A GENETIC PERSPECTIVE ON ELECTROPHYSIOLOGICAL MEASURES OF BRAIN FUNCTION

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VRIJE UNIVERSITEIT

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INTRODUCTION

The genetics of normal human behavior (such as cognitive abilities and personality traits as neuroticism) and behavioral pathology (such as depression and autism) have been extensively studied. It has become clear that individual differences in many traits and disorders are heritable, explaining in part why one person is struck with illness and the other not. For some disorders, such as autism, schizophrenia, and ADHD, heredity is the largest source of individual differences in disease risk, with up to 80% of trait variance attributable to genetic polymorphisms (Rutter, 2000; Folstein et al., 1977; Bailey et al. 1995; Derks, 2006). Various behavioral traits, such as intelligence, are highly heritable (Polderman et al., 2006; Posthuma et al., 2001, 2002; Silventoinen et al., 2006) with 50% to 80% attributable to genetic polymorphisms, and others, such as personality traits, are at least moderately heritable with 40% to 65% attributable to genetic polymorphisms (Jang et al., 1996; Rushton et al., 1986; Eaves et al., 1999).

For the genetic part of their variation, it has become clear that these traits have a complex genetic structure. Except for some highly heritable Mendelian traits or diseases that are caused by single nucleotide mutations, most traits are *polygenic* with the contribution of many genetic polymorphisms. For these traits, it has been quite hard to localize the chromosomal loci, often referred to as QTL (quantitative trait loci), that contribute to more than a single percent of the observed trait variation. In addition, once the QTL is found it is not always clear how the allelic variation causes behavioral variation (KIBRA gene; Papassotiropoulos et al., 2006).

To 'fill the gap' between genotype and phenotypes (such as traits and disease), the concept of endophenotype has been introduced (Gottesman & Shields, 1972; Gottesman & Gould, 2003; de Geus, 2002) to represent some process in the pathway from gene to its expression. Endophenotypes should fulfill the following requirements (de Geus, 2002):

1. Endophenotypes must be reliable and stable over time.
2. Endophenotypes must be heritable.
3. Endophenotypes must be correlate with the targeted phenotype (trait or disease).
4. The correlation between endophenotype and phenotype must (partly) derive from the same genetic source.
5. The association between the endophenotype and phenotype must be theoretically meaningful (causality).

Psychophysiological measures, including *electroencephalographic* (EEG) and *Event Related Potential* (ERP) measures, are generally regarded as promising candidate endophenotypes. There is a vast literature that has successfully correlated various types of psychophysiological variables to psychopathology such as ADHD (Barry,

Clarke, & Johnstone, 2003; Bresnahan & Barry, 2002; Chabot & Serfontein, 1996; Clarke, Barry, McCarthy, & Selikowitz, 2001; Clarke et al., 2003; Jasper, Solomon, & Bradley, 1938; Monastra et al., 1999; Satterfield, Cantwell, Saul, Lesser, & Podosin, 1973), schizophrenia (Blackwood, 2000; Levit et al., 1973; Verleger & Cohen, 1978), alcoholism (Ehlers & Schuckit, 1990, 1991; Gabrielli et al., 1982; Propping, 1977; Rangaswamy et al., 2002; Van Sweden & Niedermeyer, 1999; Vogel, 2000; Begleiter et al., 1984; Blackwood, 2000; Elmasian et al., 1982; Polich et al., 1994; Porjesz & Begleiter, 1990; Turetsky et al., 2000), cognitive decline in later life (Reinvang et al., 2005), and depression (Allen et al., 1993; Field et al., 2000; Gotlib et al., 1998; Reid et al., 1993; Silva et al., 2002; Bruder et al., 2001; Davidson et al., 1985; Debener et al., 2000; Henriques & Davidson, 1991; Nitschke et al., 1999; Schaffer et al., 1983; Wiedemann et al., 1999). Moreover, these candidate endophenotypes possess face validity in being intermediate phenotypes 'filling the gap' (Gottesman & Gould, 2003) between phenotype and genes.

Previous genetic studies

So far, the majority of genetic analyses of psychophysiological endophenotypes have focused on EEG oscillations and the P300 (e.g., Almasy et al., 1999; Begleiter et al., 1998; Christian et al., 1996; Hansell et al., 2001; Lykken et al., 1982; O'Conner et al., 1994; van Baal, De Geus, & Boomsma, 1996; van Beijsterveldt, Molenaar, de Geus, & Boomsma, 1996; Anokhin et al., 2004; Wright et al., 2001; Carlson et al., 2002; Katsanis et al, 1997; see van Beijsterveldt & van Baal, 2002, for a summary of twin and family studies). Most studies that investigated oscillations in the EEG signal have looked at power in the 'classical' frequency bands delta (1 to 4 Hz), theta (4 to 8 Hz), alpha (8 to 13 Hz), and beta 13 to 30 Hz), or related measures. In general, individual variation in EEG power showed high heritability, for example, heritability of alpha power showed an average of 79% across studies (van Beijsterveldt & van Baal, 2002). However, none of these previous studies have investigated whether the heritability changes gradually as a function of frequency. In addition, it remains to be investigated whether heritability of EEG power is stable across age groups. There are examples of a striking dependence of heritability on age, most notably for intelligence (Posthuma et al, 2007).

Heritability of evoked potentials such as the P300 and N1 have also been the subject of investigations in the past (Wright et al., 2001; Carlson et al., 2002; Katsanis et al, 1997; O'Conner et al., 1994; van Baal et al., 1998; van Beijsterveldt et al., 2001; Anokhin et al., 2004). P300 heritability was estimated to be 60% for amplitude and 51% for latency in a meta-analysis (van Beijsterveldt & van Baal, 2002). Most of the previous studies have investigated the simple 'overall'

parameters of P300 peak latency and amplitude. However, the P300 is no longer viewed as a single, unitary phenomenon (Dien et al., 2004) but the result of multiple waves superimposed on each other, each with a different cognitive meaning. This begs the question whether the temporal subcomponent structure is reflected in the genetics of the P300 waveform.

A few studies have investigated the genetics of connectivity between brain areas. *Coherence* is a measure that encapsulates the linear dependencies between two EEG signals. As such, it is interpreted as a measure of connectivity between the brain areas that produced these signals. Ibatoullina et al. (1994) showed low heritability of interhemispheric coherence in twins aged 5 and 6. Van Baal et al. (1998a) and van Beijsterveldt et al. (1998a) investigated coherence in 5-year-old twin and adolescent twin pairs, respectively. Both found moderate to high heritability for coherence (30% to 75%). Another measure of connectivity that has been investigated is called *Synchronization Likelihood* as introduced by Stam and van Dijk (2002). Posthuma et al. (2005) found moderate to high heritability (41% to 67%) for this relatively new measure that holds an advantage over coherence as a measure of connectivity because (i) it encapsulates not only linear, but also nonlinear statistical dependencies between two signals, and (ii) it is unbiased, whereas coherence will often be (Stam & van Dijk, 2002; Montez et al., 2006).

Although synchronization likelihood and coherence are informative about the overall degree of connectivity, they do not indicate the pattern or efficiency of brain connectivity. Recent application of graph theory to the synchronization likelihood measure allows for such qualitative assessment of connectivity patterns but no genetic analyses have been performed on these new graph theoretical measures.

To summarize, genetic studies until now have focused on relatively simple characteristics of EEG power and the P300 but have not investigated in any detail the complexity of P300 and EEG power. In addition, the extent to which heritability is different in males and females and across age groups remains underexplored. And, most importantly, many other ERP and EEG measures that have great potential as endophenotypes have yet to be explored. These include several new and recent EEG-based measures that reflect the temporal structure within EEG signals and the spatial complexity of synchronicity between the signals. **The core mission of this dissertation is to examine the genetic architecture of a set of selected EEG and ERP measures that may index important individual differences in brain structure and function.**

This dissertation

Below I provide an overview of the chapters in this dissertation and introduce the EEG or ERP measures that will be investigated.

Chapter Two revisits the heritability of the power of oscillations at various frequencies. Previous research on the topic was done mainly in children or adolescents (e.g. van Beijsterveldt & Boomsma, 1994; van Beijsterveldt & van Baal, 2002; Zietsch et al., 2007; Smit et al., 2006) and this will be extended by investigating a large adult twin sample consisting of a young and a middle-aged cohort. This sample enables the investigation of possible age differences in genetic architecture of EEG power as well as sex differences. In addition, we will investigate changes in heritability across the entire EEG power spectrum using 1-Hz frequency bins. Lastly, the chapter will look into genetic correlations between frequencies, investigating to what extent the frequencies represent the expression of the same or different genetic factors.

Chapter Three focuses on the P300 ERP, arguably one of the most studied parameters derived from EEG. The P300 is often viewed as a complex of multiple waves superimposed on each other, each with a different cognitive meaning (Falkenstein et al., 2004; Dien et al., 2004). This allows for the possibility that different genetic factors may influence the early, middle, and late parts of the P300. Chapter Three will first investigate whether there is a change in the heritability of the P300 over its time course (on a millisecond scale). Secondly it will investigate whether the P300 indeed hosts genetically different components.

Chapter Four investigates the earliest of ERP waves that can be influenced by endogenous factors such as attention: the N1. This component has been relatively neglected by geneticists so far. In the visual modality, only two studies have been performed with contrasting results. In a first study (Almasy et al., 1999) small genetic effects on variation in N1 amplitude and latency were reported for frontal leads (heritability of amplitude 19% to 31%; heritability of latency 10% to 16%) whereas larger effects were found for occipito-temporal leads (amplitude 45% to 54%, latency 3% to 12%). In the second study (Katsanis et al., 1997) only small and nonsignificant genetic effects on variation in target N1 were reported for three parietal leads. Chapter Four will separate the anterior and posterior N1 (Vogel and Luck, 2000), which may provide insight into why previous studies have reported contradictory heritability estimates for an endophenotype that may be a marker for cognitive decline in later life (Reinvang et al., 2005).

Chapter Five investigates a variable derived from alpha power that has been consistently related to depression, anxiety, and individual differences in emotional processing. *Frontal EEG Asymmetry* (FA) was first proposed by Davidson and colleagues as a biological marker of depression in the late 70's (Davidson et al., 1979). FA measures the activity of the two frontal lobes by measuring EEG

alpha activity, where higher power indexes lower activity (see Danos et al., 2001; Goldman et al., 2002; Nagai et al., 2004; Schreckenberger et al., 2004). A higher relative left frontal activity is proposed to be protective against negative affect, while higher right frontal activity is a liability for negative affect and anxiety disorder. Much support of the view has been found (Allen et al., 1993; Baving et al., 2002; Bruder et al., 2001; Davidson et al., 1985; Debener et al., 2000; Field et al., 2000; Gilbert et al., 1999; Gotlib et al., 1998; Heller et al., 1997; Henriques & Davidson, 1991; Miller et al., 2002; Minnix et al., 2004; Nitschke et al., 1999; Petruzello & Landers, 1994; Reid et al., 1993; Schaffer et al., 1983; Silva et al., 2002; Tomarken et al., 2004; Wiedemann et al., 1999; for a review see Coan and Allen, 2004), suggesting that FA is a possibly valuable endophenotype for genetic studies of depression. Finally, two studies suggested that some of the individual variation in FA is heritable (Anokhin et al., 2006; Coan, 2003). However, sex and age effects on the genetic architecture have not been explored in these studies, although the extant literature clearly suggests that the phenotypic relation between FA and anxiety/depression is more robust in females (Baving et al., 2002; Bruder et al., 2001; Miller et al., 2002; Tomarken et al., 2004).

Chapters Six and Seven present the first ever genetic analysis of two recently introduced EEG measures that are based on the patterning of EEG activity across space (ch. 6: *graph theoretical analysis*) and time (ch. 7: *detrended fluctuation analysis*). Chapter six is based on computation of the synchronization likelihood connectivity measure, that identifies linear as well as nonlinear dependencies between EEG signals. To identify a spatial patterning in the connectivity matrix between all electrodes, graph theory is then introduced that can identify a certain optimization or efficiency by the use of just two parameters. The chapter investigates whether these network efficiency parameters show evidence of heritability. Chapter seven investigates a new measure of time-based dependencies in the EEG. Within each EEG signal, patterns exist in the amplitude of oscillatory activity that reveal correlations over time. These correlations show a power law decay, and these decay values show striking individual differences (Linkenkaer-Hansen et al., 2001). We will investigate whether these individual differences, that are potentially interesting to describe individual variation in the temporal structure of brain activity, have a genetic background.

Chapter Eight investigates one ERP—the slow cortical potential—and two EEG measures—theta desynchronization and alpha synchronization—that can all be observed simultaneously in the response anticipation interval of a spatial memory *Delayed Response Task* (e.g., Birbaumer et al., 1990; Ruchkin et al., 1995; Bastiaansen et al., 2002; Hansell et al., 2001). We will establish the heritability of these phenomena and examine their phenotypic and genetic correlations. We suspect that the slow cortical potential, theta desynchronization and alpha syn-

chronization arise from a single neural substrate, and that a powerful trivariate endophenotype can be construed from these parameters.

In the final chapter the main findings of the empirical studies are summarized. These findings are discussed and evaluated with respect to the endophenotype approach. The final chapter concludes with possible directions for future research into the genetics of brain function.

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2

HERITABILITY OF BACKGROUND EEG ACROSS THE POWER SPECTRUM

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ABSTRACT

We estimated the genetic and non-genetic (environmental) contributions to individual differences in the background EEG power spectrum in two age cohorts with mean ages of 26.2 and 49.4 years. Nineteen-lead EEG was recorded with eyes closed from 142 monozygotic and 167 dizygotic twin pairs and their siblings, totaling 760 subjects. We obtained power spectra in 24 bins of 1 Hz ranging from 1.0 to 25.0 Hz. Generally, heritability was highest around the alpha peak frequency and lower in the theta and delta bands. In the beta band heritability gradually decreased with increasing frequency, especially in the temporal regions. Genetic correlations between power in the classical broad bands indicated that half to three quarter of the genetic variance can be attributed to a common source. We conclude that across the scalp and most of the frequency spectrum, individual differences in adult EEG are largely determined by genetic factors.

INTRODUCTION

Recordings of resting background EEG show striking interindividual differences (Vogel, 2000). In part, these differences can be described in a qualitative way, e.g. the presence or absence of low-voltage EEG, defined as resting EEG without rhythmic activity and with low amplitude that occurs in about 4% of the adult population; or, at the other extreme, the presence of continuous alpha waves in an estimated proportion of also about 4% of the adult population (Vogel, 1970). More common, however, is the quantitative description of the individual differences in the EEG traces by the amplitude or power spectrum.

Background EEG power has been linked with various forms of psychopathology. For example, increased theta power and theta/beta ratio is found in Attention Deficit Hyperactivity Disorder (Barry, Clarke, & Johnstone, 2003; Bresnahan & Barry, 2002; Chabot & Serfontein, 1996; Clarke, Barry, McCarthy, & Selikowitz, 2001; Clarke et al., 2003; Jasper, Solomon, & Bradley, 1938; Monastra et al., 1999; Satterfield, Cantwell, Saul, Lesser, & Podosin, 1973), and increased beta power is found in (a predisposition to) alcoholism (Ehlers & Schuckit, 1990, 1991; Gabrielli et al., 1982; Propping, 1977; Rangaswamy et al., 2002; Van Sweden & Niedermeyer, 1999; Vogel, 2000). Therefore, understanding interindividual variance in EEG power could provide clues to the underlying neurobiology of these disorders.

A first step is the partitioning of interindividual variance in EEG power into genetic and environmental parts. This can be done in twin studies that compare the intrapair resemblance between two types of sibling relationships, namely genetically identical (monozygotic twins, MZ) and non-identical twins (dizygotic twins, DZ). If MZ resemblance for EEG power is higher than DZ resemblance, this constitutes evidence for genetic influences on the EEG. A simple formula by Falconer (1960) computes the relative contribution of genetic influences to the total variance, also called heritability (h^2), as twice the difference in MZ/DZ resemblance:

$$h^2 = 2(r_{MZ} - r_{DZ})$$

where r_{MZ} and r_{DZ} quantify the intrapair resemblance for MZ and DZ twins. Observations from the early years of electroencephalography already have shown that EEG tracings of MZ twins show remarkable resemblance (Davis & Davis, 1936), and more so than those of DZ twins (Lennox, Gibbs, & Gibbs, 1945; Loomis, Harvey, & Hobart, 1936). In more recent approaches, Falconer's formula has given way to maximum likelihood techniques that can use more information than twin correlations alone (Jinks & Fulker, 1970). These models can include data from both types of twins (MZ, DZ) as well as from singleton siblings. By

fitting biometric models of sibling resemblance to observed variance-covariance matrices, the relative contribution of genetic and environmental factors can be estimated and the contribution of environmental factors can be further partitioned into factors shared by all siblings and factors unique to a single sibling (Falconer & MacKay, 1996; Neale & Cardon, 1992).

Using EEG power in the classical broad bands (delta, theta, alpha, beta), twin studies have unanimously supported the importance of genetic differences to explain individual differences (for an overview see van Beijsterveldt & Boomsma, 1994). Reliable estimates (where sample sizes have been sufficiently large) have been obtained in children and adolescents. Heritability of absolute power in the broadband frequencies (averaged over leads) ranged from 55% to 90% in 209 pairs of 5 year old twins (van Baal, De Geus, & Boomsma, 1996), and from 70% to 90% in 213 adolescent twins aged 16 (van Beijsterveldt, Molenaar, de Geus, & Boomsma, 1996). In the adult population, a large number of small-scaled twin studies correlations have suggested the importance of genetic factors in alpha amplitude measures through Falconer's h^2 calculation. However, studies employing structural equation modeling on large adult samples are still lacking. In an attempt to deal with this, van Beijsterveldt and van Baal (2002) performed a meta-analysis on the twin correlations in five smaller studies with adult samples that had assessed alpha power or similar measures. Although genetic factors significantly contributed to EEG power in each study, it was not possible to equate the results across studies into a single heritability estimate. Therefore, this study will examine a larger sample of twin families to estimate heritability of the adult EEG power spectrum. Heritability of EEG power was estimated in two different age cohorts: young adult twins and their singleton siblings with an age centered around 25 years and middle-aged twins and their singleton siblings with an age centered around 50 years.

An additional issue addressed in this paper is whether the different frequencies of the power spectrum have a similar genetic architecture. It has been shown that different frequency bands reflect different cognitive processes (Klimesch, 1999; Ray & Cole, 1985; Rugg & Dickens, 1982; Schacter, 1977). An intriguing question is whether this is reflected in a different heritability for these different frequency bands. As it may be argued that the broad bands lump together sources of information of frequency components, we examined the genetic architecture of the power spectrum in more detail by computing heritability across narrow frequency bins of 1 Hz. By plotting heritability against the frequency of the bin, we obtained the so-called 'heritability spectra'. This allowed us to investigate whether adjacent frequency bins show sharp discontinuities around the lower and upper frequencies of the broad bands. Secondly, we calculated the genetic correlations between power in the broad bands, to test how much of the genetic

Table 1. Composition of participating families.

	Number of families	
Family composition*	**MZ**	**DZ**
Both twins only	76	72
Both twins + 1 sibling	40	54
Both twins + 2 or more siblings	10	14
One twin only	3	9
One twin + 1 sibling	8	9
One twin + 2 or more siblings	-	5
1 sibling	2	4
2 or more siblings	3	-

**We based family composition on the participating offspring only. For example, a family with 'both twins only' could consist of more than two children, but these did not participate in the EEG experiment.*

variance across frequencies can be traced to a common source.

METHOD

Subjects

Subjects were recruited from the Dutch Twin Registry as part of a large project on the genetics of cognition and adult brain functions (Posthuma, Neale, Boomsma, & de Geus, 2001). Adult twins and their non-twin siblings were asked to participate in a testing protocol lasting 4.5 hour. In total, 760 family members from 309 twin families participated in the study. The complete sample consisted of two age cohorts: a younger cohort (mean 26.2 years, SD 4.1) and an older cohort (mean 49.4 years, SD 7.2). Participating families consisted of one to seven siblings (including twins). On average, 2.5 participants per family participated. Table 1 shows the frequency of families broken down by the number of twins and siblings participating, and by zygosity of the twin pair.

EEG registration

During one part of the experimental protocol, psychometric intelligence, inspection time, and reaction times were assessed. During the other, EEG was measured at rest and during various reaction time tasks. The order of the two parts of the protocol was randomized across family members. Consequently, half of EEG registration sessions were during morning hours, and half were in the afternoon.

Resting background EEG was registered for three minutes under both Eyes Open and Eyes Closed instructions, but only results from the Eyes Closed condition will be reported. Subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated, and electromagnetically shielded room. They were instructed to relax and minimize eye and body movement.

EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS; Enschede, The Netherlands) for 657 subjects and NeuroScan SynAmps 5083 amplifier for 103 subjects. Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2. Standard 10-20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2 (Jasper, 1958; American Electroencephalograpic Society, 1991). For NeuroScan subjects Fp1, Fp2, and Oz were also recorded, but not included in the analysis. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz. NeuroScan filter settings were a lowpass filter at 50.0 Hz. In principle, this suggested 30 Hz as the maximum frequency at which the systems obtained comparable data. Because the filters are not perfect, however, device-specific differences may have been introduced even before the 30.0 Hz frequency used by the TMS system, and the analyses were restricted to an upper level of 25.0 Hz for both systems.

Data processing

All EEG signals were recalculated with averaged earlobes (A1 and A2) as reference and analyzed using NeuroScan software version 4.2. The 3 minutes recordings were cut into 43 epochs of 1024 data points (4.096 s). Any linear trend was removed from EEG by fitting and subtracting the regression line for each epoch separately. Next, epochs were excluded per lead when EOG channels showed more than 400 μV and EEG more than 175 μV deviation from ground in either direction. EEG traces were then visually inspected per subject for remaining artifact due to muscle activity, swallowing, eye movement, bad recordings, and externally induced artifacts (e.g. experimenter initiated reset pulses, electrical hum). Only epochs with extreme magnitudes of muscle artifacts and eye movements

were excluded. Subjects with less than 22 valid epochs after visual inspection were considered unreliable and set to missing (22 epochs ensure at least 1 minute and 30 seconds of data per subject.) In all instances, however, data were made missing only for the particular lead. For an average lead, 741 subjects passed the criteria.

For all remaining epochs, power spectra were calculated with a Hamming window for 5% of the epoch duration at the beginning and end of the epochs. Power spectra were averaged, resulting in a single spectrum with a resolution of about 0.25 Hz (1000 / 4096 Hz). Power values across the spectrum were aggregated into 1.0 Hz bins, from 1.0 Hz up to but not including 2.0 Hz, from 2.0 Hz up to but not including 3.0 Hz, and so forth up to 25.0 Hz, thus creating twenty-four 1.0 Hz bins. Power in the classical broad bands were defined as follows: theta as the sum of all available data points from 4.0 Hz up to but not including 8.0 Hz, alpha as the sum from 8.0 Hz up to but not including 13.0 Hz, and beta as the sum from 13.0 Hz up to but not including 25.0 Hz.

Statistical analyses

Statistical genetic analysis of the power spectra was performed using Structural Equation Modeling implemented in the program Mx (Neale, 2003). Extended twin designs provide data characterized by families of variable size. Mx handles such unbalanced datasets via full information maximum likelihood, which uses the observed, raw data instead of variance covariance matrices. To evaluate how well the specified model fits the observed data, the raw data option in Mx calculates the negative Log-Likelihood (-LL) of the raw data for each family (Lange, Westlake, & Spence, 1976), as: $-LL = -k \log(2\pi) + \log|\Sigma| + (y_i - \mu_i)' \Sigma^{-1} (y_i - \mu_i)$, where k (k = 1, …, p) denotes the total number of observed variables within a family (and can vary over families), Σ (p x p) is the expected covariance matrix of family members, y_i (for i = 1,… p) is the vector of observed scores, μ_i is the column vector of the expected values of the variables, and $|\Sigma|$ and Σ^{-1} are the determinant and inverse of matrix Σ, respectively.

Twice the difference between two nested models (-2{LLfull model – LLnested model }) is asymptotically distributed as χ^2. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model. By stepwise restricting the number of parameters, the most parsimonious model for the dataset can be found. Each nested model is compared to the previous one. Additionally, a linear regression model was employed to include effects of age and sex on the observed scores: $\mu_i = \beta_0 + \beta_1 age_i + \beta_2 sex_i$, where μ_i is the expected value of individual i , age_i is the individual's age at time of measurement, sex_i is the individual's sex (0 denotes female, 1 denotes male). β_0 is the intercept, β_1 is the regression estimate of age, β_2 is the deviation of males

from females. This means model was fitted for the two age cohorts separately.

We tested for sample homogeneity by reduction of the number of parameters, as explained above, between the following groups: Twins versus other siblings, zygosity types, sexes, and cohorts. Group homogeneity was tested stepwise in this order. If groups were found not to differ significantly, parameters were equated across those groups, and the next nested model was tested. Given the large number of tests that might be involved (19 leads across 24 frequency bins = 456 tests, and more when any of the groups is found to be heterogeneous), the risk of type I error was greatly increased. Because there is no a priori reason to assume topographic differences in sample homogeneity, we restricted heterogeneity testing to the central lead Cz in four broad bands (delta, theta, alpha, and beta).

