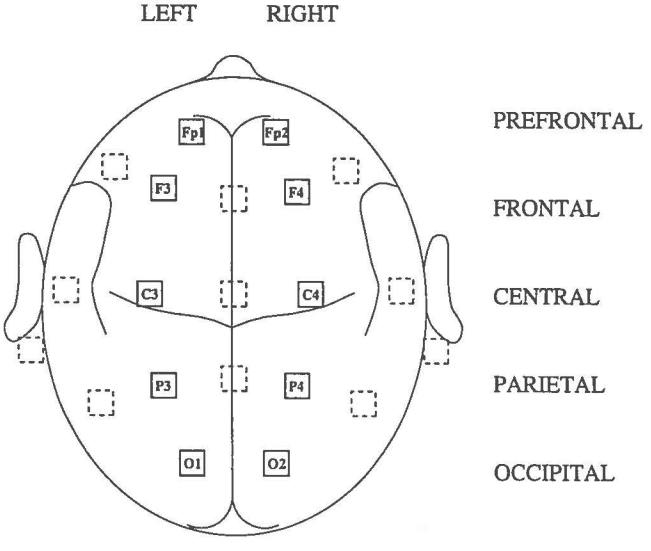

Figure 5.1 *EEG was measured at the following scalp locations: prefrontal (Fp1, Fp2), frontal (F7, F3, F4, F8), central (C3, C4), parietal (P3, P4), occipital (O1, O2) and temporal (T5 T6). This paper shows results of coherences between solid squares (i.e., Fp1 with F3, C3, P3 and O1; O1 with P3, C3, F3 and Fp1; and their right hemisphere analogs).*

Statistical analysis

Coherences were analyzed for each cortico-cortical connection separately. A number of tests were performed using structural equation modeling.

Sex- and age-differences in means

For each electrode combination eight variables were available: for each twin (oldest and youngest)and for each measurement occasion (at age 5 and at age 7) there were two coherences, one for all odd epochs and one for all even epochs. First, a saturated model

was fitted to the data, in which an estimation was made for all variances, covariances, and means in all 5 zygosity groups (MZM, DZM, MZF, DZF, DOS), without any constraints. Second, mean values were constrained to be equal for both odd- and even coherences, for oldest and youngest of a twin pair, and for MZ and DZ twins. The fit of this model was compared to the saturated model. Subsequently, sex differences in mean coherences were tested by constraining mean values of males and females to be the same, and comparing the goodness of fit of this model with the previous model. Accordingly, age differences were tested by constraining mean values at age 5 to be the same as mean values at age 7.

Reliability: Split-half correlations

To obtain estimates and test for significance of split-half correlations (i.e., the correlation between odd- and even coherences), the same approach was used. Split-half correlations were constrained to be the same for MZ's and DZ's, for oldest and youngest, and for males and females, and at age 5 and age 7. This provided one split-half correlation for each coherence at each age.

Stability: Test-retest correlations

The same model fitting approach was used to calculate test-retest correlations (stability, i.e., the correlation between coherence at age 5 and coherence at age 7). The correlations were constrained to be equal for MZ and DZ twins, for oldest and youngest twins, for odd- and for even series and for males and females. This provided 1 stability correlation for each coherence at each scalp location.

Twin- and cross-correlations

Twin-correlations (i.e., the correlation between coherence of twin 1 and coherence of twin 2, per zygosity group) and cross-correlations (i.e., the correlation between twin 1 at age 5 and twin 2 at age 7, or vice versa) were calculated using the same model fitting approach. For every measurement occasion and zygosity group (MZM, DZM, MZF, DZF, and DOS) correlations between twin 1 (i.e., firstborn, and for DOS twins male twin) and twin 2 (secondborn, and for DOS twins female twin) were constrained to be the same for both split-half series (likewise for cross-correlations). Twin- and cross-correlations provide an indication of the importance of genetic and environmental factors, and the nature of the stability between age 5 and age 7. If the correlation of MZ twins with their cotwins (rMZ) is higher than the correlation of DZ twins with their cotwins (rDZ), then genetic factors are important. Likewise, a higher cross-correlation for MZ twins than for DZ twins indicates that the stability between age 5 and 7 is influenced by

genetic factors. Different MZ correlations for males and females, and different DZ correlations for males, females and male/female twin pairs (DOS) indicate sex differences in genetic and environmental influences on EEG coherences.

Genetic model fitting

Genetic and environmental influences on interindividual differences in EEG coherences were tested using structural equation modeling. Figure 5.2 shows a path diagram of the model, which is a compilation of 3 different parts: a model for the decomposition of total observed variance into a reliable part and a part due to measurement error, shown in Figure 5.3; a model which represents the twin design to decompose variance into a genetic and environmental part (Figure 5.4); a bivariate triangular decomposition of the variance and covariance of coherence at age 5 and 7, to account for stability (Figure 5.5).

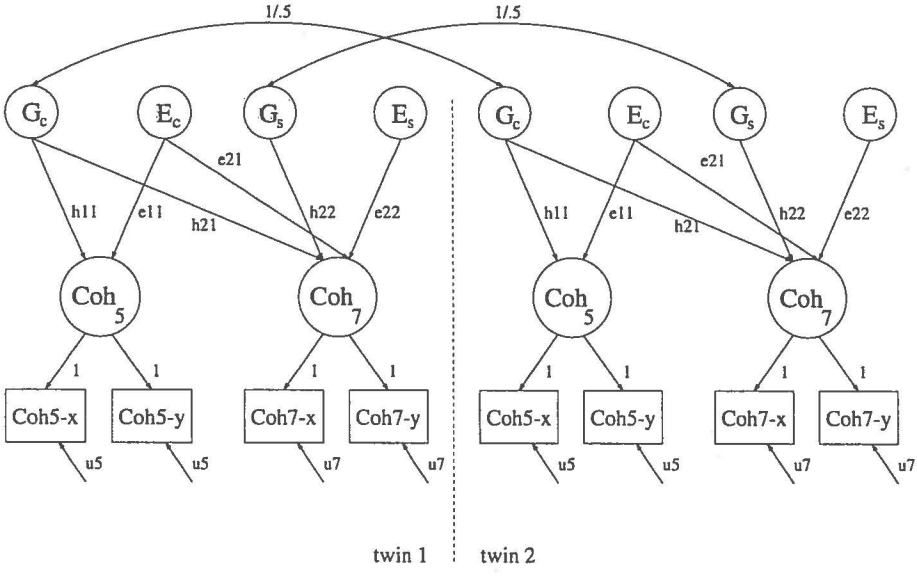

Figure 5.2 *Path model of multivariate genetic model. The model consists of 3 parts, the decomposition of observed variance into real variance and measurement error, the twin design, and the decomposition of real variance into genetic and environmental factors, common and specific to age 7.*

Figure 5.3 shows a path diagram of the decomposition of observed variance into reliable variance and measurement error. This model relies on the fact that coherence was

calculated twice at each age and for each child: once for odd epochs (coh-x) and once for even epochs (coh-y). These two variables can only differ from each other due to measurement error, all covariance between these variables is variance due to the reliable coherence. In our model measurement error was allowed to be different for males and females, and for age 5 and age 7.

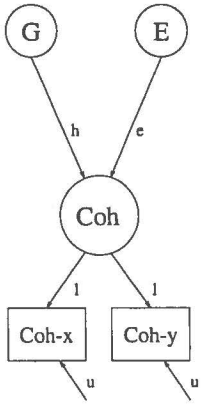

Figure 5.3 *Decomposition of total variance into measurement error and reliable variance.*

Figure 5.4 shows how the reliable variance in the EEG coherence was decomposed into genetic and environmental parts. Genetic variance can be decomposed into additive and dominant genetic variance. MZ twins share all their genes and DZ twins share 50% on average. The correlation between additive (A) and between dominant (D) genetic factors equals 1 for MZ twins, and .5 and .25 respectively for DZ twins. Environmental variance can be decomposed into common and unique environmental variances. Common environmental factors will make twins more alike, since they share the same effect from the environment. Unique environmental effects are responsible for all differences between MZ twins. The common environmental correlation is 1 in both MZ and DZ twins. Correlations for the non-shared, unique environmental influences (E) are 0 for both types of twins. This figure shows the dominant genetic and common environmental factors, but for simplicity reasons these factors are not shown in Figures 5.2, 5.3 and 5.5. Note that estimating D and C at the same time is not possible in a design using only MZ and DZ twins reared together. We therefore tested models containing A, C and E or A, D and E.

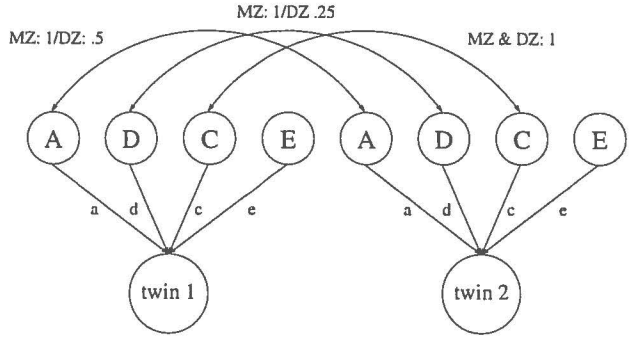

Figure 5.4 *Path diagram of a univariate twin model. Phenotypes of both twins (twin 1 and twin 2) are influenced by additive genetic factors (A), dominant genetic factors (D), common environmental factors (C), and unique environmental factors (E). Correlation between C_{twin1} and C_{twin2} is 1, correlation between A_{twin1} and A_{twin2} is 1 for MZ twins and .5 for DZ twins, correlation between D_{twin1} and D_{twin2} is 1 for MZ twins and .25 for DZ twins. Note that it is not possible to estimate influences of C and D together in a model with only MZ and DZ twins reared together.*

For each source of variation (A, D or C, and E) two factors were specified: one factor that influenced the true-coherence at age 5 and age 7 and a new factor that influenced the true-coherence at age 7 (see Figure 5.5). The first factor is common to both ages and causes covariance between true-coherence measured at ages 5 and 7. In this way, the covariance (or stability) is decomposed into a genetic and an environmental part. By constraining the factor loadings of the true-coherence at age 7 on the first set of factors to be zero, the hypothesis is tested that all genetic (or environmental) variance at age 7 is due to new genetic (or environmental) influences being expressed at that age. By constraining the factor loadings of the true-coherence at age 7 on the second set of factors to be zero, the hypothesis is tested that no new influences are expressed at age 7.

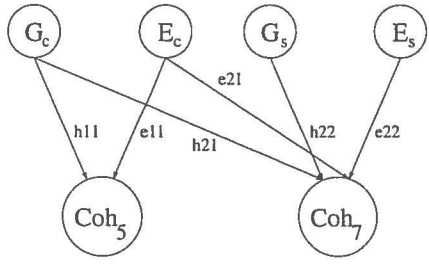

Figure 5.5 *Bivariate triangular decomposition of variance.*

In addition, an estimation was made of the amount of genetic variance that was shared at both measurement occasions. The genetic correlation, that is, genetic covariance divided by the square root of genetic variance at age 5 times the square root of genetic variance at age 7, provides an indication of this. A genetic correlation of 1 indicates that no new genetic factors emerge at age 7. A genetic correlation of 0 indicates that no common genetic factor exists for coherences at ages 5 and 7. The same was done for environmental correlations. 80% maximum likelihood based confidence intervals are obtained (Neale & Miller, in press). When the interval contains the value of 1, a new factor at age 7 was not significant. When the interval contains the value of 0, the common factor was considered significant.

The model in Figure 5.2 was fitted for each cortico-cortical connection separately. First an ADE (or an ACE) model was fitted in which parameter estimates were allowed to differ between males and females. Then, the model was reduced. Genetic and environmental variances were constrained to be the same for males and for females. Next, dominant genetic (or common environmental) variance was left out of the model (AE model). For the best fitting model genetic and environmental variances was reported, and heritability (h^2) was calculated as the percentage of additive genetic variance of total reliable variance. 80% maximum likelihood based confidence intervals were obtained (Neale & Miller, in press). Likewise, it was calculated which percentage of the observed covariance between ages 5 and 7 was due to genetic factors. When the latter is 1, then all phenotypic covariance is caused by genetic factors. When it is 0, then all phenotypic covariance is environmental.

Structural equation modeling was used to find the best fitting model. Maximum likelihood was used for parameter estimation. The goodness-of-fit of the ADE (or ACE) model was compared with the goodness of fit of a saturated model. Thus a χ^2 which indicates the goodness-of-fit of this model was obtained. To compare the fit of two nested models, hierarchic χ^2 tests were used. With these tests the log-likelihood of a nested model is subtracted from the log-likelihood of a more general model. Twice this difference is χ^2 distributed, with the difference of df's of the two models as degrees of freedom. When a constrained model does not describe the data significantly worse than the more general model, the most parsimonious model (i.e., with the least parameters) is chosen. The sample contains missing data, mainly due to difficulties during the experiment at age 5 (for example, because children cried or fell asleep), or due to the fact that not all children participated the second time (at age 7). For that reason the data could not be summarized in dispersion matrices, but the models had to be fitted directly to the raw data. The likelihood for each pedigree is calculated separately and the product of these likelihoods (i.e., the sum of the log-likelihood) is maximized.

Results

Means

Figures 5.6 and 5.7 show mean coherences (after back transformation) for males and females, at ages 5 and 7, for all electrode combinations. In Table 5.1 model fitting results for the tests of differences in mean values of coherences are reported. Except for Fp2 to F4, coherences were the same for oldest and youngest of the twins, for MZ and for DZ twins, and for the odd and even coherences. Significantly lower short distance coherences in males than in females were found for Fp1-F3, Fp1-C3, P3-O1 and Fp2-F4. This difference seemed to be most obvious in 5 year old children. Coherences of males and females became more similar at age 7. Short distance coherences between the left and right prefrontal and frontal areas increased with age in males, and remained the same in females. In both sexes, short distance coherences between prefrontal and central areas were the same at age 5 and age 7. Posterior short distance coherences in both hemispheres decreased with age. And all long distance coherences also decreased from age 5 to 7.

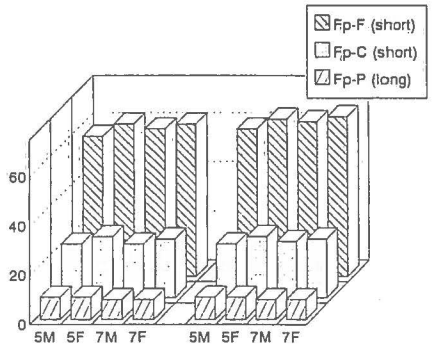

Figure 5.6 *Mean coherences for anterior to posterior axis.*

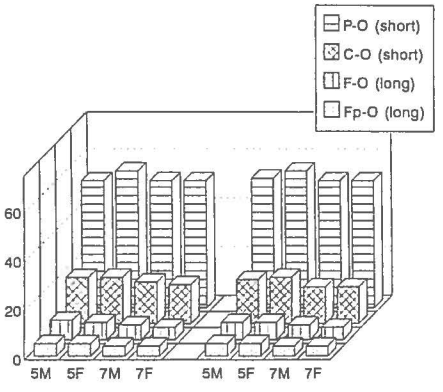

Figure 5.7 *Mean coherences for posterior to anterior axis.*

Reliability and stability

Table 5.2 shows split-half correlations and stability correlations (1st column) between coherences at ages 5 and ages 7 (calculated for coherences of all epochs). Split-half correlations across even and odd epochs were almost always the same for males and females and for ages 5 and 7. Exceptions were slightly higher correlations for males at P4-O2 and lower correlations for both sexes at age 7 for Fp1-O1 and F3-O1. Split-half

correlations became lower with increasing distance, indicating larger measurement errors for longer distances. Lowest split-half reliability was found for coherence between Fp2 and O2 (0.58), highest reliability was found for P3-O1 (0.92). Stability correlations were moderate for all coherences (0.38 - 0.48), except for the shortest distances (Fp1-F3, Fp2-F4, or P3-O1), for which they were below 0.33. Although at first sight this may seem rather low, the reader is reminded that these stability correlations are largely influenced by the reliability of the coherences at both ages (split-half correlations). Stability of the reliable phenotypes therefore will be higher. Especially for the longest distances, this will have a substantial effect: with increasing distance a larger part of the reliable variance is stable over time. No sex differences in stability were found.

Table 5.1 *Model fitting statistics for tests for differences in mean coherences for males and females at ages 5 and 7. Three models were fitted to the data: (1) A model in which means of odd- and even-averaged coherences were constrained to be equal, and for which means for oldest and youngest, and for MZ and DZ twins of the same sexes were constrained to be equal; (2) A model for which, in addition to these equality constraints, male and female coherences were constrained to be equal, but for which coherences at ages 5 and 7 were allowed to differ (m=f); (3) A model for which coherences at ages 5 and 7 were the same, but different for males and females (5 = 7). Chi-square differences between models 2 or 3 and 1 are shown. The difference in degrees of freedom is 2.*

	Left hemisphere		Right hemisphere	
	m=f**	5=7***	m=f**	5=7**
Fp-F	13.10*	5.69	9.15*	11.83*
Fp-C	8.32*	1.75	3.48	0.33
Fp-P	0.74	10.98*	0.05	10.70*
Fp-O	4.61	48.47*	0.21	50.89*
F-O	2.82	61.85*	1.06	75.15*
C-O	0.93	29.04*	2.76	45.11*
P-O	10.82*	13.48*	2.60	15.63*

* Difference is significant at alpha = .05

** Sex differences: coherences were lower in males than in females.

*** Age differences: coherences were higher at age 5 than at age 7, except for Fp2-F4, for which coherences were lower at age 5. Fp1-F3 showed a trend for lower coherences at age 5.

Twin- and cross-correlations

To get a first impression whether genetic factors influence coherences, twin correlations at age 5 and at age 7 were calculated. To obtain information about the nature of the observed stability over time in coherences, cross correlations between coherence

of twin 1 at age 5 and coherence of twin 2 at age 7, and vice versa were calculated. These correlations are shown in Table 5.2 (column 2-6). Twin correlations showed that MZ correlations were almost always larger than DZ correlations, indicating the importance of genetic factors. This applied to both short and long distances. For some short distance coherences (for example Fp2-F4, Fp2-C4) MZ correlations were notably lower than split-half correlations, indicating real unique environmental influences that cannot be due to measurement error. For long distance electrode combinations (such as for Fp1-P3, Fp1-O1) MZ correlations are only slightly smaller than split-half correlations, indicating that a large part of the reliable variance is due to genetic influences. MZM and MZF correlations are about the same, and DZM, DZF and DOS correlations also are about the same, which suggests that sex differences in genetic and environmental influences do not play an important role. For a number of electrode combinations, MZ correlations seem to change with age, pointing to a different heritability over time. MZ-cross correlations are about the same as test-retest correlations and are larger than DZ cross-correlations, which indicates that the stability between first and second measurement is mainly genetic.

Table 5.2 *Reliability and twin correlations. Reliability estimates (split-half correlations at age 5 and at age 7) and correlations between coherences at ages 5 and 7 are presented in the first column. Twin-correlations and cross-correlations (i.e., correlations between coherences of twin 1 at age 5 and twin 2 at age 7) are shown in the last 5 columns.*

Left hemisphere

		Reliability	Twin-correlations/cross-correlations				
			MZM	DZM	MZF	DZF	DOS
Fp1-F3	5 yr	.90	.45	.00	.44	.32	.36
	7 yr	.88	.43	.33	.44	-.04	-.24
	5-7 yr	.31	.23	.01	.21	.29	.22
Fp1-C3	5 yr	.86	.46	.07	.63	.12	.11
	7 yr	.85	.48	.13	.46	.24	-.27
	5-7 yr	.48	.38	.02	.36	.20	.07
Fp1-P3	5 yr	.76	.63	.11	.54	.04	-.05
	7 yr	.69	.45	-.06	.46	.22	-.32
	5-7 yr	.45	.39	-.03	.34	.13	-.12
Fp1-O1	5 yr	.67*	.45	.14	.43	-.03	.03
	7 yr	.57*	.55	.03	.44	.15	-.02
	5-7 yr	.40	.42	.13	.39	.15	.13
F3-O1	5 yr	.74*	.44	.00	.53	.01	.02
	7 yr	.65*	.58	.45	.37	.24	.14
	5-7 yr	.38	.37	.10	.40	.18	.04
C3-O1	5 yr	.86	.54	-.01	.56	.11	.25
	7 yr	.83	.71	.34	.51	.19	.19
	5-7 yr	.44	.46	-.05	.44	.09	.15
P3-O1	5 yr	.92	.30	-.14	.62	.32	.14
	7 yr	.91	.55	.16	.63	.34	.24
	5-7 yr	.33	.35	-.14	.35	.14	.09

Right hemisphere

		Reliability	Twin-correlations/cross-correlations				
			MZM	DZM	MZF	DZF	DOS
Fp2-F4	5 yr	.89**	.37	-.05	.22	.30	.31
	7 yr	.90**	.44	.07	.11	-.24	-.17
	5-7 yr	.29	.26	.17	.12	.30	-.01
Fp2-C4	5 yr	.86	.29	.22	.33	.19	.22
	7 yr	.82	.48	.33	.36	.00	-.16
	5-7 yr	.44	.30	.35	.27	.15	.08
Fp2-P4	5 yr	.75	.50	.22	.53	-.08	-.10
	7 yr	.68	.48	.07	.50	.35	-.30
	5-7 yr	.43	.37	.25	.32	.10	-.28
Fp2-O2	5 yr	.58	.38	.01	.41	.05	-.19
	7 yr	.58	.59	-.02	.47	.19	.08
	5-7 yr	.41	.47	.06	.29	.18	-.02
F4-O2	5 yr	.69	.40	-.09	.48	-.03	-.24
	7 yr	.64	.54	.24	.50	.34	-.08
	5-7 yr	.38	.34	.08	.31	.23	-.15
C4-O2	5 yr	.84	.43	.17	.57	.06	.10
	7 yr	.85	.69	.39	.65	.28	.11
	5-7 yr	.44	.35	-.05	.44	.03	.10
P4-O2	5 yr	.91***	.31	.08	.42	.02	.04
	7 yr	.91***	.72	.21	.66	.43	.36
	5-7 yr	.45	.27	-.12	.35	.06	.08

* Age differences significant.
** Males, age 5: .85; females, age 5: .93; males, age 7: .92; females, age 7: .87.
*** Males: .93; females, .90.