Next, the observed interindividual variation in power spectra was decomposed into additive genetic variation (σ^2_A), shared environmental variation (σ^2_C), or

Table 2. Stability of the frequency bands over an average period of 1.77 years.

		frequency band			
Lead	**N**	δ	θ	α	β
F7	27	0.72	0.88	0.89	0.81
F3	28	0.72	0.91	0.91	0.84
F1	27	0.71	0.91	0.93	0.82
FZ	28	0.73	0.91	0.91	0.86
F2	26	0.73	0.91	0.91	0.86
F4	27	0.73	0.90	0.91	0.87
F8	26	0.68	0.84	0.89	0.75
T7	27	0.66	0.84	0.89	0.86
C3	28	0.84	0.95	0.95	0.86
CZ	27	0.11	0.80	0.86	0.52
C4	27	0.39	0.84	0.87	0.68
T8	27	0.68	0.89	0.88	0.81
P7	27	0.87	0.96	0.92	0.90
P3	26	0.55	0.86	0.89	0.75
PZ	27	0.50	0.82	0.84	0.76
P4	28	0.87	0.95	0.96	0.89
P8	26	0.85	0.95	0.93	0.93
O1	26	0.86	0.94	0.94	0.88
O2	26	0.83	0.93	0.93	0.86
Mean		0.69	0.89	0.91	0.82

non-shared environmental variation (σ^2_E) following Neale & Cardon (1992). Sources of shared environmental variation by definition include all environmental influences that twins and siblings from the same family share, while sources of non-shared environmental variation refer to the environmental variation that is unique for an individual and that is typically not shared with other family members. For DZ twin pairs (and sibling pairs if the saturated models indicated no difference in correlation between DZ twin pairs and sibling pairs) correlation between shared environmental influences (C) was fixed at 1 and the correlation between additive genetic influences (A) at 0.5. For MZ twins correlations between additive genetic influences and between shared environmental influences were fixed at 1. Correlation between non-shared environmental influences (E), per definition, is set to zero for both MZ and DZ twins. Thus, the expectation for the total variance is $\sigma^2_A + \sigma^2_C + \sigma^2_E$ the expectation for the covariance between MZ twins is $\sigma^2_A + \sigma^2_C$, and the expectation for DZ twins/sibling pairs is $0.5 \times \sigma^2_A + \sigma^2_C$. Heritability is calculated as the proportional contribution of genetic variation to the total, observed variation ($\frac{\sigma^2_A}{\sigma^2_A+\sigma^2_C+\sigma^2_E}$). Goodness of fit of the variance decomposition models and significance of estimated parameters was, again, determined by likelihood ratio tests.

RESULTS

Temporal stability

Thirty subjects were retested after an average interval of 674 days ranging from 354 to 1322 days. Twenty-eight had valid EEG data available on any lead. Temporal stability scores (Table 2) are highest for theta and alpha. Stability of beta band power suggests more change over time than alpha and theta, and delta shows lowest stability varying from .60 to .87 with a few very low scores at Cz and C3, and only moderate scores at Pz and P3.

Sample homogeneity across groups

Assumptions of homogeneity across twin/singleton, zygosity, and sex groups were all met. We found evidence for heterogeneity of variance and/or means and/or covariances across the age cohorts (theta: $\chi^2(6) = 14.14$, $p = 0.028$; alpha: $\chi^2(6) = 20.43$, $p = 0.002$; beta: $\chi^2(6) = 16.95$, $p = 0.009$). Therefore, subsequent variance decomposition models will be estimated separately for each cohort.

Table 3. Twin and sibling correlations between identical (MZ) and any other, non-identical (DZ, SIB) sibling pairs, with heritabilities, of broadband EEG power.

	young adult												middle-aged											
	δ			θ			α			β			δ			θ			α			β		
	MZ	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**	**MZ**	**DZ SIB**	**h²**
F7	0.45	0.20	0.43	0.86	0.49	0.86	0.91	0.47	0.91	0.71	0.45	0.73	0.45	0.23	0.45	0.66	0.37	0.67	0.83	0.41	0.83	0.67	0.28	0,66
F3	0.58	0.23	0.55	0.85	0.51	0.85	0.93	0.47	0.93	0.79	0.48	0.80	0.37	0.22	0.40	0.73	0.40	0.73	0.87	0.43	0.87	0.81	0.39	0.81
F1	0.59	0.22	0.54	0.85	0.55	0.85	0.93	0.46	0.93	0.85	0.50	0.85	0.35	0.24	0.40	0.74	0.37	0.74	0.84	0.42	0.84	0.81	0.41	0.81
Fz	0.60	0.23	0.56	0.84	0.51	0.84	0.92	0.45	0.92	0.84	0.50	0.84	0.44	0.26	0.46	0.71	0.36	0.71	0.85	0.42	0.85	0.80	0.40	0.80
F2	0.59	0.22	0.54	0.84	0.52	0.85	0.93	0.46	0.93	0.87	0.50	0.87	0.42	0.23	0.43	0.72	0.37	0.72	0.86	0.42	0.86	0.80	0.39	0.80
F4	0.58	0.18	0.50	0.84	0.51	0.84	0.93	0.46	0.93	0.83	0.52	0.83	0.38	0.23	0.41	0.73	0.35	0.73	0.86	0.42	0.86	0.80	0.34	0.80
F8	0.48	0.21	0.45	0.83	0.48	0.84	0.91	0.43	0.92	0.78	0.46	0.78	0.52	0.21	0.50	0.74	0.35	0.74	0.85	0.44	0.85	0.79	0.36	0.79
T7	0.56	0.25	0.54	0.82	0.46	0.82	0.90	0.48	0.90	0.53	0.20	0.49	0.52	0.24	0.51	0.78	0.34	0.77	0.79	0.33	0.79	0.45	0.20	0.43
C3	0.71	0.32	0.70	0.88	0.48	0.88	0.92	0.44	0.92	0.86	0.46	0.86	0.55	0.25	0.53	0.77	0.34	0.76	0.84	0.39	0.84	0.77	0.32	0.77
Cz	0.78	0.54	0.79	0.87	0.51	0.87	0.93	0.48	0.93	0.88	0.52	0.88	0.55	0.26	0.55	0.78	0.29	0.77	0.85	0.40	0.85	0.83	0.39	0.83
C4	0.76	0.47	0.77	0.85	0.47	0.85	0.91	0.43	0.91	0.85	0.51	0.86	0.48	0.23	0.47	0.77	0.32	0.76	0.89	0.37	0.89	0.84	0.37	0.84
T8	0.69	0.22	0.63	0.84	0.40	0.84	0.90	0.45	0.90	0.27	0.30	0.40	0.53	0.19	0.50	0.75	0.31	0.75	0.80	0.38	0.79	0.52	0.32	0.54
P7	0.59	0.31	0.59	0.84	0.45	0.84	0.88	0.42	0.88	0.80	0.43	0.80	0.67	0.22	0.62	0.82	0.33	0.81	0.82	0.36	0.82	0.72	0.29	0.70
P3	0.74	0.53	0.76	0.88	0.51	0.88	0.89	0.49	0.89	0.88	0.55	0.88	0.65	0.25	0.62	0.80	0.32	0.80	0.88	0.39	0.88	0.82	0.37	0.82
Pz	0.68	0.51	0.71	0.82	0.48	0.82	0.89	0.52	0.89	0.78	0.56	0.80	0.69	0.34	0.69	0.79	0.34	0.78	0.87	0.38	0.88	0.82	0.43	0.82
P4	0.73	0.38	0.74	0.90	0.46	0.90	0.90	0.49	0.90	0.85	0.54	0.86	0.68	0.25	0.64	0.74	0.33	0.73	0.89	0.39	0.89	0.78	0.38	0.78
P8	0.69	0.38	0.70	0.88	0.44	0.88	0.85	0.44	0.85	0.78	0.50	0.79	0.66	0.24	0.63	0.76	0.32	0.75	0.90	0.41	0.90	0.78	0.30	0.77
O1	0.60	0.32	0.60	0.82	0.43	0.82	0.85	0.43	0.85	0.82	0.50	0.83	0.73	0.24	0.69	0.75	0.30	0.74	0.83	0.34	0.83	0.73	0.32	0.73
O2	0.69	0.24	0.66	0.85	0.38	0.85	0.85	0.43	0.85	0.82	0.50	0.83	0.70	0.22	0.65	0.75	0.32	0.74	0.87	0.39	0.87	0.70	0.35	0.70
Mean	0.64	0.31	0.62	0.85	0.48	0.85	0.90	0.46	0.90	0.78	0.47	0.79	0.54	0.24	0.53	0.75	0.34	0.75	0.85	0.39	0.85	0.75	0.35	0.75

Table 4. Phenotypic and genetic correlations (genetic variation due to a common source) between broadband frequencies on lead Cz.

	Frequency band			
	δ	θ	α	β
Young				
δ	-	0.73	0.48	0.55
θ	0.75	-	0.69	0.59
α	0.55	0.74	-	0.55
β	0.62	0.60	0.58	-
Middle-aged				
δ	-	0.58	0.37	0.45
θ	0.63	-	0.69	0.62
α	0.55	0.73	-	0.65
β	0.63	0.65	0.68	-

Note. Upper triangle are phenotypic, lower triangle are genetic correlations.

Broadband correlations and variance decomposition

Table 3 shows the twin correlations for the broad bands as estimated with Mx. These suggest a strong, additive genetic effect as the MZ correlations are high and the DZ correlations are around half the MZ correlation (Falconer & MacKay, 1996). Correlations are generally higher in the alpha band across all leads. They are also higher in the young cohort across all leads and frequencies. There is little evidence for strong topographic differences except for some lower correlations in the temporal areas in the beta range. The overall pattern of correlations does not suggest a role for common environment in EEG power. The exception may be the correlations for spectra in the young cohort, broadband theta and beta, because the DZ correlation is slightly, but systematically over half the MZ correlation. ACE vs AE model fitting, however, did not reach significance for any lead and broad band combination except for delta and beta on Pz, and for delta on P3. These significant results did not hold under the Bonferroni alpha level correction for multiple testing. Therefore, in subsequent model fitting an AE model was used for all bands and all leads.

Heritability spectra based on 1 Hz bins

Figure 1 shows the heritability spectra for each lead with cohorts plotted separately. Heritability is high for both cohorts, peaking in the alpha range. It drops

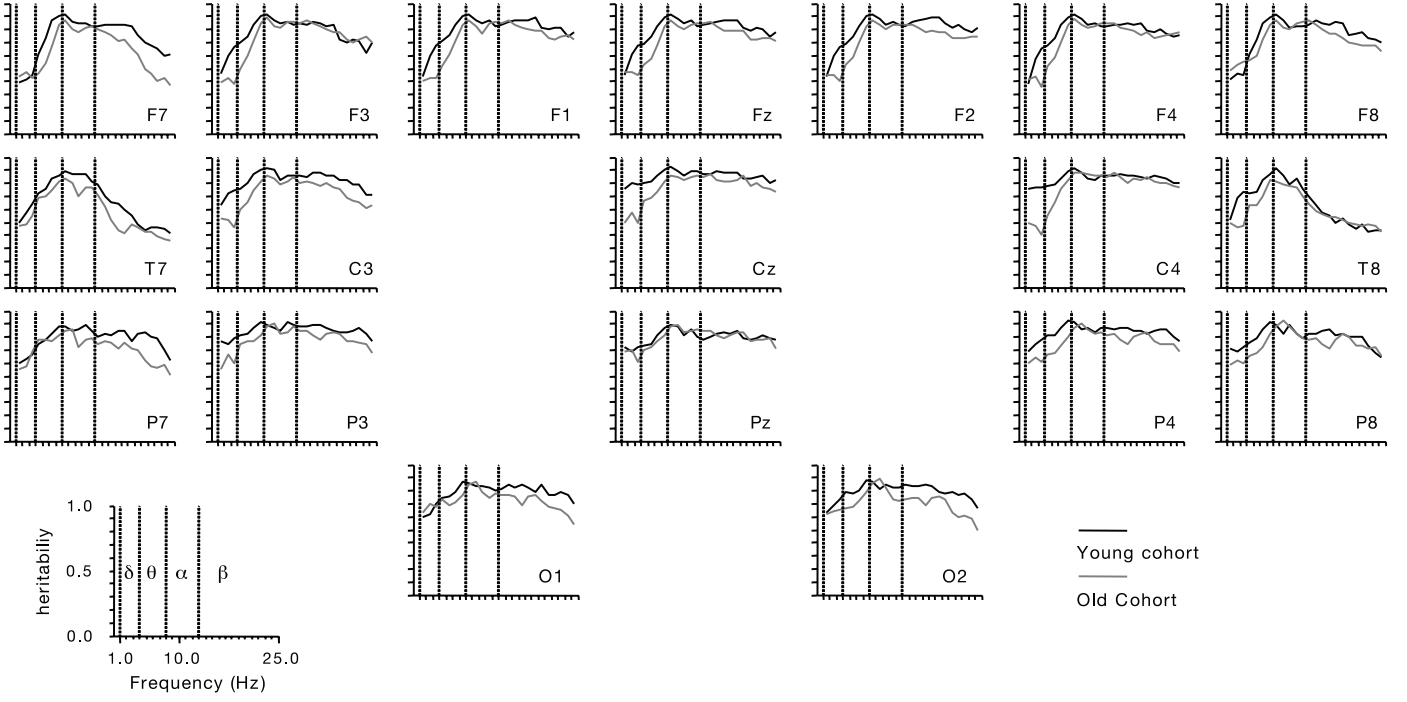

Figure 1. Heritability spectra for young (black) and middle-aged (grey) cohorts

with decreasing frequency in the theta and delta range but remains high with increasing frequency in the beta range, except for the temporal area. Cohort differences systematically showed lower heritability in the older cohort, mainly in the frontocentral regions for theta and delta, and mainly in the left hemisphere for the beta range.

Alignment of spectra on individual alpha frequency

The boundaries of the alpha band as well as of the other "classical" broad bands are based on population averaged EEG spectra that only imperfectly reflect the constituent individual EEG spectra and, consequently, their genetic determinants. In adult subjects peak alpha frequency ranges from 8 to 13 Hz. Assigning a fixed alpha band to all subjects could easily confound alpha with up to a 3 Hz bin of theta, or a 2 Hz bin of beta power depending on whether the individual's peak is high or low within the normal alpha band (Klimesch, 1999). We therefore repeated our genetic analyses on spectra that were aligned on the individual alpha peak. We defined the dominant frequency as the one with maximum attenuation of alpha power by opening of the eyes, following Klimesch. Using this "alpha blocking" definition, we were able to establish the individual alpha frequency for all but 90 subjects.

Alignment did not yield significantly different heritability estimates in the theta, alpha and beta bands on most leads after examining the 95% confidence intervals. A reduced heritability was only found in two bins surrounding peak alpha in the frontal leads of the young cohort. Overall, we conclude that alignment produces no or marginally different heritability estimates. Because many subjects were lost in the alignment procedure (no clear alpha peak), we proceeded with unaligned spectra from the Eyes Closed condition.

Genetic correlations between frequencies

To get an indication of the extent to which heritable variance of the frequency bins can be traced to a common genetic source, we calculated the genetic correlations between the broadband frequencies, i.e., the proportion genetic variance shared between any two variables. These were calculated for each cohort separately. The results are shown in Table 4. In both cohorts, 55% to about 75% of the genetic variance overlaps between the bands. The genetic correlations were all significantly different from both zero and unity suggesting that common as well as unique genetic factors contributed to each of the broad bands.

DISCUSSION

The results show that in adult subjects EEG power at rest is a heritable trait across the entire frequency spectrum. No evidence was found for common environmental influences on the EEG power spectrum. Meaningful contribution of unique environment was limited to the delta frequencies, that showed lower heritabilities down to 40%. This lower delta heritability, together with the lower temporal stability for delta, may be explained in part by larger measurement error for this frequency, due to, for example, residual eye movement artifacts. Measurement error in our modeling will show up as unique environmental influences. Alternatively, we cannot rule out that true environmental factors have more impact on low frequency EEG power than on power in the higher frequency bands. In the upper beta regions, both heritability and stability were somewhat lower in the temporal areas. Again, this might be explained either by larger measurement error or by larger sensitivity to environmental factors. It is hard to explain, however, why unique environmental factors would affect beta frequencies only in these scalp regions.

For the theta and alpha frequencies, our MZ twin correlations were similar to 5 minute test-retest correlations reported in the literature (Salinsky, Oken, & Morehead, 1991) as well as the longer term stability over a period of years reported here. Identical twins, therefore, resemble their co-twin about as much as they would themselves over a period of years. Overall, our results establish EEG power to be one of the most heritable complex traits in human subjects. This is in keeping with previous results from smaller studies of twin families (MacGuire, Katsanis, & Iacono, 1982; Lykken, Tellegen, & Iacono, 1982; Christian et al. 1988) and the large adolescent studies (van Beijsterveldt et al., 1996; van Beijsterveldt & van Baal, 2002).

The overarching suggestion of the "heritability spectra" in Figure 1 is that the separation of broad bands on the basis of EEG power has little basis in its genetic architecture. In contrast, the uniformity of the heritability spectra suggests that EEG powers at different frequencies share a common genetic source. We further tested this hypothesis by computing the genetic correlations between the broadband frequencies in a multivariate genetic model. The results indicated that a moderately high to high proportion of genetic variance was shared among the frequency bands. In both cohorts, genetic correlations varied from .55 to about .75. Therefore, a significant proportion of the heritable variance in all frequency bands must be attributed to a common genetic source. This is in concordance with genetic correlations between the broadband frequencies found in adolescents (Anokhin et al., 2001).

Genes common to all frequencies may affect EEG power through "trivial" ef-

fects on the conductive properties of the tissues surrounding the cortex. As often observed before, skull and scalp thickness, most likely heritable traits, strongly influence EEG power (Babiloni et al., 1997; Leissner, Lindholm, & Petersen, 1970; Nunez, 1981). A common genetic source for EEG may also reside in non-trivial common influences on cerebral rhythm generators like the central 'pacemaker' in the septum for hippocampal slow-wave activity (3 – 4 Hz) or the thalamocortical and corticocortical generators of cortical alpha rhythmicity (Lopes da Silva, 1991; Steriade, Gloor, Llinas, Lopes da Silva, & Mesulam, 1990). Another possible source could lie in genes directly involved in the bioelectric basis of the EEG signal itself; Genes influencing the number of pyramidal cells, the number of dendritic connections, or their orientation with respect to the scalp may directly influence the mass dendritic tree depolarization of pyramidal cells in the cortex that underlies EEG power (Ray, 1990). To resolve the genetic basis of the EEG, a whole genome scan on power in the broad bands followed by positional cloning seems the most rational approach. In view of the high heritability of EEG power, such gene finding is entirely feasible.

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3

GENETIC CONTRIBUTION TO THE P3 IN YOUNG AND MIDDLE-AGED ADULTS

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ABSTRACT

Previous studies in young and adolescent twins suggested substantial genetic contributions to the amplitude and latency of the P3 evoked by targets in an oddball paradigm. Here we examined whether these findings can be generalized to adult samples. A total of 651 twins and siblings from 292 families participated in a visual oddball task. In half of the subjects the age centered around 26 (young adult cohort), in the other half the age centered around 49 (middle-aged adult cohort). P3 peak amplitude and latency were scored for three midline leads Pz, Cz, and Fz. No cohort differences in heritability were found. P3 amplitude (~50%) and latency (~45%) were moderately heritable for the three leads. A single genetic factor influenced latency at all electrodes, suggesting a single P3 timing mechanism. Specific genetic factors influenced amplitude at each lead, suggesting local modulation of the P3 once triggered. Genetic analysis of the full ERP waveform showed that P3 heritability barely changes from about 100 ms before to 100 ms after the peak. Age differences are restricted to differences in means and variances, but the proportion of genetic variance as part of the total variance of midline P3 amplitude and latency does not change from young to middle-aged adulthood.

INTRODUCTION

The P3(00) event-related potential (ERP) is widely used to examine normal variation in cognitive function in healthy individuals as well as disturbed cognition in various clinical groups. By interspersing a low probability target stimulus (the oddball) into a sequence of a frequent nontarget stimulus, Sutton et al. (1965) and Desmedt et al. (1965) were first to elicit the P3. This 'classical' P3 component (or P3b), which peaks 300-600 ms after the target stimulus in such oddball paradigms, has a parietal distribution on the scalp and has been linked to the cognitive processes of context updating, context closure, and event categorization (Dien et al., 2004; Donchin & Coles, 1988; Kok, 2001; Verleger, 1988). For the P3 to occur it is necessary that the stimulus is relevant to the task at hand, and that the subject is conscious of this task relevancy: on missed target trials, such as in experiments on the attentional blink, the P3 is absent (Vogel & Luck, 2002; Vogel et al., 1998).

Like other ERP components, the P3 is characterized by large individual differences. These may be meaningful as markers of differences in mental health (Polich & Herbst, 2000). In normal aging, P3 latency has been found to increase and P3 amplitude to decrease as cognitive processing slows down, although the power of the P3 to differentiate between normal aging and dementia due to neural degenerative disorders such as Alzheimer's disease is inconclusive (e.g., Cohen et al., 1995; Pfefferbaum et al., 1990; Polich, 1998). Reduced P3 amplitude is also found in a variety of psychiatric and behavioral disorders, most notably schizophrenia (Levit et al., 1973; Verleger & Cohen, 1978) and alcoholism (e.g. Porjesz et al., 1980; Begleiter et al., 1984). The reduction in P3 amplitude is thought to reflect a genetic predisposition for these disorders rather than a mere functional consequence, because it is also found in unaffected relatives (Begleiter et al., 1984; Blackwood, 2000; Blackwood et al., 2001; Elmasian et al., 1982; Polich et al., 1994; Porjesz & Begleiter, 1990; Turetsky et al., 2000). A genetic influence on P3 amplitude and latency is supported by twin and family studies which indicates moderate to high heritability for both (for reviews see van Beijsterveldt & Boomsma, 1994; van Beijsterveldt & van Baal, 2002). However, the twin studies that have investigated P3 heritability investigated children or adolescent samples (Carlson et al., 2002; Katsanis et al, 1997; O'Conner et al., 1994; van Baal et al., 1998; van Beijsterveldt et al., 2001). To our knowledge, only one adult twin study with sufficient power to discriminate genetic from common environmental factors has looked at the P3 (Anokhin et al., 2004). Using a go-nogo task rather than an oddball task, P3 heritability was comparable (41% and 58% for go and no-go P3 respectively) to that in adolescent twins. However, the sample included only young adults with a maximum age of 28. In addition, the use of a

go/no-go task may have invoked a P3 which contains more of the frontocentral P3a than the parietal P3b component in comparison to the oddball task (Dien et al., 2004).

Here we examined whether the heritability estimates for the P3 found in the oddball task at young ages can be generalized to adults. Because the P3 may reflect the admixture of several different processes (Kok, 2001) along the anterior-posterior axis of the brain (Bledowsky, 2004a, 2004b) we examined whether the genetic variance underlying frontal, central, and parietal midline P3 reflected a common or separate underlying set of genes as an indication of shared underlying neurobiology. In keeping with previous studies, heritability of the amplitude of the P3 was first established at its peak latency. Secondly, as the components of the late positive complex may each have slightly different time frames, we allowed the genetic underpinnings to vary within the time course of the P3 by applying our genetic analysis to the full ERP.

METHOD

Subjects

Subjects were recruited from the Netherlands Twin Registry (Boomsma et al. 2002 b) as part of a large project on the genetics of cognition and adult brain functioning (Posthuma et al., 2001). Adult twins and their non-twin siblings were invited to participate. A total of 760 family members from 309 twin families participated in the study, and EEG data were available from 732 subjects from 305 families. Participating families consisted of one to seven siblings (including twins). For this study, we restricted the age range to young and middle-aged adulthood: only subjects in the range of 20 to 65 years were included. This resulted in a sample of 715 subjects from 303 families. The sample consisted of two age cohorts: a younger cohort (46.0% male, mean 26.5 years, SD 3.7) and a middle-aged cohort (41.3% male, mean 48.8 years, SD 6.2). Data from these cohorts will be analyzed separately. Cohort inclusion was determined on a per family basis and by the age of the twins on the day of measurement with the cut-off at 35 years. This resulted in two siblings younger than 35 being included in the middle-aged cohort on the basis of twins being over 35, and eleven siblings older than 35 being included in the young adult cohort of the basis of the twins being under 35.

Procedure

The study received prior approval by the institutional review body and ethical committee of the VU medical centre. Informed consent was obtained from each subject. They were asked to participate in a 4.5 hour lasting testing protocol. During one part of the experimental protocol, psychometric intelligence, in-

spection time, and reaction times were assessed. During the other, the subjects performed, amongst others, a visual oddball task. The order of the two parts of the protocol was randomized across family members. Consequently, half of EEG registration sessions were during morning hours, and half were in the afternoon.

During EEG recording subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated and electromagnetically shielded room. They were instructed to relax, and to minimize blinking, eye and body movement.

Stimuli

The oddball stimuli were white-on-black line drawings of cats and dogs by Snodgrass and Vanderwart (1980), balanced in the amount of physical stimulation. The dog stimuli were shown frequently (100 / 125) and were the standards. The cat stimuli were shown only infrequently (25 / 125) and were the targets. A stimulus set with an identical order of stimuli and intertrial intervals was presented to all subjects. Dog and cat stimuli were generated in an unpredictable order and trial duration varied randomly from 1500 to 2000 ms. Stimulus duration was 100 ms. Before the task, one example of each stimulus was presented. Subjects were instructed to silently count the number of targets (cats) shown on the computer screen positioned 80 cm in front of them. This distance was verified by use of a rod. The number of counted targets reported was recorded for each subject.

EEG registration

EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS, Enschede, The Netherlands) for 612 subjects and Neuroscan SynAmps 5083 amplifier (Compumedics, El Paso, TX) for 103 subjects. Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2 (Compumedics, El Paso, TX). Standard 10-20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2 (American EEG Society, 1991; Jasper, 1958). For Neuroscan subjects Fp1, Fp2, and Oz were also included. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz.

Neuroscan filter settings were a lowpass filter at 50.0 Hz and no high pass filtering. Strong DC shifts were manually reset before the start of the experiment.

Data processing

The three midline leads Pz, Cz, and Fz were selected for further analysis. The signals were recalculated with averaged earlobes as reference and analyzed using Neuroscan Edit (Compumedics, El Paso, TX). Next, if the signals were absent or the signals were deemed extremely noisy upon visual inspection the subject was excluded from further analysis. This resulted in the removal of twenty-six subjects. Signals from all leads were then reviewed for artifactual episodes (swallowing, muscle artifacts, eye movements (not blinks), and technical problems such as clipping). These episodes were removed and excluded from the analyses. Next, blink artifact reduction was performed following the procedure introduced by Semlitsch et al. (1986). Epochs were created from 100 ms prestimulus up to 700 ms post-stimulus with baseline offset correction including only epochs that did not overlap with artifactual episodes. Ten subjects with less than fifteen valid epochs in the target condition were excluded from further analysis for both target and nontarget conditions. One subject was excluded because she had counted nontargets instead of targets.