Genetic model fitting

The effects of genetic and environmental influences on coherence were studied using the model, summarized in Figure 5.2, that tested sex and age differences in these influences. Table 5.3 shows results of model fitting. First, the model with additive genetic, dominant genetic and unique environmental factors, with different parameter estimates for males and females was fitted to the data (Common environmental influences were tested in an ACE model with sex differences, but were never significant. Therefore, only analyses with the ADE models are presented). χ^2 ranged from 153 (for Fp1-P3) to 272 (for Fp2-F4, critical value at alpha = .05 = 188). Models for long distance coherences seem to fit slightly better than short-distance coherences. Next, parameter estimates were constrained to be equal for males and females. This did not significantly decrease the fit of the model, except for Fp2-F4. Leaving out dominance slightly decreased the fit of the models, especially for longer distances, but this was only significant for Fp1-P3 and for Fp2-O2. For most cases the AE model without sex differences was sufficient to describe the data. To be able to compare all the estimates of heritabilities and stabilities, irrespective of electrode distance, we used a single AE model without sex differences for all coherences. Significance of the differences in heritabilities was assessed using the confidence intervals.

Table 5.3 *Chi-squared differences for genetic models. The ADE model without sex differences (ADEno) was compared to an ADE model with sex differences. Subsequently, the AE model without sex differences (AEno) was compared to ADEno. Difdf is the difference in degrees of freedom between the model and the model with which it is compared, critval is the critical value at alpha = .05 for those degrees of freedom.*

	Left hemisphere		Right hemisphere	
	ADEno	AEno	ADEno	AEno
difdf	9	3	9	3
critval	16.92	7.82	16.92	7.82
Fp-F	7.60	0.94	22.18*	0.66
Fp-C	7.37	7.01	6.57	1.06
Fp-P	4.73	8.89*	12.14	7.07
Fp-O	2.10	6.23	5.32	8.05*
F-O	6.71	6.07	2.39	6.50
C-O	4.98	5.19	6.29	3.95
P-O	8.74	5.42	5.62	2.98

* Difference is significant at alpha = .05

Table 5.4 *Heritabilities, genetic and environmental variances at ages 5 and 7 years. 80% confidence intervals are given between brackets.*

Left hemisphere

	h^2_5	h^2_7	$V_{g\text{-}5}$	$V_{g\text{-}7}$	$V_{e\text{-}5}$	$V_{e\text{-}7}$
Fp1-F3	53% (42-63%)	36% (24-47%)	.026 (.020-.033)	.010 (.006-.013)	.023 (.019-.029)	.018 (.015-.021)
Fp1-C3	64% (52-74%)	46% (32-57%)	.029 (.022-.037)	.017 (.012-.023)	.016 (.012-.022)	.021 (.017-.026)
Fp1-P3	75% (64-84%)	56% (42-67%)	.036 (.029-.044)	.021 (.015-.027)	.012 (.008-.017)	.017 (.013-.022)
Fp1-O1	60% (46-72%)	77% (64-87%)	.024 (.018-.031)	.025 (.020-.031)	.016 (.011-.022)	.008 (.004-.012)
F3-O1	60% (46-72%)	71% (60-80%)	.029 (.021-.038)	.025 (.020-.031)	.019 (.014-.026)	.010 (.007-.015)
C3-O1	64% (54-73%)	72% (63-80%)	.033 (.026-.041)	.035 (.028-.042)	.018 (.014-.023)	.013 (.010-.018)
P3-O1	43% (30-55%)	62% (51-70%)	.015 (.010-.021)	.019 (.015-.023)	.020 (.016-.025)	.012 (.009-.015)

Right hemisphere

	h^2_5	h^2_7	$V_{g\text{-}5}$	$V_{g\text{-}7}$	$V_{e\text{-}5}$	$V_{e\text{-}7}$
Fp2-F4	37% (25-48%)	28% (15-40%)	.016 (.011-.022)	.008 (.004-.013)	.028 (.023-.034)	.022 (.018-.026)
Fp2-C4	40% (27-51%)	38% (24-50%)	.018 (.012-.025)	.012 (.007-.017)	.027 (.022-.033)	.020 (.016-.024)
Fp2-P4	64% (51-75%)	62% (49-73%)	.029 (.022-.037)	.023 (.018-.029)	.017 (.012-.022)	.014 (.010-.019)
Fp2-O2	54% (37-69%)	86% (73-96%)	.019 (.012-.025)	.028 (.023-.035)	.016 (.011-.022)	.005 (.002-.009)
F4-O2	49% (33-62%)	75% (63-85%)	.020 (.013-.028)	.028 (.022-.034)	.021 (.016-.028)	.009 (.006-.014)
C4-O2	59% (47-68%)	80% (72-85%)	.029 (.022-.036)	.041 (.035-.048)	.021 (.016-.026)	.011 (.008-.014)
P4-O2	38% (24-50%)	73% (65-79%)	.013 (.008-.018)	.022 (.018-.026)	.022 (.018-.027)	.008 (.007-.011)

Heritabilities

Table 5.4, 1st and 2nd column, displays the heritability estimates. At age 5, heritability for short distance coherence varied between 37% and 64%. For long distance coherence, heritability varied between 49% and 75%. Thus, heritability increased with electrode distance. The same picture was seen at age 7. Substantial changes in heritability were found from age 5 to age 7. Age-related changes clearly depended on electrode combination. Heritability decreased from age 5 to age 7 for all left hemispheric coherences from prefrontal to frontal, from prefrontal to central and from prefrontal to parietal locations. Analogue right hemispheric coherence heritabilities along the anterior-posterior axis remained the same. Heritability in both hemispheres increased from occipital to parietal, from occipital to central, from occipital to frontal, and from occipital to prefrontal, that is, coherence along the entire posterior-anterior axis increased.

To elucidate the changes in heritability from age 5 to age 7, we estimated total genetic and environmental variance at both ages. These are shown in Table 5.4 (column 3 to 6). Table 5.5 summarizes the age-related changes as a function of direction of coherence and hemisphere. Along the posterior to anterior axis the influence of genetic factors remained the same in the left hemisphere, but significantly increased in the right hemisphere. In both hemispheres the influence of unique environmental influences decreased. Therefore, the larger heritability for posterior to anterior connections in the left hemisphere is mainly due to a decrease in environmental variance. The larger heritability in the right hemisphere is due to both increases in genetic and decreases in environmental variances.

Table 5.5 *Summary of changes in heritabilities, genetic and environmental variances from ages 5 to 7. Plus sign indicates increase in heritability, genetic variance or environmental variance with age, minus sign a decrease with age, and the equal sign indicates no significant changes.*

	h^2		V_g		V_e	
	L	R	L	R	L	R
Fp-F	-	=	-	-	-	-
Fp-C	-	=	-	-	=	-
Fp-P	-	=	-	-	+	-
Fp-O	+	+	=	+	-	-
F-O	+	+	=	+	-	-
C-O	+	+	=	+	-	-
P-O	+	+	=	+	-	-

Coherences involving the prefrontal to frontal, central and parietal locations showed a decrease in genetic influence in both left and right hemispheres (left Fp-P not significant). Environmental variance in the left hemisphere decreased in the prefrontal-frontal connection, but it increased in the prefrontal to central (Fp1-C3) and prefrontal to parietal (Fp1-P3) connections. In the right hemisphere environmental variances decreased (right Fp-P not significant). The decreases in environmental and genetic variances in the right hemisphere are about the same, resulting in a constant heritability over age. In the left hemisphere the decrease in heritability is due to a simultaneous increase in environmental variance and a decrease in genetic variance for Fp1-C3 and Fp1-P3. For Fp1-F3, heritability decreases because of the larger decrease in environmental variance compared to the decrease in genetic variance.

Genetic and environmental factor structure

The confidence intervals of genetic and environmental correlations between coherences at ages 5 and 7 years, showed whether the changes in heritabilities coincided with a change in the sources of genetic and environmental variance, that is, the extent to which new genes or new experiences influenced coherence at age 5 and 7. In Table 5.6, genetic and environmental correlations between coherences at ages 5 and 7 are shown. When the genetic correlation is 1, no new genes are expressed at age 7. When the genetic correlation is 0, all genetic influences are new to that age, and the effect of the genes influencing coherence at age 5 is replaced by the effect of other genes. The correlation between coherences is indicated by the common genetic factor, and the effects of new genes are indicated by the specific genetic factor. If the confidence intervals include the value of 0, then the common genetic factor is not significant. Common genetic factors were always significant, and explained most of the genetic variance. Genetic correlations were .59 to .97. If the confidence intervals include the value of 1, then the specific genetic factor is not significant. Specific new genetic factors at age 7 were found for all coherences, except left and right prefrontal to occipital, right prefrontal to frontal, and right prefrontal to central (based on 80% confidence intervals of the genetic correlations). Likewise, we tested for environmental effects common to both ages, or specific to age 7. Environmental factors common to ages 5 and 7 were significant for half of the coherences, but in all cases only a small to moderate part the environmental variance was explained. Environmental correlations were .01-.47. Specific new environmental factors at age 7 were always significant.

Table 5.6 *Genetic and environmental correlations (and their 80% confidence intervals) between coherences at ages 5 and 7.*

Left hemisphere

	Genetic correlations		Environmental correlations	
Fp1-F3	.69	(.49 - .90)	.07	(.00 - .23)
Fp1-C3	.82	(.68 - .95)	.25	(.08 - .41)
Fp1-P3	.77	(.65 - .88)	.40	(.19 - .58)
Fp1-O1	.97	(.85 - 1.00)	.02	(.00 - .30)
F3-O1	.82	(.69 - .94)	.01	(.00 - .24)
C3-O1	.74	(.63 - .85)	.11	(.00 - .30)
P3-O1	.66	(.48 - .84)	.09	(.00 - .27)

Right hemisphere

	Genetic correlations		Environmental correlations	
Fp2-F4	.89	(.61 - 1.00)	.10	(.00 - .24)
Fp2-C4	.92	(.73 - 1.00)	.27	(.13 - .41)
Fp2-P4	.77	(.64 - .89)	.35	(.13 - .54)
Fp2-O2	.95	(.82 - 1.00)	.23	(.00 - .59)
F4-O2	.74	(.59 - .89)	.30	(.05 - .53)
C4-O2	.64	(.53 - .74)	.34	(.14 - .51)
P4-O2	.59	(.44 - .72)	.47	(.33 - .59)

Finally, Table 5.7 shows the relative influences of the genetic covariances on the observed covariances in coherences between ages 5 and 7. Percentages of total covariance explained by genetic covariance varied from 61 to 100%. This indicates that the observed, phenotypic stability of coherence across time was mainly genetic.

Table 5.7 *Percentage genetic covariance over total covariance between coherences at ages 5 and 7. 80% confidence intervals are given in brackets.*

Left hemisphere			**Right hemisphere**		
Fp1-F3	88%	(63 - 100%)	Fp2-F4	80%	(54 - 100%)
Fp1-C3	80%	(63 - 94%)	Fp2-C4	68%	(49 - 85%)
Fp1-P3	79%	(65 - 91%)	Fp2-P4	79%	(63 - 93%)
Fp1-O1	99%	(85 - 100%)	Fp2-O2	92%	(76 - 100%)
F3-O1	100%	(83 - 100%)	F4-O2	81%	(62 - 97%)
C3-O1	94%	(80 - 100%)	C4-O2	82%	(68 - 93%)
P3-O1	89%	(65 - 100%)	P4-O2	61%	(44 - 76%)

Discussion

This study examined EEG coherence in 209 young twin pairs twice, at age 5, and 1.5 years later, when they were around 7 years old. Thatcher and colleagues (1987; 1991) have shown this to be a period of major changes in cortico-cortical connectivity, as indexed by EEG coherence. They found that most short distance coherences (e.g., prefrontal to frontal or central) increased from age 5 to 7, whereas long distance coherences (e.g., from prefrontal to parietal or occipital) decreased. This overall pattern was substantiated by the results of the present study: we found increasing prefronto-frontal coherences, and decreasing long-distance coherences. Thatcher showed that, in comparison to the entire development of coherence from year 1 to 17, the coherence changes from age 5 to age 7 appear to constitute a clear "growth spurt" (Thatcher et al., 1987). This suggested that genetic and/or environmental influences on the brain may change in this period. The main goal of this paper was to study these changes in genetic and environmental influences on EEG coherence by using a longitudinal twin design.

As a first step heritability was established at age 5 in these twins (Van Baal et al., in press). Heritability at age 5 was tentatively suggested to be higher in long distances (> 14 cm) than in short distances. However, reliability was not integrated in the structural models as was done in the analyses of the present study. It is necessary to take reliability into account when comparing heritabilities of long and short distance coherences, because reliability decreases systematically with increasing interelectrode distance: split-half correlations ranged from .57 for longest cortico-cortical connections to .92 for the shortest cortico-cortical connections. Since measurement error may explain a large part of the variance in the signal, a model was constructed which distinguished measurement error from reliable variance. The reliable variance was then decomposed into genetic and environmental factors. Using this method it was generally confirmed that heritabilities of long distance coherences were higher compared to heritabilities of short distance coherences. Furthermore, combining the data of 5 year olds with those of the 7 year olds, the increase in heritability with increasing distance appeared to be most pronounced in the anterior to posterior direction. In the anterior to posterior direction heritabilities increased with increasing distance, whereas in the posterior to anterior direction heritabilities were very much alike. The fact that heritability was sensitive to the direction of cortico-cortical connectivity clearly shows that coherence cannot be explained by traits influencing volume conduction only. Heritability changes of these traits would than have been the same in both directions. The present results, therefore, confirm coherence to be a highly informative trait in the study of individual differences in brain development.

In all coherences, at both ages, the best fitting model to describe the individual differences was a genetic model (AE or ADE model). Coherence is determined by a number of anatomical and neurophysiological parameters, like axonal sprouting, synaptogenesis, expansion of existing synaptic terminals, myelination, pruning of synaptic connections, presynaptic changes in the amount of neurotransmitter or changes in the postsynaptic response to a given neurotransmitter. Thus, a genetic effect is not surprising. Genes may exert an influence on all of these factors, and an additive mechanism is understandable. The substantial influence of true unique environmental effects, that is, effects other than measurement error, on this "biological" index may be surprising at first sight. However, Changeux and colleagues (Changeux & Danchin, 1976; Changeux & Dehaene, 1989) proposed a theory which states that, in development, a genetically mediated overabundancy of synaptic contacts is followed by a "pruning" of the non-functional part of these synaptic contacts. The consolidation of some and elimination of others is entirely according to the demands of the environment. In this way, the gap between structure (which we would expect to be largely determined by genetic factors) and function of the brain (which depends at least partly on the environment) is narrowed by slowly sculpting and refining the micro-anatomy of the brain by experience. Environmental influences on EEG coherence therefore are essential in achieving an optimally functioning brain. In addition, the human genome is far too small to account for the large number of synaptic contacts, and constant sensorimotor experience is a requirement to establish a healthy mature brain.

With regard to the age-related changes in the determinants of interindividual differences of coherence, a decrease was found in the influences of environmental effects from ages 5 to 7, as reflected in a decreasing environmental variance in this period. Possibly this could be explained by the fact that, during this period, children have entered formal schooling. The environmental factors influencing interindividual differences in EEG coherence at age 7 are mostly new, which is reflected in the low environmental correlations between ages 5 and 7. The lower environmental variance at age 7 would then reflect the school environment, which might have a strong uniforming impact. However, the change from mostly family environment to mostly school environment would probably concern (the diminishing of) common environmental effects. No common environmental effects were found at age 5. The decrease in total environment was paired to an increase in heritability in the posterior to anterior direction, that is, from sensory input channels to executive areas and working memory in frontal and prefrontal cortex. Due to a decrease in genetic variance, heritabilities of coherence in the opposite direction, that is, from executive to sensory direction, remained the same in the right hemisphere, and actually decreased in the left hemisphere. This suggests that, although environmental

variance was smaller, its relative contribution to coherence in the anterior to posterior direction increased. If effects of unique environment on coherence indeed represent pruning (Thatcher, 1994a), this may suggest that from ages 5 to 7 pruning of connections of the prefrontal areas to more posterior areas becomes relatively more important. In a developmental perspective this corroborates the findings that the posterior brain areas mature earlier than the anterior brain areas (Stuss, 1992). The latter may continue to develop at least through adolescence. Furthermore, the selective increase in genetic variance in the right hemisphere compared to the left hemisphere, where it is already high at age 5, is in agreement with the developmental lead of the left hemisphere (Thatcher et al., 1987).

The longitudinal model that was applied to the data also provided insight into the genetic and environmental stability in EEG coherence. For unique environmental factors at ages 5 and 7 years stability was low. Other environmental influences thus come in play at age 7 compared to age 5. The within-individual stability in EEG coherence over time was largely genetic in nature. For most electrode combinations, the same genes influenced EEG coherences at ages 5 and 7. For some coherences no indications for new genetic influences existed at all (notably for prefronto-occipital connections), which would indicate that no major change in mechanism (i.e., from synaptic growth to pruning) occurred in these connections. For other connections, however, new genetic influences at age 7 were significant. Such new genetic influences can be due to any of the many neurophysiological mechanisms underlying coherence, for example changes in relative importance of myelination, axonal sprouting or pruning of either excitatory of inhibitory synapses. However, our pattern of results do not point to a massive change in genetic factors on any of the electrode combinations. Mostly the genetic factor expressed at age 5 accounted for the largest part of genetic variance at age 7. This was contrary to our expectations, because a clear change in quality of thinking (i.e., the transition from a Piagetian pre-operational to an operational stage) has been found in this age group (Piaget, 1966), and a qualitative change from synaptic growth to pruning has been suggested to account for coherence changes in this age (Thatcher, 1991). Such major changes were expected to coincide with significant changes in genetic architecture.

In summary, our results show a clear differentiation in the changes in heritability of coherence from age 5 to age 7 as a function of direction (anterior-posterior versus posterior-anterior) and hemisphere. These changes are compatible with the idea that different functional modules in the brain follow a different maturational program. However, our data also show that the same genetic factors that are present at age 5 still are the major source of interindividual differences at age 7. The growth spurt in cognition

during this period, therefore, seems to reflect regional differentiation more than the switching on of new genetic programs.

6

Summary and Discussion

The four papers in this thesis were based on a large longitudinal study of 209 twin pairs. These 418 children underwent extensive electrophysiological testing at the Vrije Universiteit in Amsterdam when they were 5 years old and again when they were 7 years. The main goal of this thesis was to examine the architecture of individual differences in a set of electrophysiological parameters that have been cited extensively in the psychophysiological literature as indices of brain maturation, and as correlates of behavioral and cognitive traits. The first aim was to establish the relative influences of genetic and environmental factors on individual differences in brain function at age 5. The twin method is a powerful method to this end. Using MZ and DZ twins allows the simultaneous assessment of genetic and shared (family) environmental influences. Thus, rather than arguing about the importance of nature and nurture for the preschool child's development, this twin study provides a solid quantification of such influences. As might have been expected, the contribution of both environment and genes was important. Nonetheless, most of the evidence implicates genes as the main source of interindividual differences in childhood EEG indices of neural functioning. In the first part of this discussion the main findings on heritabilities of EEG indices will be summarized.

In addition to the examination of the relative influences of genes and environment at age 5, an important goal was to detect changes in these influences from age 5 to age 7. In this period children enter the formal schooling system in the Netherlands, which may have a clear impact on both shared and unique environmental sources. It is also an age at which vital changes in grey and white brain matter are at their peak (Huttenlocher, 1979; 1994; Huttenlocher et al., 1982; Chugani et al., 1987; Chugani, 1994; Jernigan, 1991; Pfefferbaum et al., 1994). These changes strongly depend on proteins affecting neural growth factors, myelination, and neurotransmission. The regulation of these proteins, in turn, depends on the individuals genotype. It is not surprising, therefore, that this age has been characterized as the beginning of a gradual increase in genetic influences on individual differences in cognitive abilities (Cherny & Cardon, 1994; Boomsma & Van Baal, in press). Also, this age period is known for a clear change in the quality of cognition, that has been best described by Piaget and Inhelder (1969). Around age 6 children make a transition from a pre-operational to an operational phase, that is, they learn to notice that the quantity of something remains the same, despite transformations in its appearance. Thus, the age range of 5 to 7 is a period of change in both qualitative and quantitative differences in cognition. If changes in cognition coincide with changes in brain function, which is the central axiom of psychophysiology, then this period seems optimal for detecting changes in the genetic and environmental determinants of EEG parameters. The second part of this discussion deals with these changes across time.