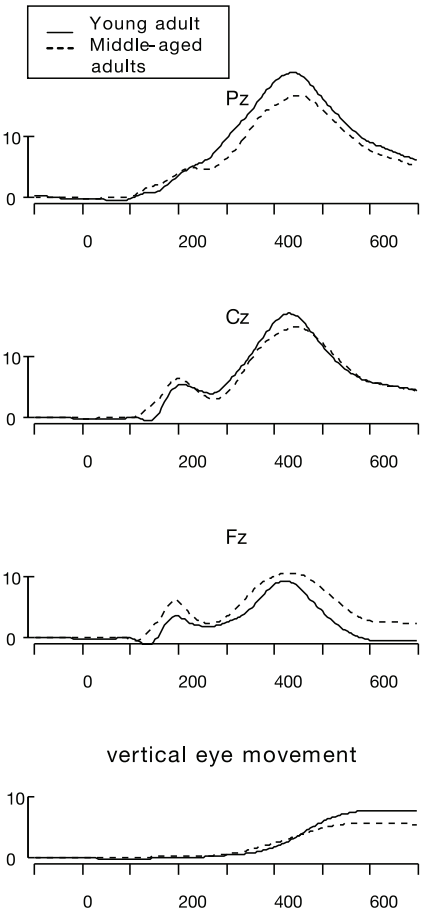

Figure 1. Fz, Cz, and Pz grand average waves for each cohort

The P3 peak amplitude and latency were extracted from each subject's average waveform for leads Fz, Cz and Pz. The time window for peak picking was determined by inspecting the histograms for latency scores. Both the lower and upper bounds of the window were adjusted to create a maximally normal distribution of latency scores across the three leads. The window was thus set from 290 to 590 ms post-stimulus.

Lower values of the lower bound resulted in a clear second peak in the histogram of latency scores on the left side of the mean that most likely reflected the erroneous picking of a P2 peak in some of the subjects. Adjusting the upper bound did not critically alter peak picking scores as shown by the latency histograms. Visual checking confirmed that the peak was correctly chosen. Subjects with no clear peak due to either multiple peaks or very low ERP amplitude on each lead were set to missing for that particular lead. Two peaks close in latency were not

Table 1. Number of families split by composition, cohort, and zygosity.

Family composition[a]	families with an MZ twin	families with a DZ twin	total
	Young cohort		
Both twins only	35	36	71
Both twins + 1 sibling	12	21	33
Both twins + 2 or more siblings	4	4	8
One twin only[b]	4	7	11
One twin + 1 sibling	5	10	15
One twin + 2 or more siblings	1	1	2
1 sibling[b]	2	4	6
2 or more siblings	1	0	1
total	64	83	147
	Middle-aged cohort		
Both twins only	31	39	70
Both twins + 1 sibling	19	13	32
Both twins + 2 or more siblings	3	2	5
One twin only[b]	11	9	20
One twin + 1 sibling	3	4	7
One twin + 2 or more siblings	1	4	5
1 sibling[b]	2	3	5
2 or more siblings	1	0	1
total	71	74	145

[a]Family composition was based on the participating offspring only. For example, a family with 'both twins only' could consist of more than two children, but these did not participate in the EEG experiment.
[b]Families with only one twin or only one sibling cannot contribute to the estimation of sibling covariance, but are retained to improve the estimation of means and variances.

considered incorrect, and the larger of the two peaks was chosen.

Genetic analyses

Resemblance (covariance) in ERP traits between twins and siblings derive from genetic relatedness or shared environmental influences (Falconer & Mackay, 1996). If the correlation between DZ twins or siblings, who share on average 50% of their genetic makeup, is half the correlation between MZ twins, who are genetically identical, this is seen as evidence for additive genetic influences (A). If the correlation between DZ twins or siblings is less than half the correlation between MZ twins this is seen as evidence for dominant (non-additive) genetic influences (D). If the correlations between MZ and DZ twins/siblings are comparable and non-zero this is evidence for shared environmental influences (C). If the correlation between MZ twins is not unity this is evidence for environmental effects unique to each individual (E). By comparing MZ and DZ/sibling correlations, using structural equation modeling as implemented in, for example, Mx (Neale, 2004), we can obtain maximum likelihood estimates of the relative contributions of each of these factors to the total trait variance. Heritability is defined as the proportional contribution of genetic effects (A + D) to the total variance (A + C + D + E). In a twin-sibling design, however, the effects of both C and D cannot be estimated simultaneously. The relative size of the DZ/sibling correlation guides which is selected. If the DZ/sibling correlation is less than half the MZ correlation, then D is modeled. If it is more than half the MZ correlation, C is modeled. For more information on genetic modeling we refer to Boomsma et al. (2002 a) and Posthuma et al. (2003).

For the peak latency and amplitude at peak latency we used a multivariate approach that looked at the P3 at multiple leads across the scalp simultaneously. This multivariate genetic analysis can be used to detect the degree of overlap in the genetic and environmental factors influencing each of the traits (Posthuma et al., 2003). For this study, we specified three genetic and three unique environmental factors that could account for P3 amplitude at the Pz, Cz, and Fz leads following a Cholesky decomposition of the genetic variance. We then restricted the model by reducing the number of genetic factors. This multivariate analysis was then repeated for P3 latency.

Finally, for each lead separately, we tested heritability of the amplitudes along the full P3 waveform by repeatedly performing a univariate genetic analysis on the amplitude at each time point. Because the amplitude at a fixed time-point is confounded with the latency of the P3 wave, we aligned the P3 waveform to individual peak latency and selected only the amplitudes in a time window from 150 ms before to 150 ms after peak latency.

Due to the large sample size and multiple tests all statistical testing was per-

Table 2. P3 amplitude and latency descriptives and effects of sex and cohort.

	Cohort													
	Young						Middle-aged							
	Female			Male			Female			Male			group differences[a]	
	N	M	SD	N	M	SD	N	M	SD	N	M	SD	Sex[b]	Cohort[c]
Amplitude														
Fz	170	12.79	5.92	138	10.62	5.79	177	13.61	4.97	124	12.23	4.40	1.83 ***	-1.23 *
Cz	173	20.83	7.19	149	16.99	6.58	182	17.71	5.78	126	16.54	5.23	2.45 ***	1.82 ***
Pz	178	24.56	6.68	146	19.90	6.64	181	19.74	5.57	130	17.50	4.72	3.37 ***	3.66 ***
Latency														
Fz	170	418.3	40.3	138	425.7	40.7	177	432.5	50.6	124	424.3	51.6	0.48	-7.52 *
Cz	173	429.2	37.9	149	435.7	38.6	182	444.9	50.6	126	436.2	45.3	1.26	-9.14 **
Pz	178	432.1	42.0	146	439.3	41.8	181	443.7	53.2	130	438.5	49.0	-0.97	-6.16

Note: Amplitude in μV and latency in ms.
[a]*Sex group effects are collapsed across cohort, and cohort group effects are collapsed across sex groups. Significance was determined with Structural Equation Modeling package Mx.*
[b]*females compared to males.*
[c]*young adult compared to middle-aged*
**p < .05; **p < .01; ***p < .001*

Table 3. Model fitting of P3 amplitude and latency

model		tested against model number	Fz -2LL	Fz df	Fz χ^2	Fz Δdf	Fz p	Cz -2LL	Cz df	Cz χ^2	Cz Δdf	Cz p	Pz -2LL	Pz df	Pz χ^2	Pz Δdf	Pz p
Amplitude																	
Cohort effects																	
0	Saturated		3691.81	596				4028.67	616				3992.48	621			
1	eq variances Y = MA	0	3702.63	597	10.83	1	0.001	4040.59	617	11.92	1	0.001	4008.71	622	16.24	1	0.000
2	eq correlations Y = MA	0	3692.94	598	1.13	2	0.567	4032.44	618	3.78	2	0.151	3998.87	623	6.40	2	0.041
Decomposition[a]																	
3	ADE + COH scalar	2	3692.94	598	1.13	2	0.567	4032.44	618	3.78	2	0.151	3998.87	623	6.40	2	0.041
4	D=0	3	3693.20	599	0.26	1	0.611	4032.55	619	0.11	1	0.742	3999.42	624	0.54	1	0.462
5	D=0, A=0	4	3739.02	600	45.82	1	0.000	4072.78	620	40.24	1	0.000	4038.71	625	39.29	1	0.000
Latency																	
Cohort effects																	
0	Saturated		6294.68	596				6445.59	616				6583.53	621			
1	eq variances Y=MA	0	6307.57	597	12.89	1	0.000	6457.05	617	11.46	1	0.001	6591.80	622	8.27	1	0.004
2	eq correlations Y=MA	0	6295.48	598	0.80	2	0.670	6446.59	618	1.00	2	0.606	6589.25	623	5.72	2	0.057
Decomposition[a]																	
3	ADE + COH scalar	2	6295.46	598	0.79	2	0.675	6447.73	618	2.14	2	0.343	6589.24	623	5.72	2	0.057
4	D=0	3	6296.50	599	1.04	1	0.308	6454.07	619	6.34	1	0.012	6590.15	624	0.91	1	0.340
5	D=0, A=0	4	6324.61	600	28.11	2	0.000	6479.85	620	25.78	2	0.000	6625.71	625	35.56	2	0.000

Note: Y = young adult. MA = middle-aged. COH = cohort. Significance was tested with an alpha level of .01. The saturated model fitted a two correlations, one variance, and a means model (one mean, one age and one sex regression) for each of the cohorts, for a total of 10 parameters.
[a]Decomposition was performed on the model allowing variances to differ across cohorts (a scalar model). All models fitted a full means model for each cohort.

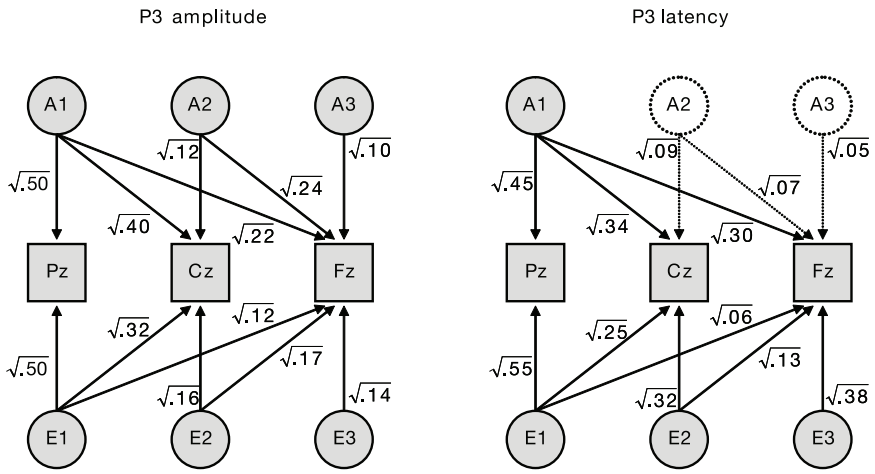

Figure 2. Structural equation models for P3 amplitude and latency each with three genetic factors ('A' circles) and three environmental factors ('E' circles), explaining variance of three observed variables (squares). The values under the root sign are standardized squared factor loadings representing proportions of variance explained by the factors. For example, environmental factor 2 explains 16% of the variance of Cz amplitude. All the arrows into one variable sum up to unity; For example, the explained variance of Pz latency is .45 + .55 = 1. Factor loadings along dotted lines were not significant.

formed against a significance level of $\alpha = .01$.

RESULTS

After EEG data cleaning and visual inspection 673 subjects from 296 families had sufficient error free data on at least one lead for genetic analyses. On average, 2.3 participants per family participated. The vast majority of 591 subjects reported the correct number of counted targets (25), 60 subjects (32 young adult, 28 middle-aged) had miscounted on a single trial, and 22 subjects miscounted on 2 or more trials. The latter subjects were removed from further EEG analyses. For the final sample of 651 subjects, Table 1 shows the frequency of families grouped by zygosity of the twin probands, the number of participating twins, and the number of participating siblings.

Effects of cohort and sex on the means

Table 2 shows Fz, Cz, and Pz amplitude and latency for each of the sex by age cohort groups. The last two columns show the mean differences between the sex groups collapsed over age cohort and cohort differences collapsed over the sexes.

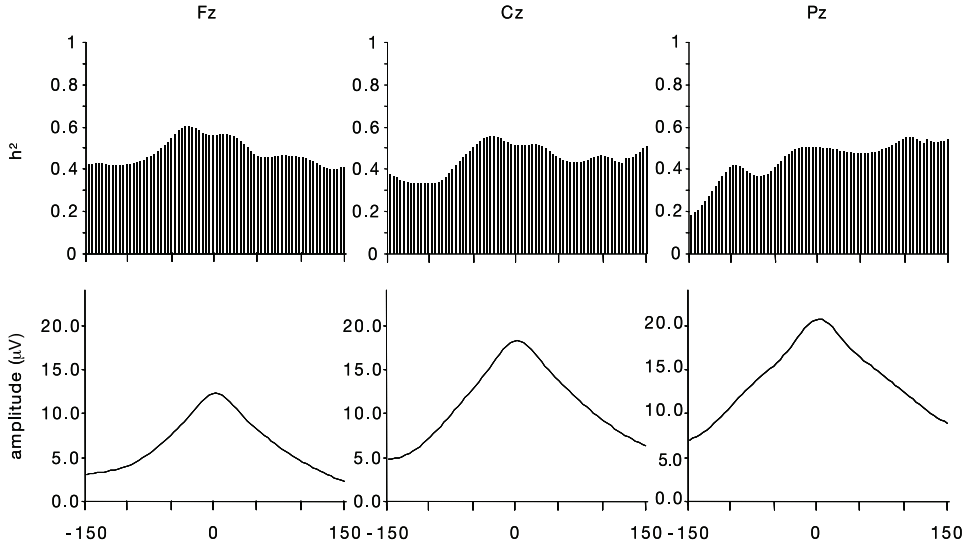

Figure 3. Grand Average P3 after alignment on individual peak latency with the corresponding heritability from 150 ms before to 150 ms after peak amplitude.

Structural Equation Modeling software package Mx was used to test significance of these differences, which allowed familial dependencies in the data to be taken into account. The older cohort showed higher P3 amplitude on Fz (χ^2 = 15.38, df = 1, $p < 10^{-4}$), but lower amplitude on Cz (χ^2 = 23.58, df = 1, $p < 10^{-5}$) and Pz (χ^2 = 43.93, df = 1, $p < 10^{-10}$). Females showed higher amplitude than males on all three leads (Fz: χ^2 = 4.34, df = 1, $p < 0.05$; Cz: χ^2 = 11.72, df = 1, $p < 0.001$; Pz: χ^2 = 45.12, df = 1, $p < 10^{-10}$). A slowing of cognitive processing with age was revealed by a significant effect of age cohort on the latency scores on two of the three leads (Fz: χ^2 = 4.67, df = 1, $p < 0.05$; Cz: χ^2 = 6.79, df = 1, $p < 0.01$). To account for these effects, sex and cohort were retained as covariates in subsequent genetic modeling.

Lead position interactions with cohort and sex were also modeled in Mx. There was no significant three-way interaction of lead by cohort by sex for P3 amplitude. The lead by cohort interaction was significant (χ^2 = 98.6, df = 2, $p < 10^{-21}$). As in the aforementioned, young adults showed higher amplitude than middle-aged adults at Cz and Pz, whereas at Fz the young adults showed lower amplitude. Also, the lead by sex interaction was significant (χ^2 = 12.50, df = 2, p = 0.002). Females showed increased amplitude compared to males, and this difference decreases from the posterior to the anterior lead.

For P3 latency no significant interaction effects with lead position were found.

Table 4. MZ and DZ/sibling correlations with heritability estimates of P3 amplitude and latency.

	Fz			Cz			Pz		
	r_{MZ}	r_{SIB}	h^2	r_{MZ}	r_{SIB}	h^2	r_{MZ}	r_{SIB}	h^2
Amplitude	0.55 ***	0.24 ***	0.56 ***	0.50 ***	0.23 **	0.51 ***	0.51 ***	0.21 ***	0.50 ***
Latency	0.45 ***	0.15 *	0.42 ***	0.52 ***	0.06	0.43 ***	0.48 ***	0.17 **	0.45 ***

Note: Sibling correlations (r_{SIB}) are based on all DZ twins, twin-sib and sib-sib pairings. Heritabilities (h^2) are derived from the trivariate models fitting on data from three leads.
**p < .05; **p < .01; ***p < .001*

Table 5. Genetic correlations of peak amplitude with pre- and post-peak amplitude.

timing relative to peak (in ms)	Genetic correlation		
	Fz	Cz	Pz
-100	0.72	0.69	0.77
-80	0.82	0.83	0.86
-60	0.92	0.92	0.92
-40	0.96	0.97	0.95
-20	0.99	1.00	0.99
0	1	1	1
20	1.00	1.00	1.00
40	0.98	0.99	0.98
60	0.91	0.96	0.96
80	0.81	0.91	0.93
100	0.73	0.82	0.87

Effects of cohort and sex on variances and correlations

In addition to the effect on the means, the cohorts showed differences in variances on all three leads for both amplitude and latency. For amplitude the middle-aged cohort showed lower variance than the young adult cohort, whereas for latency they showed larger variance. Further genetic modeling took these difference in variances into account by using a so-called scalar model (Neale & Cardon, 1992). The cohorts did not differ in MZ and DZ/sibling correlations suggesting that the relative contribution of A, C or D, and E did not differ across cohorts. No sex differences were found in either variances or sibling correlations.

Comparability of MZ twins, DZ twins, and singletons

To test whether twins are representative of the singleton population we examined if there were significant group differences for latency and amplitude on each of the three leads. Correlations between DZ twins, between siblings and between twins and siblings (that is, all fraternal sibling relationships) did not differ significantly. There were also no significant differences in variances and means

between DZ twins/siblings in any of these variables. Also, we found no differences between the means and variances of MZ and DZ twins/siblings.

Twin correlations and heritability of P3 amplitude and latency

Table 3 shows the correlations between MZ twins and DZ twins/siblings. The correlations suggest additive (A) plus dominant (D) genetic influences on both amplitude and latency as the DZ correlations are less than half the MZ correlations (Falconer & Mackay, 1996). Formal testing shows that the dominant genetic effects were not significant for any of the leads as shown in Table 3. The most parsimonious model, therefore, estimates additive genetic and unique environmental effects on the variance of each variable.

Figure 2 shows the relative contributions of the three genetic and three environmental factors in the multivariate models. Note that the factor loadings in the figure, when squared, represent proportions of variance explained by the genetic and environmental factors. For P3 amplitude, there are significant contributions from all three genetic factors on all three leads along the anterior-posterior axis (all $\chi^2 > 13.0$, ps $< .001$). For P3 latency a single genetic factor was sufficient for all of the genetic variance in all three leads. Loadings from the first genetic factor contributed significantly to the variance ($\chi^2 > 35.9$, ps $< .001$). Loadings from the second and third genetic factors did not contribute significantly (ps $> .05$). The final column in Table 4 shows the heritabilities derived from these models.

Heritability of the P3 time series

Figure 3 shows the development of heritability under the AE model over the time course of the aligned P3 component on leads Fz, Cz and Pz. Alignment of the ERP to targets results in a markedly pointier waveform indicating that alignment was successful in reducing the attenuation of the grand average P3 due to individual differences in peak latency. However, P3 heritability does not vary much around peak amplitude for all three leads. For Cz and Fz highest heritability is seen about 50 ms before and after peak amplitude, but the difference was not significant as revealed by the confidence interval around the heritability. Only at larger distances from the peak (> 100 ms), significant drops in heritability were found.

We tested whether pre and post peak amplitude were influenced by the same genes as amplitude at the peak itself. To this end, we applied a bivariate model that estimated the genetic correlations (the proportion of overlapping genetic variance) between peak amplitude and amplitude at -100, -80, -60, -40, -20, +20, +40, +60, +80, and +100 ms around the peak. Table 5 summarizes the results. Within a range of -60 to 60 ms relative to the peak the genetic correlations remained over .90. Within 80 ms of the peak the genetic correlations remained

over .80, and within 100 ms they remained at or over .69. Inspection of the 99% confidence intervals revealed that all genetic correlations were significantly different from zero.

DISCUSSION

A significant proportion of interindividual variation in adult P3 amplitude was found to be under genetic control. P3 amplitude (~50%) and latency (~45%) were moderately heritable for the three leads. A single genetic factor influenced latency at all electrodes. Specific genetic factors influenced amplitude at each lead. Genetic analysis of the full ERP waveform showed that P3 heritability barely changes from about 100 ms before to 100 ms after the peak.

No differences in heritability were found between young and middle-aged subjects. However, the age cohorts differed significantly in variances, suggesting that both genetic variance and environmental variance decreased with age for P3 amplitude, and both increased for latency. A lead by cohort interaction effect was observed consistent with the effect reported by Walhovd and Fjell (2003; but see also Polich, 1997). Across age cohorts, a relative increase of frontal P3 amplitude was found in the middle-aged cohort in comparison to the young cohort whereas a decrease was found in the parietal P3. From these data it seems that the P3 shows a shift towards the frontal/central areas with increasing age which is congruent with previously reported findings (for example, Brown et al., 1983: 0.15 μV per year decrease; Picton et al., 1984: 0.18 μV per year decrease).

Heritability for Pz amplitude at peak latency (50%) was slightly lower than the heritability estimate to targets (60%) reported in a meta-analysis by van Beijsterveldt and van Baal (2002). This slightly lower heritability may reflect the age of the subjects: it is slightly lower than large twin studies to the P3 in adolescents (van Beijsterveldt et al., 2001: 59%; Katsanis et al., 1997: 79%; Wright et al., 2001: 61%), but more comparable to twin studies in young adults (Anokhin et al. 2004: 41% at ages 18 to 28 yrs; O'Conner et al, 1994: 49%, ages 22 to 44), and a large family study in subjects 16 to 70 yrs of age (Almasy et al., 1999: 51%). Heritability of Pz latency, 45%, was also comparable to those in the extant literature. The meta-analysis by van Beijsterveldt and van Baal reported an estimated 51% heritability across studies.

It should be noted that our study differed in the exact oddball design from previous studies. P3 characteristics (amplitude, latency) are known to be sensitive to various variables such as the percentage of targets, task difficulty, speed versus accuracy instructions, and intensity and complexity of the stimulus (Pfefferbaum et al., 1988; Polich & Bondurant, 1997; Snugg & Polich, 1995; Woestenburg et al., 1983). The oddball task used in this study was somewhat different from most

oddball tasks, in terms of the visual stimuli themselves (Snodgrass figures, which are perhaps more difficult). Furthermore, in our study subjects were instructed to silently count the number of targets, whereas others used button press to signal targets. Silent-counting, rather than button press responses, may lead to higher P3 amplitude and longer latencies (Salisbury et al., 2001). Taken the sensitivity of the P3 to the antecendent task conditions, the heritability estimates across our and previous studies are surprisingly consistent.

No significant effects of common environment were found on the P3 variables. This concurs with most previous studies using a genetically informative twin design, but not many studies may have had sufficient power to detect such an influence. Ideally, two features must be present: the design must have information on identical and non-identical sibling relations and it must have a large enough sample size (Posthuma & Boomsma, 2000). Two studies, both in adolescents (van Beijsterveldt et al., 2001; Wright et al., 2002), possessed these features. Van Beijsterveldt et al., in a sample of 426 subjects, found a trend for common environmental effects in females but the effect was absent in males. Wright et al. (2002) found no evidence for common environmental influences in an even larger sample of 1023 subjects. Our current results are in agreement with their finding.

The multivariate models revealed that the genetic variance of P3 amplitude was best explained by a model with three genetic factors that revealed specific contributions to the genetic variance of each lead (straight arrows in Figure 2), but also contributions to the genetic covariance between the leads (oblique arrows in Figure 2). These findings are comparable to those found in adolescents by Wright et al. (2001). Heritabilities for Pz, Cz, and Fz in their study were comparable to our estimates in adults, and they also found three genetic factors for P3 amplitude. Regarding P3 latency, heritabilities found by Wright et al. (2001) were again comparable, but instead of a single genetic factor, a second genetic factor was found. It must be noted, however, that the second genetic factor in their model explained only 8% of the variance of Fz latency.

If the P3 wave consists of different components operating at different time points, reflecting different aspects of cognitive functioning (Kok, 2001), it could be hypothesized that the genetic underpinnings vary across the time course of the P3. The current results, however, do not seem to support such a view. Pre- and post-peak heritability is largely equivalent for the three midline leads. Heritability of amplitude scores do not differ significantly in a range of about 60 ms before of after the P3 peak. Genetic overlap is close to perfect (>90%), indicating that within this 120 ms range amplitude is influenced by the same set of genes. Only at latencies of 100 ms before or after the peak the genetic make-up of P3 amplitude differs significantly from that at the peak, and within this large range still

70% of the genes influencing individual variation in amplitude are shared with variation in peak amplitude. Two possible explanations for this result are 1) peak amplitude as well as pre- and post-peak amplitude reflect for the most part similar cognitive processes that are influenced by the same set of genes; 2) peak amplitude and pre- and post-peak amplitude reflect different cognitive processes, but are influenced by a spurious genetic factor like skull thickness.

Insofar the P3 parameters are temporally stable, their heritability classifies them as potentially useful endophenotypes (de Geus, 2002) to detect genetic influences on a number of psychiatric disorders that are associated with a deviant P3 (Cohen et al., 1995; Elmasian et al., 1982; Begleiter et al., 1984; Porjesz & Begleiter, 1990; Pfefferbaum et al., 1991; Polich & Herbst, 2000; van der Stelt et al., 1998; Iacono et al., 2003). First attempts at identification of genes which influence variation in P3 characteristics have pointed to areas on chromosomes 2, 6, and 7 as the most promising regions (Begleiter et al., 1998; Jones et al. 2004; Porjesz et al., 1998, 2002). When P3 amplitude was considered simultaneously with the liability to alcoholism an increase in the linkage signal was found on chromosome 4 around a locus known for coding alcohol dehydrogenase (Williams et al., 1999).

Finding genetic polymorphisms that influence the P3 may be helpful just for understanding downstream psychiatric disorders (Dick et al., 2006; Williams et al., 1999). However, it may also help elucidate the neurobiology of the P3 generator systems. Several competing P3 generating systems have been proposed in the literature (for reviews: Picton, 1992; Nieuwenhuis et al., 2005; Hansenne, 2000; Soltani & Knight, 2000). The recent review by Nieuwenhuis et al. (2005) stresses the role of the norepinephrinic projections from the locus coeruleus to the cortex (LC-NE) in P3 generation. It is hypothesized that the LC is recruited by input from cortical afferent projections that monitor the motivational aspects (or salience) of a stimulus. The activated LC then modulates cortical activation and information processing via coeruleo-cortical NE projections in a pathway from anterior to posterior areas (Aston-Jones & Cohen, 2005). Thus, the LC-NE system acts as a central modulator of cortical generators of the P3, which are localzed mainly in the temporal-parietal junction (TPJ) and the lateral prefrontal cortex. Nieuwenhuis et al (2005) based this hypothesis on the grounds of multiple sources of evidence, including lesion studies, covariation between LC phasic responses and P3 amplitude, and psychopharmacological evidence.