Heritability of EEG parameters in children

The recent surge in output from the field of behavior genetics has established beyond doubt that a wide range of human behaviors are influenced by genetic factors (e.g., Rose, 1995; Plomin et al., 1994; Boomsma, 1993; Heath et al., 1995; Eaves et al., 1989). This includes various measures of cognitive ability, motor skills, personality, and susceptibility for problem behavior and life style. To explain how genetic variation can account for behavioral variation, it is desirable to study intermediate phenotypes, like the functional organization of the brain (Lander, 1988). Brain functioning can be indexed by the electroencephalogram (EEG), which measures electrical activity of the brain. The EEG can be measured at rest as well as in response to a standardized stimulus, the so-called event related potentials or ERPs. If EEG is measured at different locations on the scalp, coherence of EEG between various scalp regions can be used to estimate the functional connectivity of these regions. Although some earlier studies have addressed heritability of some of these EEG phenotypes, only a small number of children were studied before we commenced this study in Dutch twins. Our study, therefore, presents the first firm data on the genetic architecture of EEG power, ERP-P3, and EEG coherence in children.

EEG Power

In studies of children, EEG power has been used as the major index for brain maturation. Around 1970, a Swedish group of researchers reported on electroencephalographic data of 561 children aged 1 to 21 years (Petersén & Eeg-Olofsson, 1971; Matoušek & Petersén, 1973). This study provided important data on development of children's EEG. It was shown that during development EEG changed from a signal containing mainly slow waves to a signal with substantially less delta and theta activity, but more alpha and beta activity. The dominant EEG frequency in adults lies in the alpha range (around 10 Hz), but is lower in young children (around 7 Hz). Relative power (the ratio of power in a certain band over total power in the signal) or the theta/alpha ratio is often used to reflect these shifts in the dominant EEG frequency components. In chapter 2 of this thesis it was shown that absolute and relative EEG power in all broad bands (delta, theta, alpha1, alpha2, beta1 and beta2) were highly heritable at age 5 (with h^2 = 70% on average). In fact, these traits are among the most heritable polygenic traits found in children. This suggests that the individual differences in rhythmic activity of cortical pyramidal cells are under strong genetic control in childhood. At first sight, this finding shows great promise to bridge the gap between genes and behavior. If the theta/alpha ratio tells us something about brain maturation, then finding specific genes influencing this ratio may help us to determine which processes are involved in the

development of the subcortical and cortical generators of these rhythms. Deviations in these processes may be reflected in behavior. With his "neurometrics" approach, John (e.g., John, Prichep, Fridman & Easton, 1988) was able to discriminate between dementia, alcoholism, unipolar and bipolar depression, based on quantitative EEG data only. However, the direct association of EEG power with behavior has not been unequivocal (Gale & Edwards, 1986). The relationship between EEG power and cognitive abilities is even less clear (Vogel et al., 1968). In fact, it has been argued (Van der Molen & Molenaar, 1994) that there is no merit in investigating EEG when the child is committed to idle contemplation bearing little or no relationship to cognition. Thus, notwithstanding the fact that EEG power does seem to track individual differences in the stage of maturation of the brain, it is unlikely that individual differences in normal behavior or cognition are well indexed by background EEG.

ERP-P3

To index brain processes underlying cognition, psychophysiological researchers have increasingly turned to the event related potentials. The P3 (also known as P300) showed particular promise for this purpose. It can be reliably evoked, even in young children, in a simple oddball paradigm. The latency of the P3 (i.e., the timing of its peak) provides a measure of mental processing speed that is independent of behavioral responding (Donchin et al., 1986). Latency gradually decreases with age until young adulthood (Courchesne, 1977; 1978; 1990; Polich et al., 1990; Friedman, 1992). Individual differences in P3 latency have been suggested to be related to faster processing speed in various tests of cognitive function (Ladish & Polich, 1990; Emmerson et al., 1990). P3 amplitude is sensitive to task relevance and (subjective) probability of the stimulus and is suggested to be proportional to attentional resources invested in the maintenance and updating of working memory (Polich, 1996). Indeed, larger P3 amplitude has been associated with superior memory performance (Fabiani et al., 1990; Noldy et al., 1990). P3 latency has been used to index individual differences in cognition, whereas P3 amplitude has been used as an indicator of clinical disorders, for example attention deficit hyperactivity disorder (Robaey et al., 1992), autism (Kemner et al., 1992) and alcoholism (Begleiter et al., 1988; Polich et al., 1994). This may indicate that P3 amplitude has a predictive value as an index of susceptibility for deviant behavior. This thesis tested the extent to which this psychophysiological marker, is of a genetic nature.

During data reduction and analysis we were confronted with a very large intraindividual variability in the P3 wave form. This was particularly problematic for targets, of which only 25 trials were available to compute the average latency and amplitude. Even after

latency correction with a Woody filter (Woody, 1967), data from all frontal and temporal leads had to be discarded because of the bad signal to noise ratio. In addition, occipital leads had to be dropped, because of the strong interference of the dominant 4-8 Hz rhythmic activity on the P3. Because of the low reliability of the P3, structural model fitting on the remaining electrodes yielded badly fitting models that explained all observed variance by unique environmental factors. Therefore, a multivariate approach was employed that allowed a distinction between reliable genetic or environmental variances and measurement error. This was done by computing two separate P3 latencies and two separate P3 amplitudes on the odd and even nontarget trials. The odd and even trials were used to estimate the measurement error on nontarget P3 and it was assumed that this measurement error was similar to that on target trials. The fit of the structural models greatly improved for both targets and nontargets, when measurement error was modeled in this way. Latency of P3 appeared to be a heritable trait in 5 year olds (h^2 = 34%). This is well in accordance with twin data from adolescents and adults. In these studies, measurement error was not accounted for, but wave forms in older subjects are generally more reliable. Taken together, adult and child twin data suggest that genetic contribution to individual differences in speed of stimulus processing is present from an early age onwards. This does not, however, preclude the possibility that different genes influence P3 latency at different ages.

Heritability of P3 latency is quite comparable to that of reaction time (Rijsdijk, 1997). Both have been associated with IQ (Chalke & Ertl, 1965; Barrett & Eysenck, 1992; Vernon, 1987). Vernon and colleagues (1993) have proposed a neural efficiency hypothesis to explain the significant relationship between reaction time and IQ: the faster the stimulus processing is completed, the more time for stimulus elaboration or processing of other stimuli is available in working memory. Similar arguments can be put forward to explain the relationship between P3 latency and IQ. In our data, low but significant correlations (.19) were found between IQ and P3 latencies. It was not tested, however, whether the genetic factor influencing IQ was the same as that influencing P3 latency. A future application of the approach in the present study is to analyze IQ and P3 latency in a multivariate genetic model.

For P3 amplitude a clear discrepancy between the heritability of targets (which was low) and nontargets (which was moderate to high) was found. The largest part of the individual differences in target P3 amplitude appeared to be explained by unique environmental factors only. Yet, according to model fitting the genetic factor influencing nontargets appeared to be the same as that influencing targets. In the discussion of chapter 3 it is suggested that this genetic factor probably affects individual differences in the first part of stimulus processing of both types of stimuli, that is, everything until the detection

of nonrelevance of stimuli. This genetic factor did not affect individual differences in the part of stimulus processing that is specific to targets. We interpreted this as being caused by the difficulty of standardizing motivational effort and general arousal in these children. However, another possibility must also be considered. Four times more nontarget than target trials were presented, improving signal to noise ratio of nontargets considerable above that of targets. This may have caused underestimation of heritability in target ERPs.

EEG Coherence

Although the P3 has a well-documented relationship to cognition, its sources in terms of neural generators are largely unknown (Johnson, 1993; Polich, 1996). In contrast, EEG coherence can be directly linked to processes like axonal sprouting, synaptogenesis, myelination, and pruning of synaptic connections (Kaiser & Gruzelier, 1996). Thatcher and coworkers (Thatcher et al., 1986; Thatcher et al., 1987; Thatcher, 1994) have suggested that coherent activity between two electrodes measures the number of cortico-cortical connections and synaptic strength of these connections between the brain areas near those electrodes. In their 'two-compartmental' model of EEG coherence, based on the structural properties of the human cortex, compartment A receives input from the short fiber system, that is, from axonal connections of neighboring pyramidal cells, whereas compartment B receives input from the long fiber system, which contains long-distance axonal connections from other parts of the brain that represent the majority of the white matter fibers. Both systems contribute to coherence at the relatively short distances (i.e., to about 14 cm), whereas coherence at the longer distances is influenced by the long fiber system only.

Analyses of coherence data were presented twice in this thesis. In chapter 4, heritability estimates on coherence at age 5 were presented. A substantial difference in measurement error of the various electrode combinations was noted, such that reliability decreased with distance. In chapter 5, reliability was accounted for in the structural model fitting. This yielded heritability estimates between 37% to 75%, depending on electrode location. In this reanalysis, the difference found in the first analyses between additive genetic factors for short distance coherence versus dominance effects in long distances was not confirmed. We did, more importantly, confirm our original finding that heritabilities of long distance coherences were higher compared to heritabilities of short distance coherences. Therefore, the heritability estimates provide support for a two compartment model. Furthermore, the increase in heritability with increasing distance appeared to be most pronounced in the anterior to posterior direction. In the anterior to posterior direction heritabilities increased with increasing distance, whereas in the

posterior to anterior direction heritabilities were very much alike. The fact that heritability was sensitive to the direction of cortico-cortical connectivity supports Thatcher's claim that individual differences in coherence reflect axonal connectivity of the brain, rather than simple volume conduction (Thatcher et al., 1986). Nonetheless, recent reports did show that volume conduction and linked ear reference, as used in the present study, may inflate coherence (Lagerlund et al., 1995) and introduce spurious individual differences. In future analyses we might, therefore, recompute coherence after a Laplacian transform of the raw EEG data. This transform reduces the effects of volume conduction.

Based on studies of dementia and callosal disconnection patients (Dunkin et al., 1994; 1995), in adults, a decrease in coherence is thought to reflect a loss of functional connectivity. In children the reverse picture is seen. Gasser et al. (1987) showed that 10 to 13 year old mildly retarded children had higher coherences than controls. Higher short distance coherences were also found in dyslectics (Leisman & Ashkenazi, 1980) and in Down's syndrome (Schmid et al., 1992). In a population of normal children, Thatcher et al. (1983) showed that a negative correlation exists between full-scale IQ and short-distance coherences. A possible explanation for these paradoxical interpretations of coherence in adults and children was provided by Kaiser and Gruzelier (1996). They proposed that coherence in childhood may increase during the formation of new connections and decrease through pruning of the non-functional part of those synapses. Decreases in short distance coherence in childhood, reflecting pruning, may thus actually improve brain function. After the last growth cycle in adolescence, during which the connections from the prefrontal regions to the rest of the brain are fully matured (Kaiser & Gruzelier, 1996), all further decreases in coherence reflect inadverted pruning of functional connections, and thus damage to normal brain function. However, caution is needed in assuming that changes in coherence solely reflect changes in synaptic density. Apart from synaptic growth, increases in coherence during childhood may reflect improved brain function through a gradual increase in myelination of the axons in cortico-cortical tracts. This process is known to continue up until young adulthood, and is at its peak in young childhood. Increases in coherence in childhood may thus reflect a double growth effect: formation of new synapses and increased myelination. If one accepts a crucial role for genes in these processes, it is not surprising that the present study has clearly established this intriguing phenotype as a genetic trait.