The current results are consistent with the role of the LC as a central timing mechanism of P3 midline activity. If the LC plays a key role in P3 generation (Aston-Jones & Cohen, 2005; Nieuwenhuis et al., 2005) P3 latency should be related to LC activity. Heimer (1983) describes how NE projections from the LC first reach the prefrontal areas before passing on to the more posterior regions. These are non-myelinated fibers and therefore relatively slow. This may explain

why the frontal P3 occurred slightly earlier than the posterior P3. In addition, the finding that P3 latency reflected a single genetic source may be more consistent with a central timing mechanism as in the proposed LC-NE system than, for example, with multiple independent cortical generators. Regarding the findings of P3 amplitude we speculate that the genetic variance common to the three midline leads reflected modulation by the LC system whereas the specific factors reflected the contribution of local P3 generators at for example the TPJ or lateral frontal cortex. Overall, we conclude that separating genetic from environmental variance has provided some insights into the biological processes underlying the P3.

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4

HERITABILITY OF ANTERIOR AND POSTERIOR VISUAL N1

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ABSTRACT

Previous studies have reported that individual variation in N1 amplitude is related to attentional problems and alcoholism. Using data from 651 twins and siblings from 292 families we examined whether variation in N1 amplitude and latency can be explained by genetic factors. In half of the subjects the age centered around 26 (young adult cohort), in the other half the age centered around 49 (middle-aged adult cohort). Two visual N1 components were identified by a spatial PCA –an early anterior component peaking from 88 to 168 ms after stimulus presentation and a posterior one peaking from 132 to 220 ms. Significant heritability was found for anterior N1 amplitude (22%) and posterior amplitude (50%), and for anterior latency (45%) and posterior latency (43%). We conclude that visual N1 amplitude and latency may serve as endophenotypes to detect genetic variation in susceptibility to psychiatric disorders.

INTRODUCTION

The N1 is an early endogenous ERP component peaking in the time range of 75-250 ms after the evoking stimulus. In cognitive psychophysiology, the N1 is thought to reflect the allocation of processing capacity to sensory input (the orienting response), or the active filtering of information (Coles et al. 1990; Altenmüller & Gerlof, 1999). As early as 1964, Eason et al. (1964) reported that attended stimuli evoke larger, more negative N1s than unattended stimuli, a finding replicated many times in selective attention tasks. In the auditory domain the N1 peaks at the vertex (Cz), whereas in the visual domain two main negative peaks can be found: an early anterior N1, and a somewhat later posterior N1 (Vogel & Luck, 2000). The N1 peak amplitude and latency have been studied widely to investigate individual differences in disease susceptibility (e.g., Olbrich et al., 2000; Winsberg et al.,1997).

The N1 can be obtained from the same oddball paradigm that is used to invoke the well-known P3 (Almasy et al., 1999). In this paradigm, frequent nontarget stimuli are interspersed with target stimuli that are infrequent but task relevant, i.e. they require some response of the subject in contrast to the frequent nontargets. Unlike the P3 component, the occurrence of the N1 is independent of the task relevance of the stimuli. To optimize the signal-to-noise ratio, the N1 is therefore usually obtained by ensemble averaging the response to the frequent nontarget stimuli.

In contrast to the P3, which has received a lot of attention from geneticists (Anokhin et al, 2001; Carlson et al., 2002; Katsanis et al, 1997; O'Conner et al., 1994; Polich & Burns, 1987; van Baal et al., 1998; van Beijsterveldt et al., 2001; Wright et al., 2001), the earlier N1 component has only rarely been subjected to genetic analyses in twin samples. In the few studies available, the results have been mixed. In the auditory domain, significant heritability was found for the N1 amplitude by some (Polich & Burns, 1987; O'Connor et al., 1994) but not by others (Surwillo, 1980). In the visual modality, only two studies have been performed with contrasting results. In a first study (Almasy et al., 1999) small genetic effects on variation in nontarget N1 amplitude and latency were reported for frontal leads (heritability of amplitude 19% to 31%; heritability of latency 10% to 16%) whereas larger effects were found for occipito-temporal leads (amplitude 45% to 54%, latency 3% to 12%). In the second study (Katsanis et al., 1997) only small and nonsignificant genetic effects on variation in target N1 were reported for three parietal leads.

The current study aims to re-examine the contribution of genetic and environmental factors to variance in the N1 amplitude and latency in a visual oddball task. To this end, we used a large sample of adult MZ and DZ twins from the

Dutch population with the addition of their non-twin brothers and sisters. Using this sample, we first established that two early endogenous components could be identified using a spatial PCA (Spencer et al., 1999), an anterior and a posterior one. For these components, we scored N1 amplitude and latency. Since variation stemming from measurement error and other unreliable trial-by-trial variation is generally of no interest in genetic analyses, we estimated the amount of variation due to such factors by analyzing the odd and even trials separately following van Baal et al. (2001). This allowed us (a) to estimate the reliability with which N1 amplitude and latency can be established, and (b) to estimate the genetic and environmental contributions to the reliable part of the N1. In addition, we investigated whether the genetic makeup of these early endogenous components is different for young versus middle-aged adults.

METHODS

Subjects

The study received prior approval by the institutional review body of the VU medical centre. Informed consent was obtained from each subject. Subjects were recruited from the Netherlands Twin Registry (Boomsma et al., 2002a) as part of a large project on the genetics of cognition and adult brain functioning (Posthuma et al., 2001). Adult twins and their non-twin siblings were invited to participate. Subjects were required to have normal or corrected-to-normal vision. Family members with previous head trauma (including concussion) or coma were not eligible.

A total of 760 family members from 309 twin families participated in the study and EEG data was available from 732 subjects from 305 families. Subjects were selected from two twin cohorts, one of about 26 years old, and one of about 49 years old. For this study, we restricted the age range to young and middle-aged adulthood: only subjects in the range of 20 to 65 years were included. This resulted in a final sample of 715 subjects from 303 families. After EEG data cleaning, 661 subjects from 292 families had sufficient error free data on at least one lead for genetic analyses, resulting in a younger cohort of 336 subjects (45.8% male, mean 26.5 years, SD 3.8) and a middle-aged cohort of 325 subjects (40.9% male, mean 48.7 years, SD 6.2). Age cohort inclusion was per family and based on the age of the twins on the day of measurement with the cut-off at 35 years. Two younger siblings under 35 were included in the middle-aged cohort on the basis of the twins being over 35, and 11 older siblings over 35 were included in the young adult cohort on the basis of the twins being under 35. Participating families consisted of one to seven siblings (including twins). On average, 2.3 participants per family participated.

Procedure

The subjects were asked to participate in a 4.5 hour testing protocol. During one part of the experimental protocol, psychometric intelligence, inspection time, and reaction times were assessed. During the other, the subjects performed, amongst others, a visual oddball task. The order of the two parts of the protocol was randomized across family members. Consequently, half of EEG registration sessions were during morning hours, and half were in the afternoon.

During EEG recording subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated and electromagnetically shielded room. They were instructed to relax, and to minimize blinking, eye and body movement. Subjects were instructed to silently count the number of targets (cats).

Stimuli

The oddball stimuli were white-on-black line drawings of cats and dogs by Snodgrass and Vanderwart (1980), balanced in the amount of physical stimulation. The dog stimuli were shown frequently (100 / 125) and were the nontargets. The cat stimuli were shown only infrequently (25 / 125) and were the targets. A stimulus set with an identical order of stimuli and intertrial intervals was presented to all subjects. In this set, dogs and cat stimuli were generated in an unpredictable order and trial duration was made to vary randomly from 1500 to 2000 ms. Stimulus duration was always 100 ms. Before the task, one example of each stimulus was presented. The computer screen was positioned 80 cm in front of the participants which was verified by use of a rod.

EEG registration

EEG was recorded with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS, Enschede, The Netherlands) for 612 subjects and Neuroscan SynAmps 5083 amplifier (Compumedics, El Paso, TX) for 103 subjects. Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2 (Compumedics, El Paso, TX). Standard 10-20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2. For Neuroscan subjects Fp1, Fp2, and Oz were also included. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG

was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz. Neuroscan filter settings were a lowpass filter at 50.0 Hz and no high pass filtering. Strong DC shifts were manually reset before the start of the experiment.

Data processing

Only nontarget trials were used in the analyses. All signals were recalculated with averaged earlobes as reference and analyzed using Neuroscan Edit (Compumedics, El Paso, TX). Next, if the signals were absent or the signals were deemed extremely noisy upon visual inspection the subject was excluded from further analysis. This resulted in the removal of twenty-six subjects. Signals from all leads were then reviewed for artifactual episodes (swallowing, muscle artifacts, eye movements (not blinks), and technical problems such as clipping). These episodes were removed and excluded from the analyses. Next, blink artifact reduction was performed following the procedure introduced by Semlitsch et al. (1986). Epochs were created from 100 ms pre-stimulus up to 700 ms post-stimulus with baseline offset correction including only epochs that did not overlap with artifactual episodes.

We expected the anterior and posterior N1 in the time range of about 50 to 250 ms post-stimulus. Data from each subject and for each time-point in this window were concatenated and subjected to a spatial PCA with Promax rotation on the resulting covariance matrix (comparable to Spencer et al., 1999). This procedure aimed to find evidence for separate anterior and posterior N1 components as reported by Luck (1995) and Vogel and Luck (2000). From these data we identified those leads that best describe the components using the factor pattern matrix from the PCA. Next, we determined N1 scoring time windows based on grand average waves for the selected leads. Finally, individual N1 peak amplitude and latency were scored in these time windows.

To estimate the reliability of N1 amplitude and latency, we separated the nontarget trials of each individual into a set of odd and a set of even trials for each of the leads analyzed. For both sets an ERP was calculated and the N1 peak-to-baseline amplitudes and the corresponding latencies were scored. A minimum of 30 trials in each set was required.

Genetic analyses

Resemblance (covariance) in ERP traits between siblings derive from genetic relatedness or shared environmental influences (Falconer & Mackay, 1996). If the covariance between DZ twins or siblings, who share on average 50% of their genetic make-up, is half the covariance between MZ twins, who are genetically

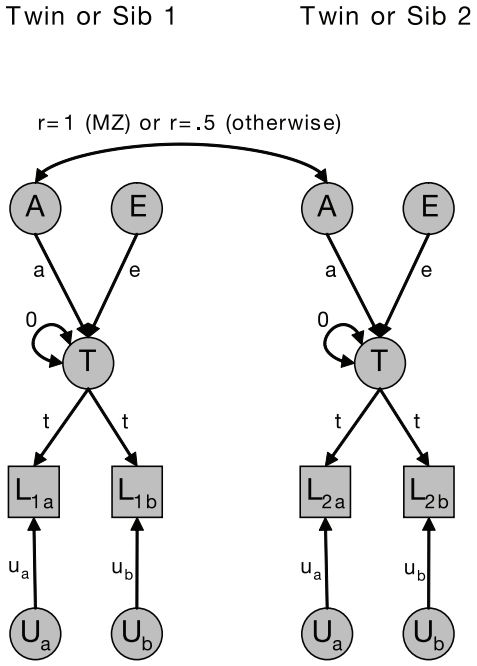

Figure 1. Path model for the genetic modeling of repeated measures (a, b) of the N1 of lead L for twins (or siblings) 1and 2. Ua and Ub represent the measurment error and is by definition zero correlated between measurement occasions (i.e., the split-halves.) The genetic modeling is performed on the factor T which represents the remaining, correlated variance which excludes the measurement error variance U. Unique environmental variance (E) has zero correlation between twins/siblings, whereas additive genetic variance (A) correlates 1 between MZ twins and .5 between fraternal twins and siblings. Note that the zero variance indicates that T is a mathematical construct which does not explain variance of the observed variables by itself but is decomposed further by factors A and E.

identical, this is seen as evidence for additive genetic influences (A). If the covariance between DZ twins or siblings is less than half the covariance between MZ twins this is seen as evidence for dominant (non-additive) genetic influences (D). If the covariances between MZ and DZ twins/siblings are comparable and non-zero this is evidence for shared environmental influences (C). If the covariance between MZ twins is not unity this is evidence for environmental effects unique to each individual (E).

By comparing MZ and DZ covariances using Structural Equation Modeling, we obtained maximum likelihood estimates of the relative contributions of each of these factors to the total trait variance. Heritability was defined as the proportional contribution of genetic effects (A + D) to the total variance (A + C + D + E). In a twin-sibling design, however, the effects of both C and D cannot be estimated in the same model. The size of the DZ correlation relative to the MZ correlation determines which component to include in the model. If the DZ correlation was less than half the MZ correlation, then D was modeled. If it was

more than half the MZ correlation, C was modeled. For more information on the basis of genetic modeling we refer to Boomsma et al. (2002b) and Posthuma and Boomsma (2005).

The genetic analyses were performed on N1 peak amplitude and latency from individual ERPs from all trials. However, the standard twin model does not allow the separation of unreliable effects such as measurement error from unique environmental effects. ERP measures generally consist of multiple registration events in the form of multiple trials that can be used to estimate measurement inaccuracy, which can in turn be used to separate out the effect from other unique environmental effects. The result is a heritability estimate that represents genetic and environmental effects while excluding all unreliable variance. We therefore repeated the genetic analyses on N1 amplitude and latency scores from odd and even trial ERPs. A repeated measures model as depicted in Figure 1 was used. In this model, measurement error is assumed to be uncorrelated across occasions, in this case N1 amplitude and latency from the odd and even trial ERPs. Measurement error is thus defined as the unreliable variation between two sets of trials that are in close proximity in time. Genetic analysis was then performed on the remaining variance, that is, the reliable, correlated variance between the odd and even trials (factor T). This variance was further decomposed into the variance components (A, C, D, and E) described above. Although this procedure halves the number of observations for each ERP, if the error is random between trials the point estimates for A, C, and D are unchanged. Heritability is still defined as the relative contribution of genetic effects (A+D) over the total variance (A+D+C+E), however, the total variance has decreased since uncorrelated variance U has been partialled out from E.

All genetic modeling was performed with Mx (Neale, 2004).

RESULTS

Preliminary analyses

Figure 2 shows the ERP grand average waves to nontargets for each of the age cohorts separately. The frontal leads show a small N1 at around 135 ms, most clearly seen in the young adult cohort. Occipital and temporal leads show a clear N1 peak at about 180 ms. The spatial PCA resulted in two components with eigenvalues larger than 1.0, explaining 58.3% and 28.5% of variance (other components explained under 5% of variance). The component loadings after Promax rotation are shown Table 1. Figure 3 shows the topographic plot for both components. The components correlated slightly at r=.05. The first component loads mainly on central and frontal leads. We chose four leads (F3, F1, F2 and F4) to

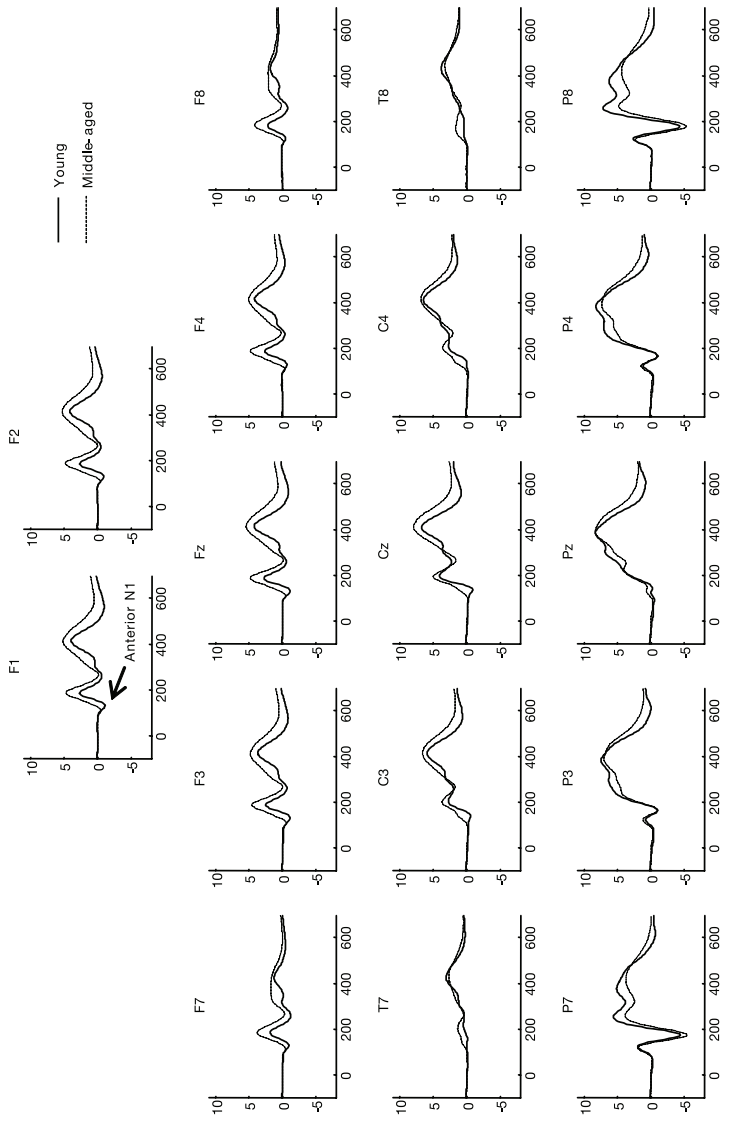

Figure 2. Grand average waveforms.

represent the anterior N1. The second component loaded mainly on occipital (O1 and O2) and lateral posterior leads (P7, P3, P4, and P8). Since P3 and P4 also loaded positively on the first component, we chose channels P7, O1, O2, and P8 to represent the posterior N1.

On the basis of the grand average waveforms peak picking windows were defined. Anterior peaks were picked in a window from 88 to 168 ms post stimulus on the selected anterior leads (F3, F1, F2, and F4); and posterior peaks be-

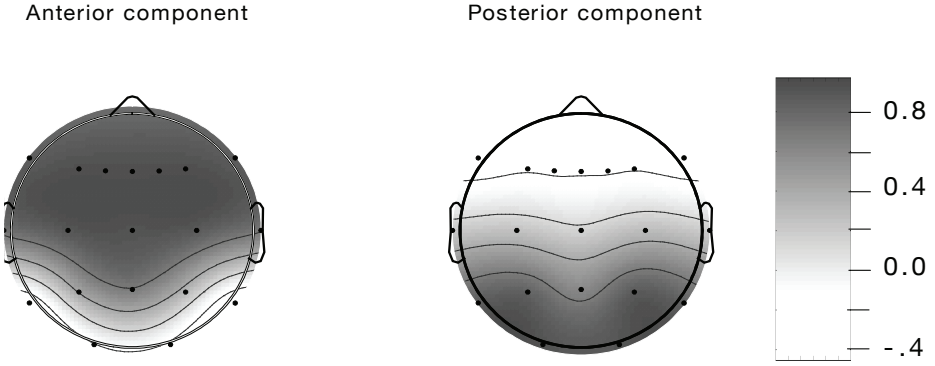

Figure 3. Component loadings topographic plots

tween 132 and 220 ms post-stimulus on selected leads P7, O1, O2, and P8. For each peak a latency and an amplitude was scored. Both anterior and posterior amplitude scores were quite normally distributed. Since we have no reason to conclude that these data points were rogue, we excluded none from the data. Latency scores were all within the limited range of the peak picking window, disallowing outliers to be identified.

Effects of age and sex on N1 amplitude and latency

Table 2 shows the amplitude and latency means averaged over the anterior and posterior leads as a function of sex and age cohort. The last columns specify the significance of sex and cohort effects on the means. The middle-aged cohort showed reduced (less negative) anterior N1 and increased (more negative) posterior N1 amplitude. Posterior N1 latency showed no significant differences between the cohorts, anterior N1 latency is shorter in the middle-aged cohort compared to the young adults. Males showed reduced (less negative) anterior N1 amplitude and longer posterior N1 latencies compared to females.

Split-half reliability

Table 3 shows the reliabilities defined as the proportion reliable variance to the total variance as determined with the N1 scores obtained from the odd and even trials. These results suggest that the posterior N1 can be measured reliably ($r > .9$), but that the anterior N1 has only modest reliability in both amplitude and latency ($r \approx .6$).

Genetic analyses

Table 4 shows the twin correlations for N1 peak amplitude and latency from the analysis on all trials and from the analysis on odd and even trials with adjust-

Table 1. Loadings of the two main spatial N1 components.

	Component	
Lead	**1**	**2**
F7	0.90	-0.30
F3	0.97	-0.18
F1	0.98	-0.19
Fz	0.98	-0.19
F2	0.98	-0.19
F4	0.97	-0.18
F8	0.91	-0.29
T7	0.77	0.21
C3	0.92	0.25
Cz	0.94	0.15
C4	0.90	0.28
T8	0.74	0.19
P7	-0.26	0.86
P3	0.44	0.82
Pz	0.70	0.59
P4	0.35	0.86
P8	-0.31	0.83
O1	-0.13	0.91
O2	-0.16	0.90

ment for measurement error. Since there was no clear difference in the pattern of male and female correlations, these were collapsed across the sexes. Overall there appear to be some differences in the correlations between the cohorts, but an omnibus test on MZ and DZ correlations showed no significance for any lead or variable (amplitude or latency). Variances differed significantly between the cohorts for anterior N1 amplitude. Subsequent genetic models were fit allowing for this difference by using a so-called scalar model. These models estimate a single heritability across both age cohorts, while allowing the total amount of variance to differ.

No significant effect of common environment or dominance were found for any of the leads/variables (all $\chi^2(1) < .74$, $p > .39$). The most parsimonious models consisted of genetic and unique environmental influences to explain all of the variance in the N1. Table 5 lists the relative contribution of these factors to the

Table 2. Anterior and posterior N1descriptives.

	Cohort													
	Young						Middle-aged							
	Female			Male			Female			Male			group main effects[a]	
Region	**N**	**M**	**SD**	**N**	**M**	**SD**	**N**	**M**	**SD**	**N**	**M**	**SD**	**Sex[b]**	**Age cohort[c]**
Anterior														
Amplitude (μV)	180	-2.07	1.66	151	-1.54	1.33	187	-1.32	1.36	131	-1.13	1.30	0.33 **	0.58 ***
Latency (ms)	180	130.0	20.5	151	127.8	21.6	187	115.7	21.8	131	117.2	20.5	0.18	-12.67 ***
Posterior														
Amplitude (μV)	180	-5.78	3.97	152	-6.36	3.63	188	-7.67	4.20	132	-6.97	3.77	0.10	-1.33 ***
Latency (ms)	180	167.9	11.4	152	174.2	11.0	188	168.0	11.3	132	176.2	10.6	7.20 ***	0.58

[a]Sex group effects are collapsed across cohort, and cohort group effects are collapsed across sex groups. Significance is derived by structural equation modeling comparing the fit of models with and without cohort and sex specific means.
[b]males compared to females.
[c]middle-aged compared to young adult.
*p < .05; **p < .01; ***p < .001

Table 3. N1 split-half reliabilities with 95% confidence intervals.

Region	estimate	95% CI
Anterior		
Amplitude	0.53	(0.50, 0.56)
Latency	0.64	(0.62, 0.67)
Posterior		
Amplitude	0.94	(0.93, 0.95)
Latency	0.91	(0.90, 0.92)

Note. Trials were split by trial number (odd, even). A minimum of 30 artifact-free trials for each set were required.

N1 for the analysis of the full set of trials and the odd/even trial analysis.

DISCUSSION

The spatial PCA of data in the early endogenous time frame of 50 ms to 250 ms post-stimulus confirmed that in a visual oddball task two components can be identified: an anterior and a posterior waveform (Vogel and Luck, 2000). The posterior waveform showed a large and clear N1 at ca. 170 ms of which amplitude and latency could be reliably measured in 30-50 trials. The anterior N1 at ca. 120 ms was much less reliably measured. Nevertheless, both the anterior and posterior N1 showed significant heritability. Posterior N1 showed 50% heritability for amplitude and 43% for latency. Adjusting for unreliable variance increased these estimates to 54% and 44% respectively. Anterior N1 showed lower heritability for amplitude (22%) but not for latency (45%). Adjusting for the unreliable variance increased these estimates to 35% and 56% respectively.

Two studies have previously reported on the heritability of the N1 in the visual domain. In contrast to our findings, Katsanis et al. (1997) found only nonsignificant or very low twin correlations for both latency and amplitude. Apart from the difference in oddball paradigm, the study by Katsanis et al. (1997) differed from the current study in several major ways which may have caused the difference in results. First, as the main topic of their article was the parietal P3, they chose to analyze the N1 on leads P3, Pz, and P4. These leads, however, do not generally show very clear visual N1 in the grand average waves, which is confirmed by inspection of the waveforms (their Figure 1). Second, they reported on N1 data

Table 4. Twin correlations with confidence intervals of average anterior and posterior N1 amplitude and latency for two age cohorts.

			Cohort							
			Young adult				Middle-aged			
			MZ (95% CI)		DZ (95% CI)		MZ (95% CI)		DZ (95% CI)	
Anterior	Amplitude (μV)	total variance	0.35	(0.07, 0.55)	0.13	(-0.02, 0.29)	0.14	(-0.09, 0.35)	0.07	(-0.07, 0.23)
		reliable variance	0.48	(0.11, 0.76)	0.21	(-0.01, 0.44)	0.26	(-0.07, 0.54)	0.16	(-0.05, 0.38)
	Latency (ms)	total variance	0.44	(0.17, 0.62)	0.22	(0.06, 0.37)	0.44	(0.22, 0.61)	0.20	(0.05, 0.35)
		reliable variance	0.60	(0.29, 0.81)	0.28	(0.08, 0.47)	0.55	(0.28, 0.75)	0.20	(-0.02, 0.41)
Posterior	Amplitude (μV)	total variance	0.36	(0.09, 0.56)	0.27	(0.11, 0.42)	0.60	(0.43, 0.71)	0.26	(0.10, 0.40)
		reliable variance	0.40	(0.14, 0.60)	0.28	(0.12, 0.44)	0.63	(0.46, 0.75)	0.29	(0.13, 0.44)
	Latency (ms)	total variance	0.45	(0.20, 0.62)	0.23	(0.08, 0.38)	0.37	(0.11, 0.55)	0.23	(0.07, 0.38)
		reliable variance	0.49	(0.22, 0.66)	0.26	(0.10, 0.42)	0.40	(0.13, 0.58)	0.26	(0.09, 0.41)

Note. All correlations were estimated using Mx after removal of age and sex effects on the means. DZ correlations include all fraternal (non-identical) siblings pairs, including opposite sex pairs.

Table 5. Heritabilities with confidence intervals of anterior and posterior N1.

		total variance		reliable variance	
Region		**h^2**	**95% CI**	**h^2**	**95% CI**
Anterior					
	Amplitude (μV)	.22	(.08, .35)	.35	(.16, .53)
	Latency (ms)	.45	(.31, .57)	.56	(.40, .71)
Posterior					
	Amplitude (μV)	.50	(.37, .61)	.54	(.42, .64)
	Latency (ms)	.43	(.30, .55)	.44	(.30, .56)

Note. The models included additive genetic (A) and unique environmental (E) factors. "Total variance" shows heritabilities from individual peak picking on the average of all trials, while "reliable variance" shows heritabilities from the reliable variance between peaks from odd and even trials.
h^2 = heritability

from the less frequent target trials rather than more frequent nontarget trials – the latter providing more reliable ensemble averages.

The second study (Almasy et al., 1999) reported N1 data from multiple scalp locations, thus allowing the separation of anterior and posterior N1. Significantly heritable N1 amplitude was found for three frontal leads (F3, Fz, and F4). Although their heritability estimates for anterior N1 amplitude (23% to 31%) are slightly higher than our unadjusted heritability estimates (22%), they remain within the 95% confidence range (see Table 4). Likewise, our heritability of posterior N1 amplitude (50%) was very comparable to posterior heritabilities reported by Almasy et al. (1999): 37% to 53%. For anterior and posterior N1 latency, however, our estimates (45 and 43% respectively) were much higher than theirs (anterior: 10% to 16%; posterior: 3% to 12%). A possible explanation for this discrepancy lies in the difference in study population. Almasy et al. (1999) studied families with a background of alcoholism, whereas the current results are from a population based, non-clinical sample. Alternatively, Almasy et al. (1999) may have used a suboptimal peak picking window for the posterior N1. They used a window of 75 to 180 ms post-stimulus irrespective of scalp location. This was adequate for the anterior N1, but, in our view, not so for the posterior leads. We consider it likely that most subjects' posterior N1 latency scores in their study were located at the upper bound of the peak picking window, causing a reduction of the variance of N1 latency and, to a lesser extent, N1 amplitude.

The N1 topography appears to be very sensitive to stimulus modality (Alten-

müller & Gerlof, 1999). In the auditory domain there is no anterior or posterior N1, but instead a component with comparable timing and morphology is found at the central leads. O'Connor et al. (1994) reported a heritability of 60% for the amplitude of this auditory N1 at lead Cz, and 56% for its latency. It is unclear whether the auditory N1 compares better to the anterior or to the posterior visual N1. A powerful way to address this would be to examine auditory and visual N1 in the same set of twins using a bivariate genetic model.

Because age effects have been reported on other ERP components other than the N1 (Curran et al., 2001; Fabiani & Friedman, 1995; Fjell & Walhovd, 2004; Polich, 1997 a, b) we explicitly tested for the effect of age cohort on N1 amplitude and latency. For the entire anterior waveforms, an upward shift was seen in the middle-aged compared to the young adult cohort beginning at about 100 ms and lasting for the length of the ERP interval (>650 ms). This caused the shortening of the latency in the middle-aged cohort while slightly decreasing the anterior N1 amplitude. The posterior waveforms showed a downward shift beginning at about 100 ms and lasting for about 500 ms into the ERP interval. This had no effect on the posterior N1 latency which was scored in a window later than the anterior N1, but caused increased posterior N1 amplitude in the middle-aged cohort. The faster anterior N1 latency is contrary to the findings in previous reports in auditory domain, where either no latency effect was found (Polich, 1997b; Beck et al., 1980), or evidence for a slower N1 response in older subjects (Curran et al., 2001). In spite of the cohort differences in amplitude and latency, the MZ and DZ correlations for all N1 measures were not significantly different across the young and middle-aged groups. We found no evidence, therefore, of age effects on the genetic architecture of the N1.

The heritability of the N1 makes it a candidate endophenotype for related psychopathology. In the extant literature it has been reported that individual differences in N1 amplitude are related to attentional problems. Satterfield et al. (1984) reported reduced N1 amplitude for hyperactive children aged 7-9 and 10-12. For ADD with combined attentive and hyperactive symptoms, Johnstone et al. (2001) reported reduced N1 amplitude at ages 8–10, 12–14, and 16–18 years, but not at 10–12 and 14–16 years. Smith et al. (2004) also reported reduced N1 amplitude in ADHD using a go/no-go task. By contrast, several other studies reported no significant group differences for amplitude in the N1 time range (Rothenberger et al., 2000; Winsberg et al., 1997). It is possible that more consistent effects may be found in the 'processing negativity', which is the negative shift seen in a selective attention task by comparing ERPs of stimuli from the attended to those of the unattended channel, appearing in a time range covering the N1, P1 and N2 (Altenmüller and Gerlof, 1999; Näätänen et al.,). Indeed, it has recently been reported that ADHD patients have a reduced processing negativity in an auditory

selective attention task (Jonkman et al, 1997; Kenemans et al., 2005).

Reduced visual N1s have also been reported in alcoholics. Cohen et al. (2002) reported the effect in the parietal region. Olbrich et al. (2000) reported reduced N1 amplitude at lead Cz. In the auditory domain, some authors reported enhanced auditory N1 in abstinent alcoholics (Ahveninen et al., 2000), whereas no such effect was reported by most (Cohen et al., 1996; 2002; Olbrich et al., 2002; Pattersen et al, 1987; Pfefferbaum et al., 1979). Overall, these findings suggest a modality specificity of the effect of alcoholism on the N1. It must be noted, however, that reduced N1 amplitude may not reflect the liability to develop alcoholism: People with a family history of alcoholism did not show affected N1 amplitude (Cohen et al. 1996), and normal N1 amplitudes were obtained before alcohol ingestion (Porjesz & Begleiter, 1990; Porjesz et al., 2005). Strong trends towards reduced amplitude in people with a family history of alcoholism were found for visual and auditory N1 by Patterson et al. (1987). Future family or twin studies may elucidate the nature of the relation between the visual N1 and alcohol use. With the correct definition of the eliciting tasks, topography, and latency, the N1 may perhaps repeat the success of the P3 as an endophenotype for alcoholism (Williams et al., 1999; Dick et al., 2005).

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5

THE RELATION BETWEEN FRONTAL EEG ASYMMETRY AND THE RISK FOR ANXIETY AND DEPRESSION

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ABSTRACT

Frontal Asymmetry of EEG alpha power (FA) may index the risk for anxiety and depression. Evidence linking FA to the underlying biological mechanisms is scarce. This is unfortunate because FA has potential as a biological marker to support gene finding in anxiety and depression. We examined the heritability of FA in 732 twins and their singleton siblings, and established the genetic and environmental contribution to the relation between FA and the risk for anxiety and depression. Multivariate models showed that FA is heritable only in young adults (males: 32% and females 37%) but not in middle-aged adults. A significant relation between FA and the risk for anxiety and depression was only found in young adult females. This relation was explained by shared genes influencing both EEG and disease risk. Future studies on asymmetry of left and right frontal brain activation should carefully consider the effects of sex and age.

INTRODUCTION

Frontal Asymmetry of EEG alpha power (FA) has been studied extensively as a correlate of individual differences in emotional responding. These studies assume that alpha power acts as an inverse index of activity: A synchronous state of oscillations reflects inactivity of the underlying neural substrate (Shagass, 1972). This assumption has been supported by fMRI and PET studies that showed a decrease in cortical blood flow with increasing alpha power (Cook et al., 1998; Goldman et al., 2000). Greater left hemispheric activity has been associated with approach related behavioral tendencies, and greater right hemispheric activity with withdrawal related tendencies. In the extant literature, therefore, it is hypothesized that FA acts as an index of the basic emotional dimension of approach versus withdrawal (Coan and Allen, 2004; Harmon-Jones, 2004). On this basis individual differences in asymmetric frontal activity are hypothesized to indicate individual differences in affective style (Davidson, 1992).

Because affective style is related to the liability to develop psychopathology such as depression and anxiety disorders a relation between FA and depression and anxiety can be expected (Coan and Allen, 2004; Davidson, 1992). In adults, many studies provided findings consistent with this view (for an overview see Coan and Allen). For example, FA has been found to differ between clinically depressed patients and non-depressed controls (Flor-Henry et al., 1979; Henriques and Davidson, 1991), responders and non-responders to fluoxetine treatment (Bruder et al., 2001), and subjects scoring high and low on a depression scales (Debener et al., 2000; Gotlib et al., 1998; Schaffer et al., 1983). The link between FA and negative affectivity also holds in infants of depressed mothers (Field et al., 1995, 2000) and seasonal affective disorder patients (Allen et al., 1993) or unipolar depression patients currently in remission (Henriques and Davidson, 1990; Gotlib et al, 1998). The latter indicates that FA is a marker for the liability for depression rather than the depressive state itself. Taken together, these studies have established a secure foothold for FA as a biological marker for depression.

As noted by Allen and Kline (2004) much of the research on FA has focused on its relation to psychopathology and other behavioral phenotypes, but evidence linking FA to the underlying biological mechanisms is scarce. This is unfortunate because FA has potential significance as a so-called endophenotype to support gene finding in depression. Endophenotypes are psychophysical or psychophysiological phenotypes that are constituents of the causal pathway from gene to phenotype. They represent the expression of a subset of genes from the whole set of genes causing the genetic part of phenotypic variation. As such, they can be useful when the great number of genes involved in the phenotype of interest reduces the statistical power in linkage and association studies. Ideally, endophe-

notypes possess several features (de Geus, 2002): i) they are stable, ii) heritable, iii) and correlated with the phenotype of interest, and iv) the relations in (ii) and (iii) share a common genetic source.

Although studies show that the requirements (i) stability and (iii) correlation to the risk for depression have been met with success (Allen et al., 1993; Gotlib et al., 1998; Henriques and Davidson, 1991; Henriques and Davidson, 1990; Schaffer et al., 1983; Tomarken et al., 1992), evidence for genetic contribution to FA and to the association between FA and depression is still scarce. Several conference abstracts reported on the genetic basis of FA. Anokhin and Rohrbaugh (1998) found a mid-parent to offspring correlation of r = .46 in a family study of alcoholic and depressed patients and controls, providing evidence for familial influences in FA. Allen et al. (1997) used a twin study to further show that these familial influences reflected genetic influences. In 60 pairs of 17 year old female twins, they found 33% of FA variability to be under genetic control. In a thesis, Coan (2003) reported that genetic influences explained a modest 22% of the variation in mid-frontal FA in 66 female twin pairs, and no significant heritability in males from a normal population. Recently, Anokhin et al. (2005) reported a modest mid-frontal FA heritability of 31% within a young adult female sample.

Here we aim to extend the knowledge base on the genetics of FA by examining FA from resting EEG in large set of twin pairs and their singleton siblings. Additionally, we aim to establish the genetic and environmental contribution to the relation between FA and the risk for anxiety and depression. Anxiety disorders have not been studied extensively in relation to FA, and the results are less conclusive than for depression (Baving et al., 2002; Heller et al., 1997; Kentgen et al., 2000; Nitschke et al., 1999; Papousek and Schulter, 2002). However, anxiety disorders are highly comorbid with depression. Moreover, the genetic variance of these phenotypes reflects for the most part a common genetic source (Jardine et al., 1984; Kendler et al., 2003; Middeldorp, Cath, et al, 2005; Middeldorp, Birley, et al., 2005). We hypothesize that the common genetic factor underlying the risk for anxiety and depression is reflected in individual differences in FA. Since sex differences in the heritability of FA as well as in the relation between FA and depression have been reported, we stratified our sample according to sex (Bruder et al., 2001; Miller et al., 2002).

METHOD

Subjects

The sample of this study was derived from an ongoing twin family study on mental and physical health in participants of the Netherlands Twin Registry (NTR). Families with adult twins have been receiving surveys on lifestyle and health ev-

ery two/three years since 1991 (Boomsma et al., 2002). Anxiety and depression data were available for 9088 twins and non-twin siblings. These were divided into two age cohorts based on the twins age on 1 January 1999: A young adult cohort (under 35) with 3879 males and 5364 females, and a middle-aged cohort (over 35) with 647 males and 1232 females. On average 2.20 siblings per family participated.

A subset of twins and siblings were invited for detailed psychophysiological study in the laboratory. For the present study, twins were invited who had previously participated in EEG or cardiovascular research. In addition, we invited their non-twin siblings. A total of 760 subjects from 309 twin families accepted the invitation to participate. As with the survey sample, the EEG sample consisted of two age cohorts based on the age of the twins: a younger cohort (M = 26.2 years, SD = 4.1) and a middle-aged cohort (M = 49.4 years, SD = 7.2). Participating families consisted of one to seven siblings (including twins). On average, 2.50 participants per family participated. Informed consent was obtained in writing for the EEG study. Both the EEG and the Questionnaire studies received approval from the appropriate ethical committees.

EEG registration

The experimental protocol and background EEG registration has been described in detail elsewhere (Posthuma et al., 2001; Smit et al, 2005), but a brief description will be repeated here. The experimental protocol consisted of two parts. During one part, psychometric intelligence, inspection time, and reaction times were assessed. During the other, EEG was measured at rest and during various reaction time tasks. The order of the two parts of the protocol was randomized across family members. Consequently, half of EEG registration sessions were during morning hours, and half were in the afternoon.

Subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated, and electromagnetically shielded room. They were instructed to relax and minimize eye and body movement. Resting background EEG was registered for three minutes under both eyes open and eyes closed instructions with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS; Enschede, The Netherlands) for 656 subjects (372 young, 284 middle-aged) and NeuroScan SynAmps 5083 amplifier for 104 subjects (24 young, 80 middle-aged). Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2. Standard 10-20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2 (Jasper, 1958; American Electroencephalograpic Society, 1991). For NeuroScan subjects Fp1, Fp2, and Oz were also recorded, but not included in the analysis.

Table 1. Number and composition of families per age cohort and zygosity of the twins.

	Young Cohort					Middle-aged Cohort				
	MZM	**MZF**	**DZM**	**DZF**	**OS**	**MZM**	**MZF**	**DZM**	**DZF**	**OS**
Families with both twins	17	19	9	13	13	16	20	9	15	10
Families with both twins + 1 sibling	9	8	9	13	9	6	14	1	9	8
Families with both twins + 2 or more sibs	2	1	4	2	3	3	3	0	1	3
Families with 1 twin only	1	2	0	1	2	2	3	2	5	5
Families with 1 twin + 1 sibling	1	4	2	2	4	3	1	1	2	1
Families with 1 twin + 2 or more sibs	0	0	0	1	2	0	0	2	0	0
Families with 1 sibling	1	1	0	0	2	0	1	0	2	0
2 or more siblings	1	1	0	0	0	1	0	0	0	0

Note. We based family composition on the participating offspring only. For example, a family with 'both twins only' could potentially consist of more than two children, but these did not participate in the EEG experiment. MZM = MZ male twins, MZF = MZ female twins, DZM DZ male twins, DZF= DZ female twins, OS = opposite sex twins.

The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz. NeuroScan filter settings were a lowpass filter at 50.0 Hz.

Data processing

Computation of FA used the EEG recorded during the eyes closed condition. Signals at leads F3 and F4 were analyzed using NeuroScan software version 4.2. The signals were recalculated with averaged earlobes (A1 and A2) as reference. The 3 minutes recording was cut into 43 epochs of 1024 data points (4.096 s). Any linear trend was removed from EEG by fitting and subtracting the regression line for each epoch separately. Next, epochs were excluded per lead when EOG channels showed more than 400 μV and EEG more than 175 μV deviation from ground in either direction. EEG traces were then visually inspected per subject for remaining artifact due to muscle activity, swallowing, eye movement, bad recordings, and externally induced artifacts (e.g. experimenter initiated reset pulses, electrical hum). Only epochs with extreme magnitudes of muscle artifacts and eye movements were excluded. Subjects with less than 22 valid epochs after visual inspection were considered unreliable and set to missing (22 epochs ensure at least 1 minute and 30 seconds of data per subject.). The number of subjects with valid data on both F3 and F4 was 732. Table 1 shows the exact composition of the final sample per age cohort and zygosity of the twins.

For all remaining, artifact-free epochs, power spectra were calculated with a Hamming window for 5% of the epoch duration at the beginning and end of the epochs. Power spectra were averaged, resulting in a single spectrum with a resolution of about 0.25 Hz (1000 / 4096 Hz). Alpha power was defined as the sum of all data points in the range from 8.0 Hz up to but not including 13.0 Hz. Frontal asymmetry is defined as

$$FA = \ln(\alpha_{F4}) - \ln(\alpha_{F3}),$$

where higher scores reflect lower left alpha power, and consequently higher left cortical activation, relative to the right cortex.

Up to twenty percent of the population may exhibit low or very low alpha synchronization (Vogel, 2000; Anokhin et al., 2005) yielding denominator values that are close to zero. This can result in an unstable and noisy FA measure. In

accordance with Anohkin et al., we repeated our analyses after excluding subjects with the lowest average frontal EEG power. This subject selection aimed to reduce the adverse effects of noise amplification due to the nature of the FA calculation.

Anxiety and Depression Surveys

Questionnaires were sent in 1991, 1993, 1995, 1997, 2000 and 2002 to twin families and their siblings who had indicated that they were willing to participate in the survey study. A detailed description of the survey content and response rates at each wave can be found in Boomsma et al. (2000). Data on trait anxiety (Anx), neuroticism (Neu), somatic anxiety (SoA), and anxious depression (Dep) from three waves were analyzed (1997, 2000, and 2002). Trait anxiety and anxious depression were collected using the Dutch versions of the Spielberger Anxiety Inventory (STAI; Spielberger et al., 1970) and the Young Adult Self Report scale (YASR; Achenbach, 1990). Neuroticism and somatic anxiety were assessed with the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970). The item content of the ABV neuroticism scale is very similar to that of the Eysenck Personality Questionnaire. From these traits a factor score was calculated after weighing each trait to maximize heritability of the factor score. As depression has repeatedly been shown to differ in genetic makeup between males and females (e.g., Bierut et al., 1999; Kendler et al., 2001, 2003), the subscale weights were calculated separately for males and females:

FS = .144xAnx+.117xNeu+.039xSoA+.064xDep, for males
FS = .133xAnx+.117xNeu+.066xSoA+.053xDep, for females

where FS is the Risk Factor Score after normalization of the Anx, Neu, SoA, and Dep scores. This factor score summarizes the genetic risk for anxiety and depression and has been found to have a heritability of about 60% (Boomsma et al., 2000). Forty-six subjects (seven young adult females) of the subjects with EEG data did not have survey data available on any of these time points.

Genetic statistical analyses

Prior to genetic model fitting we tested (1) whether the twin data could be generalized to a singleton population by comparing the means and (co)variances of twins and singleton siblings, and (2) the equivalence of means and variances across MZ and DZ twins. Significance of these differences was tested by 4 group omnibus tests, that is, for all four sex by cohort groups simultaneously.

Genetic statistical analysis of the power spectra of the sample was repeated in young males, young females, middle-aged males and middle-aged females. A linear regression model was employed to include effects of the covariate of age on the observed scores within each group, formally represented as: $\mu_i = \beta_0 + \beta_1$

age_i, where μ_i is the expected value of individual i , age_i is the individual's age in years at time of measurement, β_0 is the intercept, and β_1 is the regression estimate of age.

Structural Equation Modeling implemented in the program Mx version 1.57 (Neale, 2004) estimated the contribution of additive genetic variation (A), shared environmental variation (C), or non-shared environmental variation (E) to the observed interindividual variation in power spectra using the full information Maximum Likelihood Estimation (MLE) procedure (Neale and Cardon, 1992). Sources of shared environmental variation by definition include all environmental influences that twins and siblings from the same family share, while sources of non-shared environmental variation refer to the environmental variation that is unique for an individual and that is not shared with other family members. For two members of a DZ twin pair (and sibling pairs) who are raised in the same home and share on average 50% of their segregating genes, the correlation between shared environmental influences (C) was fixed at 1, the correlation between additive genetic influences (A) at 0.5, and the correlation between dominant genetic influences (D) at 0.25. For two members of an MZ twin pair correlations between shared environmental, additive genetic, and dominant genetic influences were all fixed at 1. Correlation between non-shared environmental influences (E), per definition, is set to zero for both MZ and DZ twins. Thus, the expectation for the total variance is A + D + C + E, the expectation for the covariance between MZ twins is A + D + C, and the expectation for DZ twins/sibling pairs is ½A + ¼D + C. Heritability is calculated as the proportional contribution of genetic variation to the total, observed variation ($\frac{A+D}{A+D+C+E}$).

The twin design with additional siblings does not allow the simultaneous estimation of dominant genetic and common environmental effects (Neale & Cardon, 1992). If the DZ correlation is larger than half the MZ correlation, this will be taken as evidence of common environmental effect and will be set to zero. If the DZ correlation is less than half the MZ correlation, this will be taken as evidence of dominant genetic effects and will be set to zero. In addition, to estimate dominant genetic effects sample sizes must be very large (Posthuma & Boomsma, 2000). We will therefore attempt to estimate this effect in the survey data only.

RESULTS

FA descriptives

FA in middle-aged subjects (M = 0.35, SD = 0.098) was higher than in young adult subjects (M = 0.54, SD = 0.111). The ANOVA showed the effect of cohort to be significant ($F(1, 728) = 6.84$, MSE = .011, $p < .01$). Neither the main effect of sex or the sex by cohort interaction were significant.

FA split-half reliability and temporal stability

The split half reliability, as an indication of measurement error, can be considered a ceiling for the MZ twin correlation. We selected 10% of the subjects at random to compute FA at odd and even epochs separately. The resulting split-half correlation was 0.87 suggesting that MZ correlations are bound by this upper value.

To compute temporal stability, 32 subjects were invited back after a period varying from 354 to 1322 days. Of those, twenty-seven had valid data available on F3 and F4 on both occasions. Temporal stability calculated as the correlation coefficient between both measurement occasions was .44. Jointly these analyses indicate that individual differences in FA are reliable and moderately stable over time.

Handedness

Generally, studies into hemispheric asymmetry limit their samples to right-handed individuals as handedness may be confounded with the lateralization of brain function. We tested this assumption by comparing FA scores of left against right-handed subjects and with a one-way univariate ANOVA with age and sex as covariates. Nine individuals indicated to be ambidextrous or did not provide an answer. Although the proportion of left-handers was slightly higher in the young adult cohort (N = 384, P = 13.3%) than in the middle-aged cohort (N = 339, P = 11.8%), this difference was not significant ($\chi^2 < 1$). Although left-handed subjects (M = 0.032) showed lower FA scores than right-handed subjects (M = 0.046), the effect did not reach significance (MSE = .011, $F(1) = 1.41$, ns). Additionally, we tested whether twin pairs discordant for handedness differed from twins concordant for handedness. If left-handedness causes a mirroring of brain function lateralization, twin pairs discordant for handedness should show a negative, or at least a reduced, intrapair correlation compared to concordant twin pairs. Maximum likelihood estimation of the correlations did not show evidence for an effect of concordance for either MZ or DZ twin pairs (both $\chi^2 (1) < 1$). From these results we concluded that handedness is not a confound of FA, and subsequent analyses used all pairs, including left-handed subjects and pairs discordant for handedness.

Table 2. Phenotypic correlations between FA and the risk for anxiety and depression.

	Subject group			
	Young adult		Middle-aged	
	Female	Male	Female	Male
All subjects	0.12	0.00	-0.09	0.07
Excluding subjects				
with the10% lowest frontal EEG power	0.17*	0.02	-0.04	0.06
with the 20% lowest frontal EEG power	0.20**	0.07	-0.03	0.04
with the 30% lowest frontal EEG power	0.25**	0.07	-0.03	0.12

*$p < .05$, **$p < .01$

Association between FA and the risk for anxiety and depression

Table 2 depicts the correlations between frontal asymmetry and the risk for anxiety and depression for each sex by age cohort group. In addition, it shows the intrapair correlations after removing the subjects with the 10%, 20%, and 30% lowest average frontal power scores (Anokhin et al., 2005). It is clear that the association was significant only in young females. Hence, (bivariate) genetic modeling proceeded in the separate age/sex groups.

Twin correlations and heritabilities for the risk for anxiety and depression

For all four groups variances and means did not differ significantly between MZ twins, DZ twins, and singleton siblings. Likewise, DZ twin, twin-sibling, and sibling-sibling correlations were not found to differ. Further analyses therefore assumed these parameters to be equal, which increases the degrees of freedom.

Table 3 shows the resulting intrapair correlations obtained for the different sex by zygosity groups. Correlations differed between the cohorts, although the effect was rather small given the large sample size ($\chi^2(5) = 15.00$, $p = .010$). Correlations did not differ between the sexes. The intrapair correlations suggested dominant genetic effects as the DZ/sibling correlations were below half the MZ correlations. These effects were significant for young adult males and middle-aged females. Heritabilities were based on the summed additive and dominant genetic effects for these groups. For young adult females and middle-aged males only additive genetic effects contributed to heratibility.

Table 3. *Twin correlations and heritabilities of FA and the risk for anxiety and depression.*

Selection	Zygosity											
	MZM		DZM		MZF		DZF		opposite sex		Male	Female
Cohort	N†	R	N†	R	N†	R	N†	R	N†	R	h^2	h^2
						Risk factor scores						
All questionnaire subjects												
Young	*555*	0.52***	*1231*	0.15***	*938*	0.60***	*1923*	0.29***	*2912*	0.22***	52%***‡	60%***
Middle-aged	*80*	0.62***	*287*	0.23**	*232*	0.57***	*630*	0.14**	*733*	0.12*	61%***	57%***‡
						Frontal asymmetry						
All EEG subjects												
Young	*28*	0.37*	*81*	-0.02	*27*	0.51***	*78*	-0.07	*97*	-0.05	32%*	37%**
Middle-aged	*25*	-0.14	*48*	0.10	*37*	0.28	*83*	-0.10	*97*	0.03	ns	ns
Excluding subjects with the 10% lowest frontal EEG power												
Young	*25*	0.29	*70*	0.40	*26*	0.50***	*63*	-0.04	*85*	-0.09	ns	38%**
Middle-aged	*21*	-0.09	*35*	0.15	*36*	0.27	*64*	-0.11	*73*	0.08	ns	ns
with the 20% lowest frontal EEG power												
Young	*24*	0.24	*61*	0.41	*22*	0.53***	*51*	0.13	*68*	-0.06	ns	43%**
Middle-aged	*19*	-0.18	*25*	0.18	*34*	0.32	*55*	-0.16	*61*	0.22	ns	ns
with the 30% lowest frontal EEG power												
Young	*19*	0.22	*52*	0.35	*17*	0.61***	*38*	0.19	*52*	-0.02	ns	44%*
Middle-aged	*16*	-0.46	*22*	0.25	*28*	0.27	*47*	-0.27	*47*	0.21	ns	ns

Note. MZM=MZ male twins, DZM=DZ male twins and same-sex siblings, MZF=MZ female twins, DZF=DZ female twins and same-sex siblings, OS=opposite sex twins and siblings. DZ correlations and OS entail all fraternal sibling relations and are corrected for intrapair age and sex differences.