In summary, the study described in this thesis provides information not previously available on the determinants of individual differences in brain function in a large, normative sample of young children. For most EEG indices (P3 amplitude to targets being the major exception) the main cause of individual differences were genetic

differences between children. More distant behavioral traits need not show the same high degree of heritability. In this same group of children, for instance, IQ at age 5, assessed by the RAKIT, interindividual variance could be explained predominantly by common environmental influences shared by children growing up in the same family (C). No evidence for shared environmental influences was found in any of the EEG and ERP indices. At face value, this may be taken to mean that "biological" traits will not be very useful to explain actual behavior. However, it has been pointed out by Plomin and DeFries (1985) that small genetically-based differences at a young age may be amplified across the life span. For instance, a small advantage in processing speed or frontal to occipital connectivity may have a small impact at age 5, but may start to make a large difference at later ages, when processing becomes more complex and the impact of small advantages in basic neural communication increases. Therefore, further research aimed at finding the actual genes influencing the EEG will contribute to our understanding of complex information processing as well as normal and aberrant behavior. In that mission, detecting linkage between a chromosomal region and EEG phenotypes would be a next logical step.

To detect linkage of a trait to a quantitative trait locus (QTL) on the chromosome, a high heritability is an advantage, because this enhances the possibility of finding a QTL that explains a substantial amount of the observed variance. The traits studied in this thesis are promising in this respect. EEG power is particularly promising, because very high heritabilities were found for this trait. In fact, this probably is the most heritable trait found in young children. A further advantage is, that EEG power is simultaneously assessed at 14 scalp locations, thus yielding the possibility of multivariate analyses, which greatly enhances the power to detect QTLs (Boomsma, 1996). This multivariate advantage also applies to P3 latencies and nontarget P3 amplitudes. Although these are slightly less heritable, they have the additional advantage of being more directly linked to cognition than EEG power. In this thesis it was shown that, prior to linkage, it is essential to account for measurement error in these indices. It also needs to be established whether the difference in heritability of P3 amplitude of targets and nontargets is real, or whether this difference may be a result of the fact that we used fewer target trials to obtain an averaged ERP than for nontargets. When the difference between targets and nontargets is real, we may question the use of target P3 amplitudes as possible genetic markers for alcoholism (Polich et al., 1994).

A disadvantage of using P3 amplitudes and P3 latencies to locate QTLs is, that we do not have a firm idea of which neurophysiological structures are responsible for the generation of the P3 wave and individual differences therein. Differences in EEG coherence, in contrast, can be more easily linked to structural aspects of the brain such

as axonal sprouting, synaptogenesis, expansion of existing synaptic terminals, myelination, pruning of synaptic connections, presynaptic changes in the amount of neurotransmitter or changes in the postsynaptic response to a given neurotransmitter. These aspects, in turn, could provide an indication of the type of protein substrates that an established QTL is coding for during follow-up studies with candidate genes. Because coherence is found to be largely heritable and because it is close to neurophysiological structure it provides an attractive starting point for linkage aimed at QTLs associated with cognitive abilities and behavior. In this regard, the recent development of techniques for evoked changes in coherence (Andrew & Pfurtscheller, 1996) is particularly promising, because it may provide phenotypes more directly linked to cognition and overt behavior than resting coherence.

Stability of genetic influences on the EEG

On average 1 year and 7 months after the first assessment at age 5, 192 of the initial 209 twin pairs returned to the laboratory for a second measurement session. This allowed the study of changes and stability of the influences on the EEG phenotypes during development. Developmental behavior genetics recognizes the importance of shifts in genetic and environmental factors on interindividual differences during development. Therefore, in addition to the questions concerning the relative influences of genetic and environmental factors on interindividual differences in electrophysiological indices of brain functioning, the changes in these influences over time were investigated. This investigation consisted of two parts: firstly, are (existing) genetic influences amplified, that is, does the relative importance of genetic over environmental influences, as expressed by heritability (h^2) change over time? Secondly, do new genetic influences emerge, that is, are different genes expressed at ages 5 and 7?

The 5 to 7 age span was chosen for a number of reasons. In developmental psychology it is well recognized that from infancy to adulthood, human brain and behavior undergo large changes. Part of these changes consists of continuous growth, but in addition, a number of periods during childhood can be identified that express more pronounced development. These periods are commonly indicated as growth spurts. One of these growth spurts is seen between ages 5 and 7 years. In this period remarkable stagewise development in cognition is seen (Piaget, 1966; Piaget & Inhelder, 1969): the transition from pre-operational to concrete-operational stage is made, in which the child learns the concept of conservation. Transition from one stage to another always involves

qualitative changes in the child's cognition, and is not reversible. Therefore, children at age 5 are in a qualitatively different phase than children at age 7.

Biological psychology provides a second perspective on change during childhood. Converging evidence from morphometric, PET and MRI studies (Huttenlocher, 1979, Huttenlocher et al., 1982; Chugani et al., 1987; Jernigan et al., 1991) suggests that early brain development from childhood to adolescence is characterized by a gradual decrease in grey matter and an increase in white matter. The decrease in grey matter, starts at about 4 years (Pfefferbaum et al., 1994), and is thought to reflect a pruning of synaptic contacts, such that only connections incorporated into functional networks survive, whereas random connections are eliminated. The increase in white matter may reflect the ongoing myelination of the many cortico-cortical connections. Both these effects contribute to a better differentiation and integration of functionally distant brain areas.

Finally, research in behavior genetics itself provides an indication of development in the age span of 5 to 7 years. In the longitudinal data from the Colorado Adoption Project (Cherny & Cardon, 1994) heritability of childhood IQ was shown to increase after 4 years of age. More importantly, new genetic factors were emerging somewhere between the ages 4 and 7. This, again, may indicate qualitative differences between young children (4 or 5) and somewhat older children (age 7).

In the physiological realm significant developmental changes can be non-invasively indexed by a number of EEG parameters. Substantial empirical evidence from these maturational EEG indices suggests that, on top of continuous growth, various periods of growth spurts in brain activity can be observed. Growth spurts were particularly demonstrated for EEG mean relative power (Hudspeth & Pribram, 1992) and for EEG coherence and phase (Thatcher, 1991; 1992; 1994a; 1994b; Thatcher et al., 1987). Hudspeth and Pribram (1992), using data of Matoušek and Petersén (1973), showed that, in addition to clear continuous growth, growth spurts in mean relative EEG power of four distinctive brain areas existed. They suggested 5 periods of increased development: around ages 4, 8, 12, 15 and 19 years. Their data show a period of relatively little changes between ages 5 and 7. Growth spurts were also found in EEG coherence and EEG phase by Thatcher and colleagues (Thatcher, 1991; 1992; 1994a; 1994b; Thatcher et al., 1987). Their studies concerned a group of 577 children aged 1 to 17 years, for whom EEG was collected on 19 scalp locations. Figure 6.1 (adapted from Thatcher et al., 1987) shows biennial changes in coherences for left and for right intrahemispheric coherences from prefrontal to more posterior scalp locations. The largest increase in coherence was found around age 6. Other peaks were at 10 and 13 years. This suggests that the large changes in coherences are systematically found after the large changes in relative EEG power. They may be related to the same phenomenon, and both Thatcher

and colleagues and Hudspeth and Pribram suggest that the growth spurts in the EEG are probably related to Piagetian stage transitions.

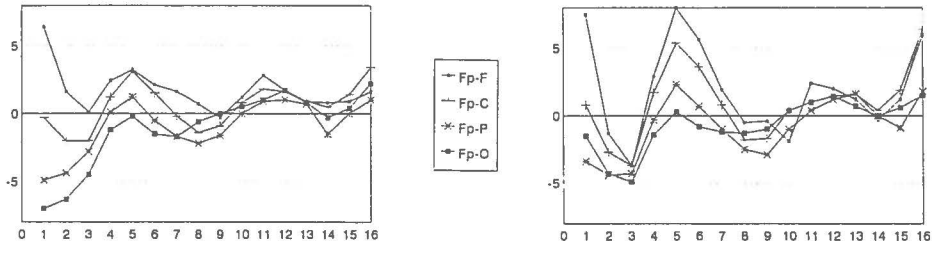

Figure 6.1 *Biennial differences in EEG coherence from ages 1 to 17 years, for left and for right hemispheres, for coherences between prefrontal scalp locations and frontal, central, parietal or occipital scalp locations. Periods with strong increases in coherence are interspersed with periods of less increase, or even decrease in coherence. (Figure adapted from Thatcher et al., 1987).*

In this thesis the development of electrophysiological indices, and possible growth spurts therein, were approached from a behavior genetics viewpoint. Specifically, we tested whether heritability changed over time and whether we could detect discontinuities in the genetic architecture of EEG power, P3 and coherence.

EEG power

Figure 6.2 shows that the power spectra of 7 year old children clearly differ from power spectra of 5 year olds. The alpha peak is more prominent at age 7, and especially delta and theta activity seem to have decreased with age. Although complete analysis of longitudinal EEG power data was not pursued in this thesis, in favor of analyses on ERP and coherence, some preliminary analyses were done for this discussion. Decomposition of variances and covariances into genetic and environmental factors showed that the high heritabilities reported in chapter 2 remained high in 7 year olds. Phenotypic correlations between EEG powers at age 5 and age 7 were about .8 (absolute alpha1-band) and cross-correlations were about twice as high for MZ twins compared to DZ twins, suggesting that the stability between ages 5 and 7 is mainly genetic. No information is yet available about the emergence of new genetic factors.

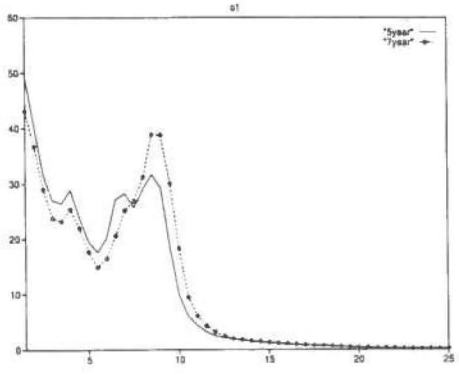

Figure 6.2 *Power spectra averaged over all subjects during rest with eyes closed for left occipital electrode position for 5 year old children and for 7 year old children. The power spectrum of 5 year old children shows more EEG power in the delta and theta frequency bands (.5 to 7.5 Hz), but less EEG power in the alpha frequency bands (8.0 to 12.5 Hz) than the power spectrum of 7 year old children.*

ERP-P3

P3 was chosen as a first target for longitudinal genetic analyses. In chapter 3 the results of genetic and environmental influences on the stability of P3 amplitude and P3 latency were presented. Contrary to our expectations, our results suggest that the maturation of P3 is a continuous process. This is surprising because P3 appears to closely mirror the time-course of development of grey and white matter (Courchesne, 1977; Courchesne et al., 1987). Changes in P3 amplitude are tentatively interpreted as a result of differences in synaptic density, whereas changes in P3 latency are probably related to changes in myelination (Courchesne et al., 1987). Since myelination affects neural speed, a decrease in P3 latency over time might reflect an increase in information processing speed (Reed & Jensen, 1984). Previously, a relationship between P3 latency and IQ had been reported (Chalke & Ertl, 1965; Barrett & Eysenck, 1992). Since new genetic factors emerge for IQ data in the 5 to 7 age range (Cherny & Cardon, 1994), new factors were expected to emerge for P3 latency. Instead, a single genetic factor found at age 5 still had large effects on P3 latency at age 7 on all leads. A significant additional genetic factor emerged only for P3 latency at Cz and P3 scalp locations, but they explained only a small part of the interindividual variance. Therefore, clear emergence of new genetic factors, as had previously been found for IQ in children of this age, was

not found for P3 latency. However, in the same twins as used in this study, Boomsma and Van Baal (1997) showed that interindividual differences in general intelligence at ages 5 and 7 are also influenced by the same genetic factors. No new genetic influences emerged for IQ at age 7. Thus, the absence of such influences on P3 latency in these twins is less surprising.