†N represents the total sum of all possible sibling pairs, e.g., a family with 1 sister and two MZ brothers has two opposite sex sibling pairs, and one male MZ twin pair. Note that during statistical analyses these sibling relations within a single family were not treated as independent.

‡Heritability (h^2) was modeled including dominent genetic effects (ADE). In all other cases, only additive genetic effects and unique environmental effects were modeled (AE).

**p < .05, **p < .01, ***p < .001, ns: p > .05*

Twin correlations and heritabilities for FA

As with the risk for anxiety and depression, no differences were found in the means and variances between the zygosity groups (MZ, DZ) and in the means, variances and correlations between DZ twins and siblings.

Table 3 shows the sibling correlations for male MZ twins, female MZ twins, male DZ twins plus all other non-identical male-male sibling relations, female DZ twins plus all other non-identical female-female sibling relations, and opposite sex twin and sibling relations. These intrapair correlations are given for the full EEG sample including those subjects without questionnaire data available on any occasion. In the young cohort, DZ/Sibling correlations were less than half the MZ correlations. This pattern of twin correlations suggests the presence of genetic dominance, but in view of the sample size for FA genetic dominance was not explicitly modeled (Posthuma & Boomsma, 2000).

Common environmental variation did not contribute significantly to the observed variation in FA and we proceeded by fitting a model with additive genetic and unique environmental influences only (AE). Under this model, FA heritability was significant only in the younger cohort (32% males; 37% females). These models were refitted after excluding the 10, 20, and 30 percent of subjects that scored lowest on the average of F3 and F4 power. Heritability estimates in the middle-aged cohort remained non-significant. In the young males, evidence for a genetic contribution disappeared.

Genetic and environmental contribution to the association between FA and the risk for anxiety and depression

Bivariate genetic analyses were used to determine whether the observed correlation between FA and the factor score for anxiety and depression is due to genes or environmental factors shared between the two variables. That is, insofar FA and anxiety and depression correlate, how much of that shared variance can be attributed to genetic sources, and how much to environmental sources. Since a significant correlation between FA and the risk for anxiety and depression was only found in young females, we limited the bivariate genetic analysis to this group. As the best fitting models in young females estimated additive genetic and unique environmental effects (AE) on both the risk factor score and FA in the univariate cases, the bivariate models, too, estimated AE effects only.

The results showed that environmental correlations were close to and not significantly different from zero at all selection criteria (all $\chi^2 < 1$, ns). The genetic correlations were not significant in the full sample ($\chi^2(1) = 2.18$, $p = .14$), borderline significant after 10% of the subjects with the lowest frontal alpha were excluded ($\chi^2(1) = 3.54$, $p = .06$), and significant after 20% and 30% of the subjects with the lowest frontal alpha were excluded ($\chi^2(1) > 5.17$, $p < .05$). These results

suggest that the observed correlation between FA and the risk for anxiety and depression in young females can be explained by shared genetic sources and not by an overlap in environmental influences.

DISCUSSION

FA has been put forward as a biological marker for the risk for anxiety and depression (for reviews: Coan and Allen, 2004; Davidson, 1992). Anxiety and depression are heritable disorders and have found to be influenced by overlapping genes (Jardine et al., 1984; Kendler et al., 1986; Middeldorp, Cath, et al., 2005). FA has therefore great potential to be used as a so-called endophenotype in studies searching for genes that influence the shared neurobiological pathways that are affected in these disorders. Two requirements, however, are that FA is heritable and that the genes influencing FA also influence the risk for anxiety and depression. Here we explored this question in male and female twins and their siblings in two different age cohorts.

Our results show that frontal asymmetry was only heritable in young adulthood and was higher in young females (37%) than in young males (32%). FA heritability in young females rose slightly after selecting subjects with sufficient frontal alpha power (Anokhin et al., 2005), whereas in young males heritability disappeared. It may therefore be concluded that heritability is more robust in young adult females. These results are consistent with the previous twin studies on FA in young female adults that reported heritabilities of 33% (Allen et al., 1997) and 31% (Anokhin et al., 2005). The only twin study thusfar to include males (Coan, 2003) found a heritability of 22% in females and no significant heritability in males. With regard to cohort differences, none of these previous studies had included subject groups with mean ages older than twenty-one, so we cannot compare our results in adults to previous work.

The heritability of young adult FA seems rather low, but it must be appreciated that resting FA consists of a mixture of trait and state components (Hagemann, 2004). Using Structural Equation Modeling on data recorded during four recording sessions four weeks apart, Hagemann et al. (2002) estimated about 40% of total FA variance to be state and 60% trait variance. Heritability of FA, therefore, was bound by a maximum of 60%. The contribution of state and trait variance may be unequally distributed across gender. Females may be more reactive to the experimental procedures invoved in the EEG recordings perhaps in interaction with traits that are known to modulate FA, like defensiveness (Kline et al., 1998, 1999). If the EEG recording environment is more anxiogenic in women than in men, and this reactivity is genetically determined, then trait variance in women will show larger heritability estimates.

To index the risk for anxiety and depression we used a factor score obtained from multiple scales at multiple measurement occasions. As reported previously, this factor score is about 60% heritable in both males and females (Boomsma et al., 2000). The relation between the factor score and FA was not significant in older subjects or in young adult males. As with heritability, young females were the positive exception. Only in this group, the relation between FA and the risk for anxiety and depression became significant after excluding subjects with the lowest average frontal EEG power as suggested by Anokhin et al. (2005).

The finding that the relation between FA and anxiety and depression is restricted to females is in keeping with much of the previous literature. Studies that related FA to psychopathology were often limited to female subjects, or included a majority of females in their samples. This can be observed in the exhaustive summary of studies relating FA to psychopathology by Coan and Allen (2004; table 3 in their paper), and has been explicitly noted by others (Miller et al., 2002). Of those that included an adult sample, five report exclusively on females (Allen et al., 1993; Field et al., 2000; Gotlib et al., 1998; Reid et al., 1993; Silva et al., 2002). Seven report on samples with a majority of females (Bruder et al., 2001; Davidson et al., 1985; Debener et al., 2000; Henriques & Davidson, 1991; Nitschke et al., 1999; Schaffer et al., 1983; Wiedemann et al., 1999). Four studies included males and females in about equal proportions (Heller et al., 1997; Miller et al., 2002; Minnix et al., 2004; Tomarken et al., 2004). By contrast, only two studies report on a majority of males (Gilbert et al., 1999; Petruzello & Landers, 1994).

A stronger case for sex differences comes from studies that directly compared results from males and females (Baving et al., 2002; Bruder et al., 2001; Miller et al., 2002; Tomarken et al., 2004). Bruder et al. (2001) found increased right frontal activity only in depressed females not responsive to fluoxetine treatment and no effects in males or responsive females. Miller et al. (2002) found an effect in males opposite that of females, that is, higher left frontal activity for males with family history of depression. Tomarken et al. (2004) found significant interaction effects between depression liability and sex in an ANOVA predicting FA depending on the reference montage: with vertex (Cz) as reference, FA was related to increased liability of depression in females, and not in males. In the field of anxiety, Baving et al. (2002) found greater right frontal activity in anxious 8- and 10-year-old girls and greater left frontal activity in 11-year-old boys. Similar sex differences have been reported in studies investigating defensiveness as measured by the Eysenck L-scale (Kline et al., 1998, 1999). These results, plus the results presented here, provide evidence for sex differences in the relation between FA and anxiety or depression.

The summary of studies on the FA by Coan and Allen (2004) clearly reveals

that while infants, adolescents, and especially young adults have been studied extensively, older adults are underrepresented. Henriques and Davidson (1990), reporting on subjects of 37 years on average, found evidence of group differences between depressed and non-depressed subjects congruent with the FA hypothesis. Baehr et al. (1998) found similar results with a measure related to FA in a sample of 43-57 years. Urry et al. (2004) found that self-reported well-being was related to greater left fronto-central activity in subjects 57-60 years. Kline et al. (1998, 1999) reported similar findings between a young adult and an elderly age group in the relation between FA and defensiveness. Other studies included both younger and older subjects, but did not report their data separately for the age groups (Bruder et al., 2001; Debener et al., 2002; Davidson et al., 2000; Jacobs & Snyder, 1996; Minnix et al., 2004). In contrast to these previous results, the current results showed no evidence of a relation between FA and the risk for anxiety and depression in a large middle-aged Dutch sample. In view of the genetic analyses, this should not be surprising. In the middle-aged cohort variance in FA only reflected the accumulated effects of environmental factors and life events unique to family members. Because the young cohort showed that FA and the factor score were correlated entirely by underlying genetic fators, the lack of a correlation between FA and the risk for anxiety and depression simply may simply reflect the absence of heritable influences on FA in this age range.

In short, the relation between FA and the risk for anxiety and depression is most robust in young females. This relation was fully explained by shared genes influencing both EEG and disease risk. At least in young females, FA may be a valid endophenotype that can support future gene finding for these disorders (de Geus, 2002), provided subjects are selected who have sufficient resting alpha power on the frontal leads. The most striking conclusion deriving from this study may be that future studies on asymmetry of left and right frontal brain activation should carefully consider the effects of both sex and age.

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6

HERITABILITY OF 'SMALL-WORLD' NETWORKS IN THE BRAIN: A GRAPH THEORETICAL ANALYSIS OF RESTING-STATE EEG FUNCTIONAL CONNECTIVITY

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ABSTRACT

Recent studies have shown that resting-state functional networks as studied with fMRI, EEG and MEG may be so-called small-world networks. We investigated to what extent the characteristic features of small-world networks are genetically determined. To represent functional connectivity between brain areas, we measured resting EEG in 574 twins and their siblings and calculated the synchronization likelihood between each pair of electrodes. We applied a threshold to obtain a binary graph from which we calculated the clustering coefficient C (describing local interconnectedness) and average path length L (describing global interconnectedness) for each individual. Modeling of MZ and DZ twin and sibling resemblance indicated that across various frequency bands 46% to 89% of the reliable individual differences in C and 37% to 62% of the reliable individual differences in L are heritable. It is asserted that C, L, and a small-world organization are viable markers of genetic differences in brain organization.

INTRODUCTION

Brain connectivity is likely to have evolved under the constraints of optimized processing capacity while maintaining cost efficiency and resilience to loss of substrate (Bassett & Bullmore, 2006; Achard & Bullmore, 2007). To describe such properties of neural networks, recent studies have applied graph theoretical methods on data obtained with MRI, fMRI, MEG, and EEG (e.g., Achard, Salvador, Whitcher, Suckling & Bullmore, 2006; Bassett & Bullmore; 2006; Micheloyannis et al., 2006a, 2006b; Ponten et al., 2007; Stam, 2004). Graph theory describes mathematical methods applied to representations of networks reduced to their essence: vertices (nodes) and edges (connections). In their ground-breaking article, Watts and Strogatz (1998) calculated two parameters to describe graphs derived from biological as well as non-biological networks. The first describes the amount of local interconnectedness – or cliquishness – called *clustering coefficient C*. It takes a value between 0 and 1 indicating the proportion of neighboring vertices that are interconnected amongst each other. That is, if a neighbor is defined as a vertex that is one step removed, how many of the neighbors of one vertex are not only connected with that vertex, but also with each other. The second parameter describes global interconnectedness and is called the *average path length L*. It is a value simply indicating the average number of steps required to go from each vertex to all others taking the shortest route. Figure 1 shows a graphical explanation of the calculation of C and L.

Watts and Strogatz (1998) showed that C and L parameters represent non-trivial aspects of connection patterns along the dimension ranging from highly ordered graphs (lattices, regular networks) to fully randomized graphs. Ordered

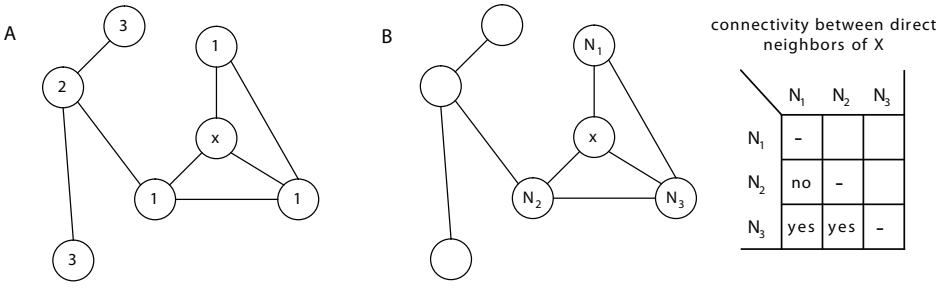

Figure 1. *A:* Average path length L for X is the average number of steps from node X to all other nodes. L_X = (1+1+1+2+3+3)/6 ≈ 1.8.

B: Clustering coefficient C for X describes proportional connectivity between the nodes neighboring node X. C_X = 2 out of 3 possible connections = 0.67.

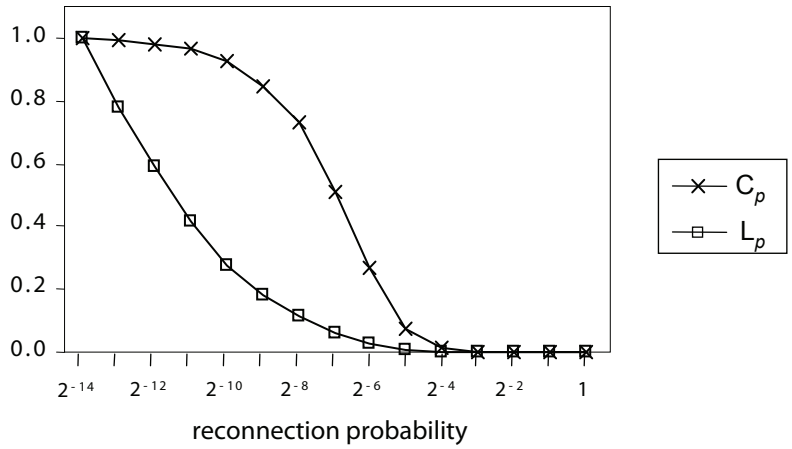

Figure 2. Development of C and L as a function of the probability of randomly rewiring of the edges of a 100 vertex ordered network with degree K=8. Both C_p and L_p are scaled to a minimum of zero (a fully randomized network) and a maximum of one (a fully ordered network).

graphs are characterized by high C and long L. Random graphs have short L and low C. By starting from ordered graphs and randomly reconnecting single edges with a rewiring probability *p*, Watts and Strogatz showed that while the average path length L drops quickly, clustering coefficient C showed resilience against reconnection. Figure 2 shows the development of C and L against reconnection probability for a simulated 100 vertex graph with degree K=8, where K represents the average number of edges per vertex. Ordered graphs, therefore, require only a few random long distance connections to drastically shorten the path length while maintaining a high clustering. Such efficient networks are designated "small-world", referring to the phenomenon that it takes surprisingly few steps to contact any two people in the world (Milgram, 1967) or to connect any actor to Kevin Bacon (wikipedia entry: Six degrees of separation; Bassett & Bullmore, 2006). Many types of existing networks have been shown to possess small-world features, including power grids, the world wide web, and, as indicated, human societies.

In the realm of neural networks, a small-world topology has also been shown to exist in the neural network of *C. elegans* (Watts & Strogatz, 1998), in the brain anatomy in macaque and cat cortex (Hilgetag et al., 2000), and recently also in connectivity derived from cortical thickness measured in humans (He, Chen & Evans, 2007). Besides stationary anatomical connectivity, however, the brain also shows nonstationary *functional* connectivity between brain areas. Due to the high

temporal resolution, EEG and MEG are particularly useful to study this kind of connectivity. Statistical interdependencies between EEG/MEG signals may serve as indices to these temporary connections between brain areas (or 'effective connectivity'; Aertsen et al., 1989). Although coherence is the most widely used *linear* measure of connectivity of EEG/MEG patterns, *nonlinear* measures of coupling may be more appropriate since brain activity is perhaps better modeled as an ensemble of coupled nonstationary, nonlinear dynamical subsystems (Friston, 2000; Pereda et al., 2005). A relatively new measure that captures both the linear as well as non-linear dependencies is Synchronization Likelihood (SL). With this measure, Stam and co-workers (Stam & van Dijk, 2002; Stam et al., 2003; Montez et al., 2006) have found that both linear and nonlinear synchronization are indeed present in normal background EEG/MEG. In addition, they suggested clinical usefulness of SL by showing that synchronization increased during and slightly before epileptic seizures; also, alpha, beta and gamma band SL was decreased in Alzheimer's disease patients (for reviews see: Stam, 2005; 2006).

SL is based upon the concept of generalized synchronization as introduced by Rulkov et al. (1995). Generalized synchronization is said to exist between two dynamical systems X and Y if there exists a continuous one-to-one function F such that the state of one of the systems (the response system) can be mapped onto the state of the other system (the driver system): $Y = F(X)$ (Abarbanel et al., 1996; Kocarev & Parlitz, 1996; Rulkov et al., 1995). Intuitively, this means that generalized synchronization exists between two systems X and Y if the following holds: if X is in the same state at two different times i and j, Y will also be in the same state at times i and j. By deriving the state of the system X from one EEG/MEG signal at a certain time point and the concurrent state of system Y, we can find evidence for connectivity between the brain areas whose activity is reflected in the signals. In sum, SL has the advantage over coherence as it will not show spurious connectivity between bandpass filtered white noise—in which case SL will assume the fixed, predefined value p_{ref}—, and is able to detect complex nonlinear coupling patterns. Detailed calculation procedures are presented in the Methods section.

The result of calculating SL between all possible pairs in a set of EEG/MEG signals can be interpreted as a general (linear and nonlinear) measure of connectivity strength in a functional network of brain areas. By application of a threshold, a sparsely connected graph can be created that is suitable for further graph theoretical analysis as proposed by Watts and Strogatz. Both C and L calculated from these graphs can be interpreted as measures of—local and global—efficiency (Lago-Fernandez et al., 2000; Latora & Marchiori, 2001; Barahona & Pecora, 2002; Masuda & Aihara, 2004). Short L reduces the time or effort needed to connect two vertices, allowing efficient information exchange between, in this case,

more distant brain regions. High C lowers the cost of building and maintaining localized networks and increases error tolerance in the case of loss of connectivity (Bassett & Bullmore, 2006; Achard & Bullmore, 2007). Therefore, these parameters may reflect biologically important characteristics of the network.

The main focus of the current article is to determine whether individual differences in the network properties C and L, derived from resting state EEG functional connectivity, have a biological basis by establishing them as heritable traits. Heritability will be assessed by comparing C and L scores of MZ and DZ twins and their singleton siblings, who, having varying degrees of genetic relatedness, provide information on the amount of variation that can be attributed to genetic or environmental sources of variation (Fisher, 1918; Falconer & MacKay, 1996; Boomsma, Bushjan, et al., 2002).

Since it has been suggested that a small-world network architecture may be optimal for synchronizing neural activity between different brain regions, it seems plausible to hypothesize that individual differences in network properties C and L may be correlated with overall processing capacity or cognitive performance. Indeed, using EEG measured during a working memory task (the 2-back task), higher educated subjects showed less of a small-world phenomenon than subjects with less education with almost the same behavioral performance (Micheloyannis et al., 2006a). We will therefore investigate whether the same relation can be found between 'small-worldness' and measures of general cognitive ability in the context of resting state EEG functional connectivity.

METHOD

Subjects

The sample for this study was derived from an ongoing twin family study on cognition (Posthuma et al., 2001) in twins and family members from the Netherlands Twin Registry (Boomsma, Vink, et al., 2002). Twins and siblings were invited for detailed psychophysiological study in the laboratory. The EEG sample consisted of 760 subjects from 309 families divided into two age cohorts based on the age of the twins: a younger cohort (M = 26.2 years, SD = 4.1) and a middle-aged cohort (M = 49.4 years, SD = 7.2). Participating families consisted of one to seven siblings (including twins). On average, 2.50 participants per family participated. Informed consent was obtained in writing for the EEG study. The study received approval from the appropriate ethical committees.

Intelligence testing

IQ was measured with the Dutch adaptation of the WAISIII-R (WAIS-III, 1997). In accordance with the WAIS guidelines, the following four dimensions were calculated: Verbal Comprehension (the mean percentage correct of subtests information, similarities, and vocabulary), Working Memory (the mean percentage correct of subtests arithmetic and letter-number sequencing), Perceptual Organization (the mean percentage correct of subtests block design, matrix reasoning, and picture completion), and Processing Speed (the number of correct items per 60 seconds of subtest digit symbol substitution). The validity of these four dimensions was confirmed by a reanalysis of the WAIS manual data by Deary (2001). From these dimensions the combined full scale IQ was determined.

EEG recording

The experimental protocol for background EEG registration has been described in detail elsewhere (Posthuma et al., 2001; Smit et al, 2005), but a brief description will be repeated here. EEG was measured at rest. Half of registration sessions were during morning hours, and half were in the afternoon. Subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated, and electromagnetically shielded room. They were instructed to close their eyes, relax, but stay awake and minimize eye and body movement. EEG was registered for three minutes with 17 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS; Enschede, The Netherlands) for 657 subjects (381 young, 280 middle-aged) and NeuroScan SynAmps 5083 amplifier for 103 subjects (24 young, 80 middle-aged). Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2. Standard 10-20 positions were F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2. The vertical electro-oculogram (EOG) was recorded bipolarly between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz. NeuroScan filter settings were a lowpass filter at 50.0 Hz.

EEG Data processing

The signals were recalculated with averaged earlobes (A1 and A2) as reference. All EEG was automatically and visually checked for bad channels such as absence of signal, hum, clipping, persistent muscle tone artifacts, and external noise. Subjects without the full set of 17 leads were excluded. This procedure resulted in the exclusion of 186 subjects leaving 574 subjects. Next, the data were cut into 16 s epochs with 8 second overlap. For each subject, artifactual episodes were identified automatically using the EEGLAB (Delorme and Makeig, 2004) 'reject by threshold' and 'reject by spectra' option. Threshold settings for all leads was +/- 200 μV. The spectral analysis procedure identified deviant epochs by comparing each epoch's power spectrum to the spectrum averaged over all epochs. Epochs with more than 10 dB excess power within the frequency range below alpha (1.0 – 8.0 Hz) or above alpha (13.0 – 30.0 Hz) were marked artifactual. If less than four non-overlapping epochs were available, the (quite strict) criterion of 10 dB was relaxed until exactly four were obtained. The average level of the dB criterion was 16.8 (SD 3.4). No subject reached a criterion level over 36 dB. Visual inspection revealed that this procedure successfully selected epochs without artifacts.

Each epoch was baseline corrected and filtered using theta (4.0 – 8.0 Hz), lower alpha (8.0 – 10.0 Hz), upper alpha (10.0 – 13.0), lower beta (13.0 – 18.0 Hz) and upper beta (18.0 – 25.0 Hz) bandpass filters. Frequencies above 25.0 Hz were disregarded as the discrepancies in hardware filter settings between TMS and Neuroscan registered subjects may lead to spurious results.

Table 1. Synchronization Likelihood parameters per frequency band

band	LF	HF	L	m	W1	W2
theta	4	8	8	9	72	472
lower alpha	8	10	6	6	36	436
upper alpha	10	13	5	6	30	430
lower beta	13	18	3	7	21	421
upper beta	18	25	3	7	16	416

Note. LF = low frequency filter setting. HF= high frequency filter setting. L = Lag. m = embedding dimension. W1 = minimum window distance. W2 = maximum window distance. LF and HF are in Hz, all other parameters in number of samples.

SL calculation

The state of a driver system – here, an EEG signal – is operationalized with the embedded vector $\boldsymbol{X} = \{x_i, x_i+1\cdot L, x_i+2\cdot L, \ldots, x_i+(m-1)\cdot L\}$ where L is the lag and m the embedding dimension. The elements of $\boldsymbol{X}$ are m samples taken from the signal spaced L apart. The vector is taken to represent the state of the system at time i. Within the same signal recurrences are sought at times j that reflect a similar state: A threshold distance ε is chosen such that a fixed proportion (p_{ref}) of comparisons are close enough to be considered in a similar state. Next, the same comparison is made for a different system $\boldsymbol{Y}$ at the same time points i and j and with the same value for p_{ref}. Now the synchronization likelihood S_i between $\boldsymbol{X}$ and $\boldsymbol{Y}$ at time i is defined as follows:

$$S_i = \frac{1}{N}\sum_j \theta(\varepsilon_y - |Y_i - Y_j|)\theta(\varepsilon_x - |X_i - X_j|)$$

where θ is the Heaviside step function returning 0 for all values < 0 and 1 for values $>= 0$. N represents the number of recurrences in signal $\boldsymbol{X}$, i.e.:

$$\sum_j \theta(\varepsilon_y - |X_i - X_j|)$$

Overall SL between $\boldsymbol{X}$ and $\boldsymbol{Y}$ is the average over all possible i. To withhold the system to compare X_i and X_j while they represent the same state, only values for j are considered that are at sufficient time distance. The value of this distance, $W1$, is the Theiler correction for autocorrelation (Theiler, 1986). The value for $|i-j|$ is upper bound to create a window ($W1 < W2 < N$) to sharpen the time resolution of S_i. More details on SL calculation can be found in several other publications (Montez et al., 2006; Posthuma et al., 2005; Stam & van Dijk, 2002). The parameter settings L , m, $W1$ and $W2$ were chosen based on the filter frequency settings. This approach, as put forward in Montez et al. (2006), determines the lag L in sampling the embedded vector on the high frequency parameter of the filter, and the embedding dimension m on the ratio of the high and low frequency parameters. From these, windowing parameters $W1$ and $W2$ are chosen such that embedded vectors are not too close in time to avoid autocorrelation effects, while allowing enough estimations to be made. Table 1 shows the parameter settings for each frequency band. The remaining, free parameters $W2$ and p_{ref} were fixed at fixed values of $W1$+400 and 0.01. These values reflect similar choices from the previous literature (Ponten et al., 2007; Stam et al., 2006). Using data from 51 randomly selected subjects revealed that increasing the value of W2 from 430 to 830 did not change the results in the upper alpha band (r=.99). Increasing p_{ref} from 0.01 to 0.05 also yielded similar results (r=.85), adding to previous observations that variation of p_{ref} yields similar results (Stam & van Dijk, 2002).