A priori, there was good reasons to expect a change in the genetic contribution to P3 amplitude. Previous studies suggested a discontinuity in P3 topography as a function of cognitive developmental stage. Stauder and colleagues (Stauder, 1992; Stauder et al., 1993; 1995) have claimed, using dipole analyses, that different P3 sources are found for children in the Piagetian preoperational phase than for children in the operational phase, a phase transition that occurs somewhere between ages 5 and 7. In this context, our finding of a common set of genes for both ages and no new genetic influences is not consistent, since it is unlikely that a new source of P3 would only be influenced by new environmental effects. It must be noted, however, that the present study did not allow for a detailed topographical analysis due to the unreliable signals at occipital and frontal locations. In the oddball task of Stauder et al. (1995) P3 amplitude of children with the ability of volume conservation differed from children without volume conservation only at lead Fz. It is possible that with the emergence of operational thinking new genetic factors emerge only in the frontal P3 generators.

EEG coherence

For coherence between most electrode combinations, the genetic factor expressed at age 5 accounted for the largest part of genetic variance at age 7, which indicates that no massive changes in genetic sources were found. However, additional new genetic factors were shown to emerge for 10 out of a total of 14 cortico-cortical coherences. These new genetic influences were hypothesized to be caused by changes in the relative importance of synaptic growth and synaptic pruning between age 5 and age 7. In an attempt to distinguish between two qualitatively different periods, a period of synaptic growth and a period of synaptic pruning, we simultaneously looked at changes in heritability as well as changes in total genetic and environmental variance (table 5.5). Changeux and colleagues (Changeux & Danchin, 1976; Changeux and Dehaene, 1989) proposed a theory which stated that, in development, a genetically mediated overabundancy of synaptic contacts is followed by a "pruning" of the non-functional part of these synaptic contacts. The consolidation of some and elimination of others is entirely according to the demands of the environment. Genetic influences affect interindividual differences in a number of anatomical and neurophysiological parameters underlying coherence, like axonal sprouting, synaptogenesis and myelination, whereas environmental

influences are believed to account for the final process of pruning. Changes in total genetic or environmental variances from age 5 to 7 thus may point to changes in the relative importance of these mechanisms. Such changes were clearly observed in this study.

A general decrease in environmental variance was found for almost all cortico-cortical coherences. The decrease in total environmental variance coincided with an increase in heritability in coherences in the posterior to anterior direction, that is, from sensory input channels to executive areas and working memory in frontal and prefrontal cortex. This appears contrary to the expectation that from age 5 to 7 pruning would become more important. This expectation was based on Thatcher (1991), who interpreted his coherence data for children between ages 5 and 7 as representing a qualitative change from a stage of synaptic growth to a stage of pruning. However, heritabilities of coherence in the opposite direction, that is, from executive to sensory direction, remained the same in the right hemisphere, and actually decreased in the left hemisphere due to a decrease in genetic variance. Therefore, although environmental variance was smaller, its *relative* contribution to coherence in the anterior to posterior direction actually increased. If effects of unique environment on coherence indeed represent pruning, this may suggest that from ages 5 to 7 pruning of connections of the prefrontal areas to more posterior areas becomes relatively more important. From a developmental perspective this corroborates the findings that the posterior brain areas mature earlier than the anterior brain areas (Stuss, 1992). The latter may continue to develop at least through adolescence. Furthermore, the selective increase in genetic variance in the right hemisphere compared to the left hemisphere, where it is already high at age 5, is in agreement with the developmental lead of the left hemisphere (Thatcher et al., 1987).

This is the first longitudinal study on changes in genetic and environmental influences on electrophysiological indices of neural development in children. Using models in which the unreliability of the measures at each occasion was taken into account, it was shown that interindividual differences in these indices were largely influenced by genetic factors, and that the stable part of the variance is mainly genetic. EEG power and EEG coherence for long connections were highly heritable, EEG coherence for short connections, P3 latencies and (nontarget) P3 amplitudes were moderately heritable. For EEG powers and P3 amplitude and latency these heritabilities did not change much from age 5 to age 7. For a number of cortico-cortical coherences heritabilities changed significantly from age 5 to age 7. This was shown to be due to changing environmental variances or changes in genetic variances, depending entirely on hemisphere and on the direction of the cortico-cortical connection. Little evidence was found for qualitative differences in brain electrophysiology in this period: Although new

genetic factors emerged at age 7 for a number of cortico-cortical coherences and for P3 latency at Cz and P3, these new genetic factors only accounted for a small part of the genetic variance at age 7.

These findings suggest two important directions of further research attempting to connect brain and behavior: The high heritability of these indices of brain functioning make them promising phenotypes to detect quantitative trait loci (QTLs). Once candidate genes have their role in brain function confirmed, neurophysiological study of the protein and its effect on brain structures can be pursued. A second direction of future research is the use of quantative genetic model fitting to link cognitive abilities, personality and psychopathology of children to EEG power, EEG coherence and P3 amplitudes and latencies. Such modelling allows the assessment of associations between these domains, simultaneously testing whether the association is genetically or environmentally mediated. Two possibilities are directly suggested by the present thesis: because P3 latency has been associated with neural speed and IQ (Chalke & Ertl, 1965; Barrett & Eysenck, 1992), one may try to establish the nature of this association by using the structural model that corrects for measurement error in P3 latency. Secondly, the relation between EEG coherence and Piagetian conservation ability could be directly tested. Since IQ data and conservational ability of the twins who participated in this study are available (Boomsma & Van Baal, in press) these questions will be pursued in the future.

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Samenvatting

De ontwikkeling van de hersenen in genetisch perspectief

Elektrofysiologische indices van hersenfunctioneren in vijf en zeven jaar oude tweelingen

De vier artikelen in deze dissertatie zijn gebaseerd op een grote longitudinale studie naar interindividuele verschillen in hersenfunctioneren van 209 jonge tweelingparen. Deze 418 kinderen ondergingen een uitgebreid elektrofysiologisch onderzoek aan de Vrije Universiteit te Amsterdam op zowel vijf- als zeven-jarige leeftijd. Met dit onderzoek werd inzicht verkregen in de genetische architectuur van individuele verschillen in een aantal elektroencefalografische parameters die worden beschouwd als indices van rijping van de hersenen en als mogelijke correlaten van gedrag en cognitie. Een tweetal vragen stond centraal. Ten eerste, wat is de relatieve bijdrage van erfelijke en omgevingsinvloeden op interindividuele verschillen in de elektrofysiologische parameters van hersenfunctioneren in jonge kinderen? Ten tweede, hoe veranderen deze invloeden in een periode in de kindertijd die bekend staat als een periode van versnelde ontwikkeling?

Als eerste werden de relatieve invloeden van genetische en omgevingsfactoren op hersenfunctioneren vastgesteld op vijf-jarige leeftijd. De tweelingmethode is hiertoe zeer geschikt. Gegevens over een-eiige (MZ) en twee-eiige (DZ) tweelingen kunnen gebruikt worden voor het vaststellen van zowel erfelijke als (gezins-)omgevingsinvloeden op interindividuele verschillen. De verschillen in een bepaalde eigenschap binnen MZ tweelingparen worden vergeleken met de verschillen binnen DZ tweelingparen. MZ tweelingen zijn 100% genetisch identiek, terwijl DZ tweelingen gemiddeld slechts 50% genetisch verwant zijn, net als gewone broers en zussen. Verschillen tussen de twee kinderen van een MZ tweeling kunnen dus alleen verklaard worden door verschillen in unieke omgevingsfactoren, terwijl verschillen tussen de kinderen van een DZ tweeling veroorzaakt kunnen worden door de unieke omgeving, maar ook door verschillende genen. Voor beide paren geldt dat overeenkomsten binnen een tweelingpaar veroorzaakt kunnen worden door gemeenschappelijke erfelijke factoren, maar ook door systematische effecten van een gedeelde (gezins)omgeving. Deze onderlinge relaties vormen de basis voor het bepalen van de relatieve invloed van genetische en omgevingsinvloeden op interindividuele verschillen in een gemeten eigenschap. De genetische invloed wordt vaak uitgedrukt in de erfelijkheid van een eigenschap (h^2), ofwel de proportie genetische variantie van de totale variantie.

Met de tweelingmethode kan op kwantitatieve wijze worden beschreven wat de relatieve invloed is van 'nature' en 'nurture' op indices van hersenontwikkeling in kinderen. Zoals

te verwachten was, bleken zowel de bijdragen van omgeving als van erfelijke factoren van belang voor de interindividuele verschillen in *alle* elektroencefalografische indices van hersenfunctioneren. Echter, de invloed van de genetische factoren bleek voor de meeste indices veel groter dan de invloed van de omgeving.

In hoofdstuk 2 werd een hoge tot zeer hoge erfelijkheid gerapporteerd voor de absolute en relatieve power van zes verschillende frequentiebanden in het EEG-spectrum. Absolute EEG-power van een frequentieband geeft aan hoeveel variantie in het EEG-signaal toe te schrijven is aan cyclische variaties met frequenties die in die band vallen. Relatieve EEG-power drukt uit wat het relatieve belang van die frequentieband is ten opzichte van de vijf andere frequentiebanden. Het is bekend dat het EEG sterk verandert met de leeftijd, van een signaal met vooral veel langzame golven in jonge kinderen, naar een signaal met wat snellere golven in volwassenen. De dominante EEG-frequentie ligt rond 10 Hz in volwassenen, terwijl dit in kinderen rond 7 Hz ligt. EEG-power wordt daarom vaak gebruikt als maat voor hersenrijping (maturatie). Op zowel vijf- als zeven-jarige leeftijd bleken absolute en relatieve EEG-parameters zeer sterk erfelijk te zijn: gemiddeld was de erfelijkheid 70%. EEG-power (absoluut en relatief) behoort daarmee tot de meest erfelijke karakteristieken gevonden in kinderen.

In hoofdstuk 3 bleken interindividuele verschillen in amplitude en latentie van de P3 Event Related Potential (ERP) te worden beïnvloed door zowel genetische als unieke omgevingsfactoren. ERP's zijn elektrofysiologische hersenreacties op een bepaalde gebeurtenis, zoals het zien van een plaatje of het horen van een toon. De P3 treedt op bij de evaluatie van de relevantie van een stimulus, zoals in de in dit onderzoek gebruikte visuele 'oddball' taak. In deze taak worden frequente, nontarget-stimuli ('hondjes') afgewisseld met meer zeldzame, target-stimuli ('katjes') die door de kinderen moeten worden geteld. Het bleek dat de scoring van de P3 bij vijf-jarige kinderen, en in mindere mate ook bij zeven-jarige kinderen niet eenvoudig was. Dit resulteerde in een vrij hoge meetfout. Omdat meetfouten van de P3 de schatting van erfelijke en omgevingsinvloeden ongunstig beïnvloeden, werd in een multivariaat genetisch model een schatting gemaakt van het betrouwbare deel van deze invloeden. Gebruik makend van dit model werd gevonden dat de erfelijkheid van P3 amplitudes op target-stimuli zeer laag was, terwijl de erfelijkheid van P3 amplitudes op nontarget-stimuli juist redelijk hoog was. Dit kan er op wijzen dat responsen op zeldzame gebeurtenissen (selectieve attentie) meer door de omgeving beïnvloed worden dan frequente gebeurtenissen. De erfelijke effecten op target P3 amplitudes werden echter wel veroorzaakt door dezelfde genetische factoren als de erfelijke effecten op nontarget P3 amplitudes. Omdat P3 amplitude vaak is gebruikt als indicator van klinische afwijkingen, zoals autisme of alcoholisme, impliceert dit dat een genetische marker voor dit gedrag makkelijker kan worden gevonden met P3

amplitudes in reactie op nontarget-stimuli dan op target-stimuli. De erfelijkheid van latentie van de P3, een maat die samenhangt met individuele verschillen in snelheid van informatieverwerking, was matig tot redelijk hoog (gemiddeld 34% voor vijf-jarigen en 70% voor zeven-jarigen). Dit gold zowel voor targets als voor nontargets, die door dezelfde genetische factoren werden beïnvloed.

In hoofdstukken 4 en 5 werd de genetische architectuur van EEG-coherentie bestudeerd. Coherentie is de overeenkomst tussen twee EEG-signalen op verschillende elektrodeposities. Het wordt gebruikt als index van functionele verbindingen tussen verschillende regio's van de hersenen, bijvoorbeeld tussen de frontale gebieden onderling (Fp1-F3) of tussen de frontale gebieden en de visuele schors (Fp1-O1). Uit de resultaten bleek een onderscheid in de invloed van genetische factoren op lange en korte afstanden: voor coherenties tussen elektrodes die relatief dicht bij elkaar lagen was de erfelijkheid lager dan voor de coherenties tussen elektrodes die verder van elkaar lagen. De gemiddelde erfelijkheid van coherentie op beide leeftijden op de kortste afstand (7 cm) was 46%, terwijl dat op de langste afstand (28 cm) 69% was. Ook werd een verschil in type genetische invloed gevonden tussen lange en korte afstand coherenties, hoewel dit alleen afdoende getoetst is op vijf-jarige leeftijd. De interindividuele verschillen in coherentie op korte afstand werden alleen door additieve genetische factoren beïnvloed, terwijl interindividuele verschillen in coherentie over langere afstand ook door dominante genetische factoren werden beïnvloed (dominante genetische factoren zijn factoren die interactie-effecten tussen genen aangeven). De verschillen in erfelijkheid van lange en korte afstand coherentie kunnen worden opgevat als een bevestiging voor het twee-compartiment model van Thatcher (Thatcher et al., 1986) waarin coherentie wordt verklaard uit de interactie tussen lokale neuronale netwerken en axonale verbindingen uit verder weg gelegen gebieden. Als de elektrodes relatief dicht bij elkaar liggen (tot 14 cm) dan wordt de coherentie tussen twee gebieden door beide compartimenten bepaald. Als ze verder weg liggen, leveren alleen de lange cortico-corticale verbindingen een bijdrage aan de coherentie.

Het bovenstaande geeft duidelijk aan dat erfelijkheid een belangrijke rol speelt in hersenfunctioneren. Echter, de hoofdvraag van deze dissertatie was of er in de periode van vijf tot zeven jaar belangrijke veranderingen zouden optreden in de genetische architectuur. In deze periode gaan de kinderen naar de basisschool, wat de invloed van zowel gezamenlijke als unieke omgevingsfactoren kan veranderen. Tevens valt deze periode samen met een sterke rijping van de grijze en witte hersenstof. Voorts is bekend dat in deze periode belangrijke kwalitatieve veranderingen in cognitie optreden en dat de genetische architectuur van intelligentie verandert. De kwalitatieve veranderingen in cognitie zijn beschreven door Piaget. Rond het zesde levensjaar maken kinderen een

cognitieve groeisprong, waarin ze bijvoorbeeld gaan begrijpen dat het volume van water niet verandert als het van een hoog smal glas in een lang breed glas wordt overgegoten (volume-conservatie). In dezelfde periode blijkt de erfelijkheid van IQ toe te gaan nemen en treden nieuwe genetische invloeden op IQ aan het licht. De leeftijd van 5 tot 7 jaar lijkt dus optimaal te zijn om veranderingen in de determinanten van interindividuele verschillen in hersenfunctioneren te onderzoeken. Twee vragen stonden daarbij centraal: Ten eerste, hoe veranderen de relatieve bijdragen van genetische (h^2) en omgevingsinvloeden van leeftijd 5 jaar naar leeftijd 7 jaar? Ten tweede, zijn er nieuwe genetische factoren, die geen invloed hadden op interindividuele verschillen in EEG-parameters van vijf-jarige kinderen, maar die wel invloed hebben op interindividuele verschillen in EEG-parameters van diezelfde kinderen op zeven-jarige leeftijd?

Gemiddeld 1 jaar en 7 maanden na de eerste meting werd het EEG van 192 van de 209 tweelingparen een tweede keer gemeten. Uit hoofdstuk 3 blijkt dat de erfelijkheid van P3 amplitude niet veranderde in de leeftijd van 5 tot 7 jaar. Ook waren er geen aanwijzingen voor het optreden van nieuwe genetische invloeden op de P3 amplitude op zeven-jarige leeftijd. Wel leek de erfelijkheid van de P3 latentie toe te nemen van 5 naar 7 jaar. Mogelijk hing deze toename samen met het optreden van nieuwe genetische invloeden op 7 jaar, hoewel die slechts voor 2 van de 6 bestudeerde elektrodeposities (Cz, P3) significant waren. Het grootste gedeelte van de genetische factoren die op zeven-jarige leeftijd van invloed waren op de P3 latentie, werden reeds op vijf-jarige leeftijd gevonden. Al met al werd er dus geen sterke aanwijzing gevonden voor kwalitatieve veranderingen in de P3 generatoren in deze leeftijdsperiode.

In hoofdstuk 5 werden de veranderingen in de erfelijkheid van coherentie van 5 naar 7 jaar getoetst. Bovendien werd gekeken of de totale genetische en de totale omgevingsvariantie in de tijd veranderde. Dit leverde een verrassend patroon op, dat is samengevat in Tabel 5.5. De veranderingen in erfelijkheid en varianties in de coherenties tussen prefrontale elektrodes en elektrodes meer naar het achterhoofd waren anders dan die in de coherenties tussen de occipitale elektrodes en elektrodes meer naar het voorhoofd. Afhankelijk van de richting waarin de coherentie werd gemeten was er ook een verschil tussen de linker en rechter hersenhelft. Erfelijkheid nam in beide hersenhelften toe voor coherenties van achter naar voor op het hoofd. Erfelijkheid nam in de linker hersenhelft af van voor naar achter op het hoofd, maar bleef hetzelfde in de rechter hersenhelft. Dit patroon sluit aan bij het idee dat de linker en rechter hersenhelft verschillend rijpen en dat de connecties van frontaalschors naar parietale en centrale regio's later rijpen dan de connecties van de occipitale schors naar parietale, centrale en frontale regio's. Individuele verschillen in deze rijping kunnen een weerspiegeling zijn van verschillen in processen als myelinizatie en ontstaan en afbraak van synapsen. De duidelijke

genetische invloed op die verschillen is niet verwonderlijk omdat deze processen worden gereguleerd door eiwitten waarvan de regulatie zelf weer afhankelijk is van het genotype van het individu.

Net als bij de P3, bleken de genetische invloeden op de coherentie in de leeftijd van 5 tot 7 jaar grotendeels stabiel. Nieuwe genetische factoren op leeftijd 7 jaar werden gevonden voor 10 van de 14 elektrodecombinaties. Echter, deze nieuwe genetische factoren verklaarden slechts een klein deel van de genetische variantie in coherentie: de genetische invloeden op leeftijd 5 jaar waren nog steeds verantwoordelijk voor het grootste gedeelte van de genetische variantie op leeftijd 7 jaar. Dit suggereert dat er van 5 tot 7 jaar geen overheersende kwalitatieve veranderingen in verbindingen tussen de hersengebieden optreden.

Deze dissertatie suggereert verschillende mogelijkheden voor vervolgonderzoek. Allereerst blijkt duidelijk dat EEG-indices goed gebruikt kunnen worden om zogenaamde QTL's (Quantitative Trait Loci) te vinden, die een invloed uitoefenen op interindividuele verschillen in hersenfunctioneren. Dit linkage-onderzoek is de eerste stap op weg naar de identificatie van individuele genen en hun effect op eiwitten die een rol spelen bij neurofysiologische processen. Linkage wordt beter mogelijk naarmate de bestudeerde eigenschap meer erfelijk is. De EEG-indices die besproken zijn in deze dissertatie voldoen aan die voorwaarde, met uitzondering van P3 target amplitude. Bovendien neemt de statistische power voor linkage toe als een multivariaat design gebruikt kan worden. Bij EEG-indices is dit goed mogelijk omdat het EEG op verschillende elektrodelokaties verzameld wordt. Ten tweede kan in vervolgonderzoek worden gekeken naar de relatie tussen veranderingen in EEG-parameters en cognitieve vaardigheden. Bij de tweelingen in dit project zijn bijvoorbeeld ook een intelligentie-test en een test voor volume-conservatie afgenomen. Het is goed mogelijk de gebruikte genetische modellen uit te breiden om de aard van de relaties tussen EEG-parameters en cognitieve vaardigheden vast te stellen. Wanneer zo'n relatie overwegend erfelijk blijkt te zijn, kunnen de chromosomale regio's waarin QTL's voor elektrofysiologische maten werden gevonden zelfs als startpunt worden genomen om genen te lokaliseren voor cognitie.

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