C and L calculation

SL was computed between each pair of electrodes resulting in a (17, 17) matrix

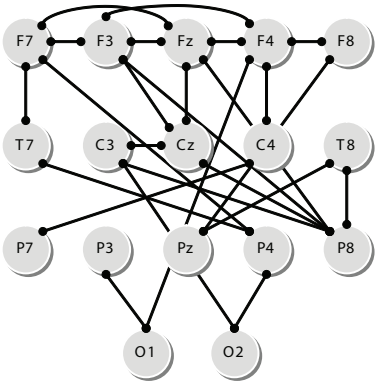

Figure 3. An example of a graph derived from the Synchronization Likelihood matrix for a single epoch.

where the values on the diagonal will be ignored. To correct for individual differences in overall SL this value was subtracted from the matrix of SL connectivity. Using this matrix to represent 'edge strength', a binary graph was formed by applying a threshold θ such that the average number of edges per vertex was fixed at five different levels ($K \in \{3,4,5,6,7\}$). An actual example of a graph extracted from a single epoch is provided in Figure 3.

C and L were calculated as explained in the introduction and indicated in Figure 1 with the following extension. Standard C and L calculation requires that the graph is fully connected (Watts and Strogatz, 1998; Latora and Marchiori, 2001). Many EEG epochs, however, resulted in graphs with at least one vertex unconnected. To accommodate for real world applications where unconnected nodes are unavoidable, we followed Newman's (2003) proposal to assign the value of $+\infty$ to the path length involving unconnected nodes and use the harmonic mean:

$$L = \frac{N}{\sum_{i=1}^{N} \frac{1}{L_i}}$$

For each graph we created 50 randomized graphs by randomly reconnecting edges, preserving the symmetry of the matrix. The average C and L values from these graphs were used to calculate standardized parameters (Humphries, 2006):

$$\gamma = \frac{C}{C_{ran}}$$

and

$$\lambda = \frac{L}{L_{ran}}$$

The small world parameter was then calculated as $\sigma = \frac{\lambda}{\gamma}$. Since C_{ran} and L_{ran} are fixed numbers for graphs with the same degree K, there is no need to repeat the correlational analyses for γ and λ. Therefore, these analyses will be restricted to C, L, and σ.

Reliability and Twin Correlations

For all statistical modeling the freely available software package Mx version 1.65a was used (Neale et al., 2004).

A tetravariate repeated measures structural equation model as depicted in Figure 4 was used, which allows the estimation of the reliability of the measurement. Variance of the four occasions (epochs) is split into a correlated part F (the 'true' scores) and an uncorrelated part U which we may assume represents measurement error. Reliability was then defined as the proportion of non-error variance to the total:

$$R_{epoch} = \frac{f^2}{\left(f^2 + u^2\right)}$$

However, these single-epoch reliabilities should be corrected using the total number of epochs actually measured to represent reliability for the full 4 x 16 s

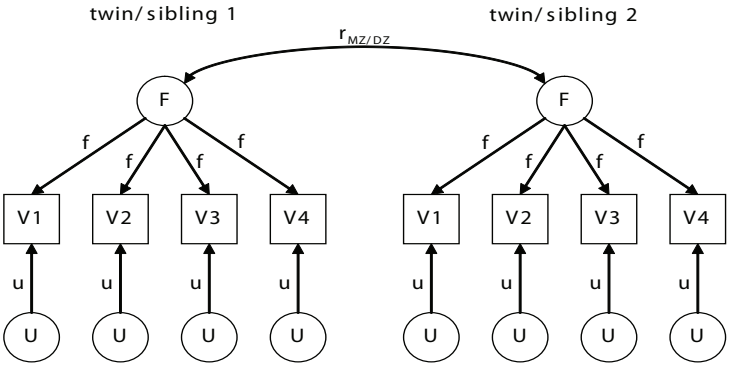

Figure 4. Path model describing the relation between any pair of siblings. V1, V2, V3, and V4 represent the observed variable C, L, or SW from four epochs. Factors U are uncorrelated and represent unreliable factors such as measurement error. F are 'true' factor scores representing the remaining variance. Depending on the relation between the two subjects, an r_{MZ} (MZ twins), r_{DZ} (DZ twins and siblings) is modeled.

duration using the following formula:

$$R_{total} = \frac{kR_{epoch}}{1 + (k-1)R_{epoch}}$$

where k = 4.

The correlation r in Figure 4 will be estimated to have the value r_{MZ} or r_{DZ}, depending on the nature of the relation between the two individuals. For twins and siblings members either an MZ or DZ/sibling correlation will be estimated. Note that the resulting twin correlations represent the relation between 'true' factors F, and are thus corrected for measurement error.

Means were modeled with cohort and sex and the cohort by sex interaction as covariates. Variances were tested for heteroscedasticity between the sexes and the cohorts. When significant, heteroscedasticity was modeled by using a scalar model. These models use a scalar close to 1.0 to equalize the variances in one group (e.g., males) to the other (e.g., females). Significant differences in error variances were modeled similarly.

Genetic analyses

Resemblance (covariance) in psychophysiological traits between twins and siblings derive from genetic relatedness or shared environmental influences (Falconer and Mackay, 1996; Boomsma, Bushjan et al., 2002). If the correlation between DZ twins or siblings, who share on average 50% of their genetic make-up, is half the correlation between MZ twins, who are genetically identical, this is seen as evidence for additive genetic influences (A). If the correlation between DZ twins or siblings is less than half the correlation between MZ twins this is seen as evidence for dominant (non-additive) genetic influences (D). If the correlations between MZ and DZ twins/siblings are comparable and nonzero this is evidence for shared environmental influences (S). If the correlation between MZ twins is not unity this is evidence for environmental effects unique to each individual (E). By comparing MZ and DZ/sibling correlations, using structural equation modeling as implemented in, for example, Mx (Neale et al., 2006), we can obtain maximum likelihood estimates of the relative contributions of each of these factors to the total trait variance. Heritability is defined as the proportional contribution of genetic effects (A + D) to the total variance (A + S + D + E). In a twin-sibling design, however, no information is available to estimate the effects of both S and D simultaneously. The relative size of the DZ/sibling to the MZ correlation guides which is selected. If the DZ/sibling correlation is less than half the MZ correlation, then A + D + E are modeled. If it is more than half the MZ correlation, A + S + E are modeled. For more information on genetic modeling we refer to Boomsma, Busjahn et al. (2002) and Posthuma et al. (2003).

WAIS correlations

Correlations were calculated between C, L, and σ and scores on the four subscales Verbal Comprehension, Working Memory, Perceptual Organization, and Perceptual Speed. Since dependencies exist between family members, statistical inference from straightforward correlations between traits would be incorrect. We therefore modeled the correlations while allowing for within family correlations (i.e., MZ and DZ/sibling correlations, and cross-twin-cross-trait correlations). As with all other modeling, the correlations were modeled on the 'true', non-error variance of C, L, and σ.

RESULTS

Unconnected vertices

It may have been possible that one lead resulted in an unconnected vertex in most subjects, suggesting that this lead should be removed from the analysis. Although T7 and T8 showed quite a high proportion of epochs which resulted in unconnected vertices, still in about 60% of the epochs they were connected. Therefore, we decide not to exclude these leads.

Descriptives

Table 2 shows the descriptives for parameters C and L, and the derived variables γ, λ and σ. Since the distributions for λ were in many cases right skewed, median values are shown to define central tendency. Overall, these parameters are consistent with a small-world organization of the functional network, since λ is relatively close to unity, whereas γ is larger than that (see Table 2). For all levels of K, γ was clearly and significantly larger than unity. However, increasing K resulted in reduced levels of γ (as can be expected). The median value for the variable λ is only slightly larger than unity for higher degrees of K ($K \geq 5$). Lower values of K showed clearer deviation from unity with values above 1.1 in all frequency bands.

Levels of K

To explore the nature of the dependency of the graph variables C and L on degree K we calculated the correlation of the graph descriptors C and L between all levels of K. Next, we averaged the correlations (using the Fisher transform) to obtain marginals representing the strength of correlation between a each level of K with all other levels. Table 3 shows the average correlations for C and L in all frequency bands. For C, the correlations are somewhat smaller than those for L, but still moderate to high (ca. .5 for K=3 but .6 to .7 for other levels). For L, cor-

Table 2. Medians and interquartile ranges for graph theoretical parameters of functional connectivity.

	C		L		γ		λ		σ	
band	**Me**	**Quart. range**	**Me**	**Quart. range**	**Me**	**Quart. range**	**Me**	**Quart. range**	**Me**	**Quart. range**
K=3										
theta	0.34	0.31 - 0.38	2.67	2.49 - 2.92	2.23	2.00 - 2.45	1.20	1.12 - 1.31	1.80	1.66 - 1.97
lower alpha	0.37	0.33 - 0.40	2.90	2.62 - 3.17	2.33	2.12 - 2.55	1.31	1.19 - 1.44	1.74	1.60 - 1.88
upper alpha	0.36	0.33 - 0.39	2.80	2.57 - 3.09	2.30	2.08 - 2.50	1.27	1.17 - 1.40	1.76	1.62 - 1.92
lower beta	0.35	0.32 - 0.38	2.67	2.51 - 2.87	2.20	2.01 - 2.39	1.21	1.14 - 1.30	1.76	1.63 - 1.93
upper beta	0.35	0.32 - 0.38	2.78	2.58 - 3.07	2.23	2.04 - 2.44	1.26	1.17 - 1.39	1.72	1.57 - 1.86
K=4										
theta	0.41	0.38 - 0.44	2.06	1.98 - 2.17	1.78	1.67 - 1.91	1.10	1.06 - 1.16	1.59	1.49 - 1.70
lower alpha	0.44	0.41 - 0.47	2.27	2.09 - 2.50	1.91	1.77 - 2.05	1.21	1.12 - 1.34	1.54	1.43 - 1.66
upper alpha	0.44	0.41 - 0.47	2.19	2.07 - 2.40	1.89	1.75 - 2.03	1.17	1.11 - 1.28	1.55	1.45 - 1.67
lower beta	0.43	0.40 - 0.46	2.13	2.03 - 2.27	1.85	1.73 - 1.98	1.14	1.08 - 1.22	1.59	1.49 - 1.69
upper beta	0.44	0.41 - 0.47	2.24	2.07 - 2.44	1.89	1.74 - 2.01	1.20	1.11 - 1.31	1.54	1.43 - 1.65
K=5										
theta	0.46	0.44 - 0.50	1.76	1.72 - 1.81	1.52	1.44 - 1.62	1.04	1.02 - 1.08	1.44	1.36 - 1.52
lower alpha	0.51	0.48 - 0.54	1.89	1.80 - 2.05	1.67	1.57 - 1.76	1.13	1.07 - 1.22	1.44	1.37 - 1.53
upper alpha	0.51	0.48 - 0.54	1.84	1.77 - 1.98	1.66	1.56 - 1.77	1.10	1.06 - 1.18	1.47	1.39 - 1.55
lower beta	0.50	0.47 - 0.53	1.82	1.74 - 1.92	1.63	1.53 - 1.74	1.08	1.04 - 1.14	1.48	1.40 - 1.55
upper beta	0.51	0.47 - 0.54	1.88	1.79 - 2.02	1.65	1.54 - 1.75	1.12	1.06 - 1.20	1.44	1.36 - 1.51
K=6										
theta	0.50	0.48 - 0.53	1.60	1.59 - 1.63	1.37	1.30 - 1.45	1.02	1.01 - 1.04	1.33	1.28 - 1.39
lower alpha	0.57	0.54 - 0.59	1.69	1.63 - 1.78	1.53	1.45 - 1.61	1.08	1.04 - 1.14	1.39	1.33 - 1.46
upper alpha	0.56	0.54 - 0.59	1.66	1.62 - 1.74	1.52	1.44 - 1.60	1.06	1.03 - 1.11	1.41	1.34 - 1.47
lower beta	0.55	0.53 - 0.59	1.64	1.60 - 1.73	1.50	1.42 - 1.59	1.05	1.02 - 1.10	1.40	1.34 - 1.47
upper beta	0.56	0.53 - 0.60	1.71	1.62 - 1.81	1.52	1.43 - 1.61	1.09	1.03 - 1.16	1.38	1.31 - 1.44
K=7										
theta	0.55	0.53 - 0.57	1.49	1.49 - 1.50	1.25	1.20 - 1.31	1.01	1.00 - 1.01	1.24	1.20 - 1.29
lower alpha	0.62	0.58 - 0.65	1.54	1.50 - 1.60	1.41	1.34 - 1.47	1.04	1.01 - 1.08	1.33	1.28 - 1.38
upper alpha	0.61	0.59 - 0.65	1.52	1.50 - 1.57	1.40	1.34 - 1.47	1.03	1.01 - 1.06	1.34	1.29 - 1.40
lower beta	0.60	0.57 - 0.65	1.51	1.49 - 1.59	1.38	1.30 - 1.47	1.02	1.01 - 1.07	1.32	1.26 - 1.38
upper beta	0.62	0.58 - 0.66	1.56	1.50 - 1.65	1.41	1.33 - 1.50	1.05	1.01 - 1.11	1.32	1.26 - 1.38

relations between levels are high. Degree K=3 seems to correlate the worst with all other levels, whereas K=5 seems to correlate best for both C ($r > .67$) and L ($r > .83$) in all frequency bands.

Overall, K=5 seems to be the best representation of most of the variation shared between all levels of K. In addition, C and L at degree K=5 seems to show small world network properties as mentioned in the previous paragraph. To reduce the

amount of further testing, we chose to restrict the genetic and phenotypic analyses to graphs with degree K=5.

Effects on means and variances

Sex differences in L were found in the lower frequency bands such that males show shorter L (theta: $\beta_{sex} = 0.026$, $\chi^2(1) = 7.70$, $p = 0.006$: lower alpha: $\beta_{sex} = 0.045$, $\chi^2(1) = 8.30$, $p = 0.004$). No other significant mean sex differences were found. The middle-aged cohort showed significantly lower C for the theta band ($\beta_{coh} = -0.011$, $\chi^2(1) = 8.53$, $p = 0.003$), but higher C for upper alpha ($\beta_{coh} = 0.011$, $\chi^2(1) = 8.52$, $p = 0.004$). Middle-aged adults showed longer L in both

Table 3. The correlations between scores on each level of K and scores on all other levels which were averaged using Fisher transform, variables C and L.

		K				
		3 with 4,5,6, and 7	**4 with 3,5,6, and 7**	**5 with 3,4,6, and 7**	**6 with 3,4,5, and 7**	**7 with 3,4,5, and 6**
C						
	theta	0.52	0.59	0.68	0.65	0.63
	lower alpha	0.54	0.64	0.69	0.69	0.63
	upper alpha	0.50	0.60	0.67	0.67	0.63
	lower beta	0.45	0.59	0.69	0.67	0.65
	upper beta	0.52	0.65	0.72	0.71	0.66
L						
	theta	0.67	0.79	0.83	0.81	0.74
	lower alpha	0.78	0.85	0.88	0.87	0.82
	upper alpha	0.79	0.86	0.89	0.88	0.80
	lower beta	0.79	0.87	0.89	0.89	0.84
	upper beta	0.75	0.85	0.89	0.88	0.82

these bands (theta: $\beta_{coh} = 0.028$, $\chi^2(1) = 8.53$, $p = 0.003$; upper alpha: $\beta_{coh} = 0.077$, $\chi^2(1) = 24.4$, $p = 1\times10^{-6}$). In the theta band this resulted in a significantly lower value of the small-world variable sigma for the middle-aged ($\beta_{coh} = -0.029$, $\chi^2(1) = 8.14$, $p = 0.004$).

Variances differed between the sexes only for L in the theta band ($\chi^2(1) = 17.5$, $p = 3\times10^{-5}$). In males 'true' variance was larger than in females. Large differences

Table 4. Reliable, non-error variance as proportion of total variance.

	C	L	σ
theta	0.48	0.71	0.33
alpha low	0.53	0.77	0.41
alpha high	0.52	0.77	0.41
beta low	0.52	0.86	0.41
beta high	0.62	0.87	0.47

Note. For some variable ICCs differed between cohorts and/or sexes due to heterogeneity of variance or heterogeneity of error variance. Average values are shown. Reliabilities are based on the correlations between all epochs, then corrected to represent reliability of the total of four epochs.
C=clustering coefficient
L=average path length
σ=Small World variable γ/λ.

Table 5. Twin correlations for C, L, and σ for graphs with degree K=5

	C		L		σ	
	MZ	**DZ**	**MZ**	**DZ**	**MZ**	**DZ**
theta	0.60	0.18	0.93***	0.12	0.74	-0.01*
lower alpha	0.51	0.10	0.55***	0.14	0.52	0.28
upper alpha	0.49	0.10	0.81***	-0.01*	0.26	-0.22
lower beta	0.66*	0.26	0.49***	0.34	0.58	-0.03
upper beta	0.53*	0.28	0.70***	0.35	0.47	0.14

Note. All correlations were estimated using Mx after removal of age and sex on the means, and significant cohort and sex effects on variance terms by use of a scalar model. DZ correlations include all fraternal (non-identical) siblings pairs, including opposite sex pairs.
C=Clustering coefficient
L=Path length
σ=Small World variable γ/λ.
**=p<.01; **p<.001; ***p<.0001*

in true variance were also found between the cohorts (theta: $\chi^2(1) = 57.0$, $p = 4\times10^{-14}$; upper alpha: $\chi^2(1) = 17.8$, $p = 2\times10^{-5}$), such that the older cohort had larger variances in L than the younger cohort.

Reliabilities

Reliabilities were calculated using the repeated measures model and corrected to represent reliability of the full measurement as specified in the methods. Table 4 shows that the reliabilities were, in general, moderate for C and σ. This indicates

Table 6. Heritabilities for C, L, and σ

	C	L	σ
theta	0.50 *	0.89 *	0.37
lower alpha	0.37	0.46 ***	0.51
upper alpha	0.39	0.78 ***	0.00
lower beta	0.62 **	0.53 ***	0.27
upper beta	0.54 ***	0.70 ***	0.39

Note. Heritability was modeled using additive genetic and unique environmental effects, except for: Dominant genetic effects were significant for theta L and upper alpha L. For these, heritability (h^2) includes both additive and non-additive (dominant) genetic effects.
C = clustering coefficient
L = average path length
σ = Small-World variable γ/λ.
**p<.01 **p<.001 ***p<.0001*

that measurement error covered a substantial proportion of the variance of C (~50%) and slightly more of σ (~65% to 50%). This proportion was larger for the lower frequency bands. The proportion measurement error was markedly less for L than for C and σ. Note that in some cases heterogeneity of variance and/or error variance were found (as aforementioned). Since these differences resulted in different reliabilities for sex or age cohort groups, Fisher-z transformed averages (transformed back) are shown in the Table in those cases.

Genetic analysis

Genetic models included aforementioned significant effects on the means and variances. Men and women and the older and younger cohorts did not differ in MZ and DZ/sibling correlations. The resulting twin correlations – collapsed across cohort and sex – are shown in Table 5 along with 95% confidence intervals.

Although the twin correlations suggested effects of shared environment for theta band L, this effect was not significant. Significant dominant genetic effects were found for L for the theta and upper alpha bands ($\chi^2(1)=8.9$, p=.0003 and $\chi^2(1)=10.1$, p=.002, respectively). For these variables broad heritabilities were estimated, that is, including both dominant and additive genetic variance, and a two degree of freedom test determining the significance of both the effects of A and D simultaneously. All heritabilities are shown in Table 6. Highly significant heritability could be established for L in all frequency bands (lowest significance for theta: $\chi^2(2)=18.5$, $p=1\times10^{-4}$). Significant heritability for C was found in the theta and beta frequency bands (theta: $\chi^2(1)=8.5$, p=.004; lower beta: $\chi^2(1)=14.9$,

$p=1\times10^{-4}$; upper beta: $\chi^2(1)=17.6$, $p=3\times10^{-5}$). Heritability estimates for σwere all positive but did not reach significance.

WAIS correlations

No significant correlations were found between the WAIS subscales Verbal Comprehension, Working Memory, Perceptual Organization, and Perceptual Speed on the one hand and the graph theoretical measures C, L, and σon the other.

DISCUSSION

The results for parameters λ (L/L_{ran}), γ (C/C_{ran}), and $\sigma = \frac{\lambda}{\gamma}$ derived from functional connectivity graphs with degree K = 5 are comparable to those reported in the existing literature and summarized in Table 7. Functional connectivity graphs derived from fMRI, MEG and EEG have shown values for λ in the range of 1.0 to 1.5, but are typically around 1.1 which is highly comparable to our 1.04 to 1.13. Values for γ from the same studies ranged from 1.58 to 3.77. The values reported here (1.52 to 1.67) are at the lower end of this previously reported range, although this seems consistent with the results from the other EEG studies. Small world parameter σin the literature ranged from 1.56 to 2.79. Here, too, our results were at the low end of the dimension with 1.44 to 1.48. However, the small world requirement defined as $\lambda=1.0$ while $\gamma>>1.0$ seems to be met.

Modeling of MZ and DZ twin and sibling resemblance indicated that across various frequency bands 46% to 89% of the individual differences in C and 37% to 62% of the individual differences in L are heritable. Overall, these results suggest that there are consistent individual differences in functional connectivity patterns that have a firm biological basis. Graph theoretical analysis of background EEG connectivity reveals systematic individual differences in average path length L and clustering coefficient C, which makes them viable biomarkers for individual differences in brain functioning. However, unreliable variance constitutes a large part of the total variance of mainly C. If the unreliable variance can indeed be attributed to measurement error, that this finding indicates the importance of measuring many epochs of sufficient length to obtain reliable estimates of individual scores. In the present study, measuring four epochs did not yield sufficient power to establish significance of the heritability estimates for the overall network efficiency as measured with σ.

Since it has been argued that C and L are parameters that describe network efficiency, it was reasonable to hypothesize that efficient connectivity patterns would be related to cognitive performance measures. However, we found that WAIS was not significantly related to either C, L, or small-world parameter σ. Although individual differences in C and L may simply not reflect those in cogni-

tive performance, the absence of significant correlation may also have been the result of suboptimal measurement evidenced by the substantial amount of error variance. In addition, the application of a threshold to the SL matrix resulted

Table 7. Previous findings for l, g, and s from functional connectivity graphs.

study	sample	measurement	condition	λ	γ	σ
current study	*healthy subjects*	*EEG theta, low alpha, high alpha, low beta, high beta, K=5*	*resting state*	*1.09*	*1.63*	*1.45*
Achard et al., 2006	healthy subjects	fMRI .0007-.45 Hz[b]	resting state	1.14	2.18	1.94
Bartolomei et al., 2006	healthy subjects	MEG theta, beta, gamma	resting state	~1.5	~4.0	2.67
Bassett et al., 2006	healthy subjects	MEG 1.1-75 Hz[b]	resting state			1.92
			finger tapping			1.86
Eguiluz et al., 2005[c]	healthy subjects	fMRI	music listening and finger tapping	1.0 - 2.9	168 - 325	
Micheloyannis et al., 2006	schizophrenia	EEG theta, alpha, beta, gamma	WM task	1.15	1.64	1.43
	healthy subjects			1.15	1.80	1.56
Ponten et al., 2007	epileptic patients	Depth electrode EEG, 1 to 48 Hz synchronization	interictal periods	1.1	1.72	1.56
Salvador et al., 2005	healthy subjects	fMRI	resting state	1.09	2.08	1.91
Stam et al., 2004	healthy subjects	MEG theta, gamma[a]	resting state	1.41	3.76	2.68
Stam et al., 2006	Alzheimer's	MEG beta	resting state	1.12	1.6	1.43
	healthy subjects			1.07	1.58	1.48

Results as summarized by the respective authors, except:
[a]Stam, 2004 did not find small-world organization in the alpha and beta frequency bands (omitted here).
[b]No averages reported by the authors, values averaged across frequencies are shown.
[c]The degree K was much smaller than ln(N), which may have caused the anomalous results (see Bassett & Bullmore, 2006).

in the loss of possibly valuable information regarding the connectivity strength between brain areas. To make use of this source of information, alternative approaches for investigating network efficiency to the one presently used have been proposed. For example, Latora and Marchiari (2001) proposed a method to describe local and global efficiency computed from what have been called weighted graphs. It stands to reason that such an approach could result in better estimations of overall network efficiency, and hence, better estimates of phenotypic correlations. Ultimately, though, this remains an empirical question. Another possible source of bias is that volume conduction in the EEG signals could have falsely increased connectivity strengths of nearby electrodes. Therefore, recently proposed measures that take volume conduction into account, such as the phase lag index (Stam et al., 2007), could possibly alleviate this problem. Lastly, using a single degree K for all individuals may have disregarded 'true' differences between subjects, that is, some subjects may simply have a more sparsely connected functional network. Again, this too remains an empirical question.

In conclusion, graph theory is a useful tool for describing functional connectivity of the brain. The pattern of twin correlations for C, L, and σestablish them as viable markers of genetic differences in brain organization.

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7

GENETIC CONTRIBUTIONS TO LONG-RANGE TEMPORAL CORRELATIONS IN ONGOING OSCILLATIONS

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ABSTRACT

The amplitude fluctuations of ongoing oscillations in the electroencephalographic (EEG) signal of the human brain show autocorrelations that decay slowly and remain significant at time scales up to tens of seconds. We call these long-range temporal correlations (LRTC). Abnormal LRTC have been observed in several brain pathologies, but a biological basis has not been firmly established. We recorded the ongoing EEG during eyes closed rest in 390 monozygotic and dizygotic twins and investigated the temporal structure of ongoing oscillations in the alpha and beta frequency bands using detrended fluctuation analysis (DFA). The strength of LRTC was more highly correlated in monozygotic than in dizygotic twins. Genetic modeling attributed up to ~60% of the variance in DFA to genetic factors, indicating a high heritability for the temporal structure of amplitude fluctuations in EEG oscillations. Importantly, the DFA and EEG power were uncorrelated and had a different genetic architecture; DFA was influenced by nonadditive genetic influences whereas power showed only additive influences. LRTC in ongoing oscillations are robust, heritable, and independent of power, suggesting that LRTC and oscillation power are governed by distinct biophysical mechanisms and serve different functions in the brain. We propose that the DFA method is an important complement to classical spectral analysis in fundamental and clinical research on ongoing oscillations.

INTRODUCTION

Oscillations are ubiquitous in neuronal systems and are believed to play an important role for working memory or neuronal representations (Engel et al., 2001; Varela et al., 2001; Buzsaki, 2006). The phase of network oscillations is known to bias the spike timing of individual neurons, which may mediate coordinated activity in spatially distributed networks with millisecond temporal resolution (Laurent, 1996; Fries, 2005). Without an oscillation, however, there is obviously no phase, suggesting that the amplitude modulation of oscillations may be equally important for coordinated neuronal activity (Canolty et al., 2006). Indeed, parametric increases in the amplitude and the duration of oscillatory activity in the theta, alpha and beta frequency bands have been observed in frontal, temporal and parietal regions during mnemonic and attentional tasks lasting up to 10 seconds (Raghavachari et al., 2001; Jensen & Tesche, 2002; Jensen & Lisman, 2005).

These findings suggest that the stability of oscillations or their slow amplitude modulation may be critical for cognitive processes that require "binding" of temporally distributed activity on time scales of several seconds (Linkenkaer-Hansen et al., 2005). Stably oscillating networks, however, may not possess the flexibility that is required for swiftly adapting to new tasks or behaviors (Chialvo, 2007). Several groups have suggested that the optimal compromise between uncorrelated neuronal activity with no memory and strongly correlated activity patterns with little flexibility may be found in a so-called critical state (Chialvo & Bak, 1999; Linkenkaer-Hansen et al., 2001; Beggs & Plenz, 2003; Freeman, 2004; Drew & Abbott, 2006; Kinouchi & Copelli, 2006; Plenz & Thiagarajan, 2007). The critical state is characterized statistically in terms of powerlaw decaying correlations in space and time (Bak et al., 1987).

Several groups have observed that amplitude fluctuations in ongoing neuronal oscillations are characterized by slowly decaying autocorrelations of a powerlaw form on time scales from seconds to minutes in humans (Linkenkaer-Hansen et al., 2001; Linkenkaer-Hansen et al., 2004; Nikulin & Brismar, 2004; Nikulin & Brismar, 2005) and monkeys (Leopold et al., 2003). Clinical studies have observed changes in LRTC in distinct brain regions and in epilepsy (Parish et al., 2004; Monto et al., 2007), and major depressive disorder (Linkenkaer-Hansen et al., 2005).

In view of the accumulating evidence that amplitude fluctuations in ongoing oscillations carry valuable information about the state of the underlying neuronal networks, it is important to gain a better understanding of the factors that may cause subjects to differ in this temporal structure. Here we quantify the LRTC in EEG alpha and beta oscillations on time scales from 1 to 20 s using detrended

fluctuation analysis (DFA) and determine the genetic contribution to LRTC using the twin design (Boomsma et al., 2002). The results indicate that the amplitude dynamics are highly heritable and that the biological mechanisms underlying LRTC are distinct from those that determine the oscillation power. The findings demonstrate unambiguously that the amplitude modulation in ongoing oscillations cannot be explained by uncontrolled experimental variables, but rather reflect genetically determined physiological parameters that influence the intrinsic dynamics of neuronal networks.

MATERIALS AND METHODS

Subjects and data acquisition.
Dutch twins (n = 390, 16.5–19.5 years, 194 females) were drawn from a community based sample of subjects participating in a large epidemiological study of health related behaviors and measured with EEG at the Psychophysiology Laboratory at the Vrije Universiteit Amsterdam, The Netherlands. Zygosity was determined by genotyping for 114 same sex twins and by a questionnaire completed by the mother of the twins. Agreement between zygosity diagnoses based on questionnaire and genotyping was 95%. Previous papers have investigated these subjects in order to estimate the genetic influence on EEG coherence and the P300 component (van Beijsterveldt et al., 1998; van Beijsterveldt et al., 2001).

The spontaneous EEG was recorded during eyes closed rest for 3 min (n = 332) or 4–6 min (n = 58). Brain electric activity was recorded using Electrocap electrodes placed according to the international 10–20 system (n = 14 electrodes). Linked earlobes were used as references according to the method described in Pivik et al. (Pivik et al., 1993), but re-referenced offline to common average electrode. Electrode impedances were kept below 5 kOhm, the sampling rate was 250 Hz and the data were bandpass filtered at 1–35 Hz. Eye movements were recorded with the horizontal and vertical electro-oculograms.

Artifact Rejection
Independent component analysis (ICA) as implemented in the EEGLAB toolbox was performed in order to identify maximally independent processes in the EEG data (Delorme and Makeig, 2004). Independent components corresponding to eye movements or blinks, heart beat or breathing were manually identified based on their characteristic scalp maps and spectral signatures, and projected out of the data (Jung et al., 2000). Finally, the data were inspected for transient artifacts, e.g., muscle movements, and these were manually removed. Generally, only a small amount of data (< 10 s) was lost from removing periods of nonperiodic artifacts.

Four subjects were excluded from further analysis because of severe muscle ar-

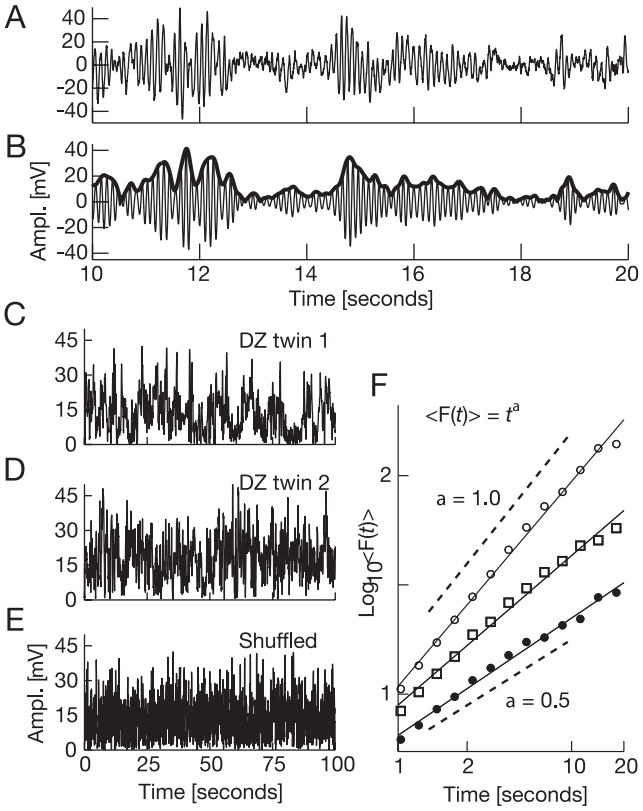

Figure 1. From broadband EEG to the DFA of narrow-band ongoing oscillations. A 10-s segment of EEG from occipital electrode O2 showing alpha oscillation bursts before (A) and after (B) band-pass filtering in the alpha-frequency range (8–13 Hz). We analyze the temporal structure of the amplitude envelope of the oscillation, which is indicated with a thick line in (B). Ongoing oscillations in the human EEG generally exhibit long-range temporal (auto-)correlations (LRTC), which is identified qualitatively as a large variation in the duration and magnitude of the amplitude envelope over time as seen in the dizygotic (DZ) twin siblings 1 and 2 (C,D). The temporal structure and correlations of the signal in (C) may be removed by randomly shuffling the signal in windows of 100 ms (E). The DFA exponent, a, provides a quantitative measure of LRTC, and the stronger correlations in DZ twin 1 (F, circles) compared with DZ twin 2 (F, squares) is reflected in a value of a closer to 1 (0.94 vs 0.68). The lack of temporal structure and correlations in (E) is reflected in the DFA exponent having the value of ~0.5, which is characteristic of an uncorrelated random process (F, dots).

tifacts and 7 subjects were identified as outliers because of having DFA exponents higher than 1.05 in the alpha band. Whenever a subject was removed from the database, so was the twin brother/sister. The final data comprised 80 monozygotic (MZ) and 104 dizygotic (DZ) twin pairs (n = 368 subjects in total).

Analysis of oscillation power and long-range temporal correlations.
We analyzed the amplitude fluctuations of alpha (8–13 Hz) and beta (15–25 Hz) oscillations. The extraction of the instantaneous amplitude of the oscillations was performed by bandpass filtering and subsequent computation of the analytic signal based on the Hilbert transform (Nikulin & Brismar, 2005). We used finite impulse response (FIR) filters with a Hamming window and filter order of 58. The oscillation power was determined as the squared mean amplitude after bandpass filtering and Hilbert transform.

The temporal correlations of the amplitude fluctuations in the time range from 1 to 20 s were quantified using detrended fluctuation analysis (DFA; Peng et al., 1994; Peng et al., 1995). The DFA measures the scaling of the root-mean-square fluctuation of the integrated and linearly detrended signals, F(t), as a function of time window size, t. For signals that are uncorrelated or have persistent power-law correlations, the average fluctuation $<F(t)>$ is of the form $<F(t)> = t^{\sigma}$, where $\sigma$is the DFA scaling exponent. If $0.5 < \sigma \leq 1.0$, this indicates power-law scaling behavior and the presence of temporal correlations, whereas $\sigma = 0.5$ indicates the ideal case of an uncorrelated signal. Details on the temporal correlation analysis of the amplitude modulation of ongoing oscillations have been published previously (Linkenkaer-Hansen et al., 2001; Linkenkaer-Hansen et al., 2004). The major steps from broadband EEG data to the DFA exponent of amplitude fluctuations are shown in Figure 1.

We also performed autocorrelation function (ACF) and power spectral density (PSD) analyses to confirm that these classical techniques agreed with the slow power-law decay of correlations as indicated by the DFA (Fig. 2). The PSD of the amplitude envelope of the oscillations was determined by means of the Welch technique with the Hamming window; it reveals the contribution of different frequencies to the total power of the signal. Uncorrelated so-called "white noise" signals contain equal power at all frequencies, whereas long range correlated signals have log-log linear power spectra with a nonzero power-law exponent β (a so-called $1/f\beta$ type signal). Periodic signals have peaks in the spectrum at frequencies that are inverses of these periods. Thus, in our analysis of the amplitude envelope, a peak would point to oscillations having a characteristic scale of modulation (e.g., (Linkenkaer-Hansen et al., 2004)). The autocorrelation function (ACF) gives a measure of how a signal is correlated with itself at differenttimelags. A normalized ACF attains its maximum value of one at zero time

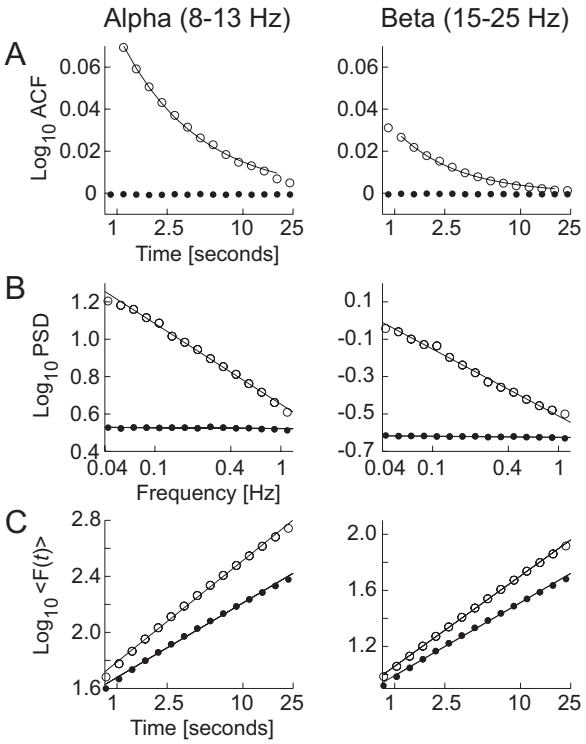

Figure 2. Power-law decaying correlations in the amplitude fluctuations of alpha- and beta-frequency band oscillations. Three complementary autocorrelation analyses were performed: the autocorrelation function (ACF, A), the power spectral density (PSD, B), and the detrended fluctuation analysis (DFA, C). Each analysis was performed on the amplitude envelope of alpha and beta oscillations and averaged across the 368 subjects and 14 electrodes (open circles, see Materials and Methods). The data have been fitted with a power-law function on time scales from 1 to 20 s (black lines). The ACF is plotted semi-logarithmically, because the autocorrelation function can attain negative values; PSD and DFA are plotted in log-log coordinates. Each analysis points to a slow decay of correlation or LRTC. The dots indicate the analysis of computer-generated white noise that is filtered identically to the EEG data. The auto-correlation function is zero at all time lags, the power spectrum is flat and the DFA exponent is close to 0.5 for both frequency bands, showing that the band-pass filters did not introduce autocorrelations in the amplitude time series on the long time scales investigated here.

lag, decays towards zero with increasing time lag for finite correlated signals, and fluctuates close to zero at time lags free of correlations. Signals that are modulated at a characteristic scale produce autocorrelation functions that are also modulated with the period of the characteristic scale. DFA provides a more robust estimate of the decay of correlations with increasing time scales than the ACF or PSD analyses and is less sensitive to the often apparent non-stationarity of electrophysiological time series (Rangarajan & Ding, 2000; Kantelhardt et al., 2001). Therefore, we used the power law exponent from DFA to quantify the LRTC in individual subjects and channels.

Maximum likelihood estimations of phenotypic correlations were computed between DFA exponents and power for each EEG lead (n = 14) and frequency band (n = 2). Because of the many tests performed, the significance level was set to 0.01.

Influence of signal-to-noise ratio on DFA exponents.
It has been shown previously that the amplitude envelope of narrow bandpass filtered electromagnetic laboratory noise is temporally uncorrelated on time scales from 1–20 s (Linkenkaer-Hansen et al., 2001). It is therefore expected that the strength of long range temporal correlations is underestimated in ongoing oscillations that have a low signal-to-noise ratio (SNR). To estimate the SNR at which the bias of the DFA exponent becomes significant, we performed simulations on correlated signals with different amounts of uncorrelated noise added. The SNR was defined as the ratio between the mean amplitude of correlated and uncorrelated signals. It was found that signals with DFA exponents of ~0.7 become significantly underestimated—and thus biased by the uncorrelated noise—when the SNR < 1, whereas SNR > 2 was sufficient for an accurate estimation of the DFA exponent (data not shown). From the large variation in oscillation power among the individual subjects (Fig. 3A) it is evident that not all subjects have ongoing oscillations with a high SNR in all frequency bands and electrode locations. As a conservative estimate of the level of background noise in a given frequency band in our EEG laboratory, we chose the power from the subject with the smallest signal at F3 (generally the electrode with the smallest signal). All analyses related to DFA exponents have been tested for their robustness against excluding channels that did not meet the criterion of a SNR > 2.

Statistical genetic analysis. A twin design is used to partition the variance of EEG measures into additive genetic (A), nonadditive genetic (D), and common environmental effects (C) (Falconer & MacKay, 1996; van Beijsterveldt & van Baal, 2002; Posthuma et al., 2003). The contribution of each of these factors can be inferred from the size of the DZ correlation relative to the MZ correlation: If the DZ correlation equals the MZ correlation this evidences C, if it equals

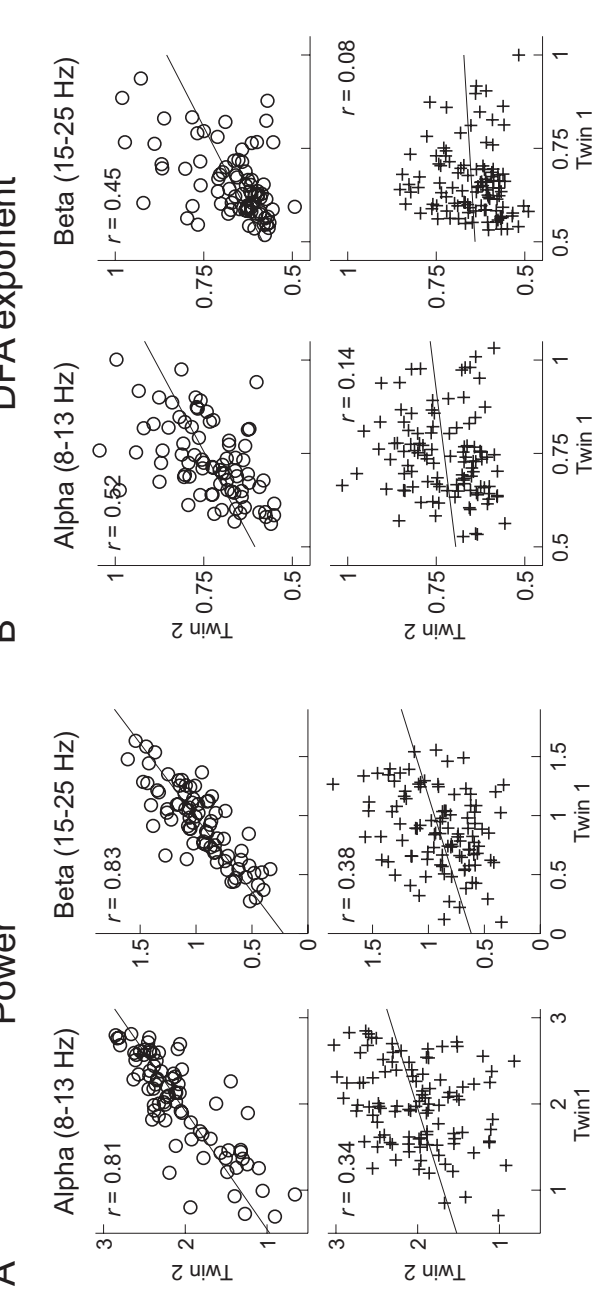

Figure 3 Genetic factors shape the power and the temporal correlation structure of ongoing oscillations. The scatter plots show the logarithmically transformed power (A) and the DFA exponents (B) of MZ (circles, n = 80 pairs) and DZ (plusses, n = 104 pairs) twin pairs for alpha and beta at occipital electrode 'O2'. That both power and DFA of alpha and beta oscillations are heritable traits is indicated by the more than two times higher correlation between MZ twins than between DZ twins. Twin correlations, r, are indicated in the plots.

half the MZ correlation this evidences A, and if it equals one fourth the MZ correlation this evidences D. In between levels of the DZ correlation are modeled by a linear admixture of either A and D (for values lower than half the MZ correlation) or A and C for values larger than half the MZ correlation. Residual variance between MZ twins left unexplained by A, D, and C is called unique environment (E) and includes effects such as measurement error. Heritability is defined as all genetic variance (A+D) divided by the total variance (A+D+C+E). We used structural equation modeling package Mx (Neale MC et al., 2003) to obtain likelihood estimates of models with the relative contributions of the factors A, D, and E to the total trait variance. Significance of the heritability can be tested by the subsequent calculation of the likelihood of a model with the influence of the factors of interest (A and D) constrained to zero. Sex was included as a covariate; however, the age was not, because of the narrow age range in our subjects (16.5–19.5 years).

RESULTS

Power-law decay of autocorrelations in the alpha and beta-frequency bands

The amplitude envelope of alpha and beta oscillations was extracted using band-pass filtering and the Hilbert transform (Fig. 1AB, Materials and Methods). The temporal structure of fluctuations was observed to exhibit complex fluctuations over time and varied across subjects (Fig. 1C,D), suggesting that the decay of autocorrelations is to some extent individually determined. At the group level and averaged across EEG leads, the autocorrelation function (ACF), power spectral density (PSD), and detrended fluctuation analysis (DFA) all indicated that temporal correlations of the amplitude fluctuations of ongoing oscillations exhibit a slow and smooth decay governed by a power law (Fig. 2). This finding is in agreement with previous studies (Linkenkaer-Hansen et al., 2001; Nikulin & Brismar, 2004; Parish et al., 2004; Monto et al., 2007), albeit that the duration of the EEG recordings in the present study only allowed an investigation of LRTC on time scales up to 20 s (~1/10 of the data length). The ACF or PSD analyses of individual subject data sets provide a noisy estimate of the decay of correlations with increasing time scales compared to DFA (Kantelhardt et al., 2001). The power-law exponent from DFA, by contrast, provides a robust index of the strength of long range temporal (auto) correlations and, therefore, was used to quantify the LRTC in individual subjects and channels in the remaining analysis.

Genetic factors shape the complex time structure of ongoing oscillations

Comparing the resemblance between MZ twins for a trait with the resemblance between DZ twins offers a powerful means of estimating the extent to which

Table 1. Results related to oscillation 'power' and 'DFA' exponents in the alpha and beta bands at all electrode locations. MZ and DZ twin correlations are denoted r(MZ) and r(DZ), respectively. The heritabilities (h2) of power represent additive genetic effects because r(MZ) ~ 0.5□r(MZ), those of DFA exponents represent both additive and nonadditive genetic effects because r(DZ) < 0.5□r(MZ). Additive effects represent the summation of genetic effects over all contributing loci, nonadditive effects (genetic dominance) represent interactions between alleles within, and possibly across loci. Both were based on the univariate model. The r indicates the phenotypic correlation between power and DFA exponents as given by maximum likelihood estimations. The rightmost three columns indicate number of subjects (n) that pass the SNR criterion at a given electrode and the resulting heritability and phenotypic correlation from this subset of subjects. Significance levels as derived from likelihood ratio tests are: * < 0.01; ** < 0.001.

		All subjects (N=368)							selected subjects	
		Power			DFA			Power--DFA	DFA	Power--DFA
Alpha		r(MZ)	r(DZ)	h^2	r(MZ)	r(DZ)	h^2	r	N	h^2	r
	fp1	0.82	0.44	*82%* **	0.60	0.11	*60%* **	0.1	257	*57%* **	0.07
	fp2	0.81	0.39	*81%* **	0.58	0.14	*58%* **	0.11	256	*56%* **	0.09
	F3	0.83	0.42	*83%* **	0.56	0.16	*56%* **	0.13	270	*49%* **	0.06
	F4	0.86	0.35	*86%* **	0.51	0.15	*51%* **	0.17 *	283	*49%* **	0
	F7	0.80	0.41	*80%* **	0.43	0.16	*43%* **	0.11	235	*44%* *	0.11
	F8	0.81	0.33	*81%* **	0.52	0.15	*52%* **	0.13	247	*52%* **	0.07
	C3	0.83	0.47	*83%* **	0.41	0.16	*41%* **	0.18 **	274	*34%*	0.03
	C4	0.85	0.45	*85%* **	0.44	0.19	*44%* **	0.13	283	*31%*	0.04
	P3	0.86	0.40	*86%* **	0.54	0.14	*54%* **	0.12	308	*48%* **	0.09
	P4	0.85	0.44	*85%* **	0.52	0.17	*52%* **	0.06	322	*48%* **	0.13
	O1	0.86	0.33	*86%* **	0.53	0.11	*53%* **	0.02	349	*51%* **	0.04
	O2	0.81	0.34	*81%* **	0.51	0.14	*51%* **	0.03	354	*49%* **	0.02
	T5	0.80	0.37	*80%* **	0.46	0.13	*46%* **	0.02	303	*34%* *	0.12
	T6	0.85	0.51	*85%* **	0.38	0.14	*38%* **	0.07	334	*33%* *	0.18 *
Beta											
	fp1	0.62	0.37	*62%* **	0.44	0.13	*44%* **	0.04	181	*39%*	0.03
	fp2	0.60	0.41	*62%* **	0.40	0.25	*43%* **	0.02	192	*19%*	0.04
	F3	0.74	0.38	*74%* **	0.50	0.15	*51%* **	0.23 **	269	*43%* *	0.14
	F4	0.75	0.26	*75%* **	0.45	0.20	*45%* **	0.32 **	297	*44%* **	0.22 **
	F7	0.39	0.32	*42%* **	0.32	0.14	*33%* *	0.03	170	*63%* *	0.03
	F8	0.54	0.26	*54%* **	0.37	0.18	*37%* **	0.11	222	*54%* **	0.07
	C3	0.80	0.33	*80%* **	0.50	0.07	*50%* **	0.24 **	251	*43%*	0.07
	C4	0.83	0.35	*83%* **	0.48	0.19	*48%* **	0.23 **	283	*45%* **	0.13
	P3	0.86	0.33	*86%* **	0.54	0.11	*54%* **	0.12	294	*45%* **	0
	P4	0.83	0.40	*84%* **	0.45	0.17	*47%* **	0.11	309	*43%* **	0.01
	O1	0.83	0.34	*83%* **	0.48	0.00	*47%* **	0.02	347	*46%* **	0.03
	O2	0.83	0.38	*83%* **	0.45	0.08	*44%* **	0.03	353	*44%* **	0.02
	T5	0.72	0.30	*72%* **	0.49	0.11	*49%* **	0.04	293	*41%* *	0.13
	T6	0.83	0.47	*83%* **	0.39	0.14	*39%* **	0.05	320	*36%* *	0.13

genetic variation determines phenotypic variance of that trait (Boomsma et al., 2002). In agreement with previous reports, we observed that the power of alpha and beta oscillations was highly correlated between genetically identical twins and about twice as strongly correlated as between fraternal twins (Fig. 3A, Table 1), suggesting strong additive genetic influences on these traits (van Beijsterveldt & van Baal, 2002). MZ correlations of DFA exponents of alpha and beta oscillations (Fig. 3B, Table 1) were also significantly larger than zero indicating that LRTC is heritable, albeit slightly less than power in the same frequency bands. Importantly, the twin correlations were largely the same when the analysis was performed only on the subjects and channels with a high SNR and heritability estimates remained relatively high (~50%, Table 1).

DZ twin correlations of DFA were less than half the MZ correlations, indicating that non-additive genetic factors contributed to individual variation in LRTC. This is in contrast to power, which generally show MZ twin correlations about half that of MZ twin correlations (Table 1), and suggests that power and DFA have different genetic architectures. The shared rearing environment played no significant role in either power or DFA. The deviation of heritability from unity can be attributed to unique environmental factors, measurement noise and uncertainty of DFA estimates caused by the finite amount of data. Our data only allowed for nine independent segments of the longest time scale included in the DFA (20 s), compared with ~1800 independent oscillation cycles of alpha for the power analysis, which suggests that the heritability of DFA has been underestimated relatively to power (van Beijsterveldt and van Baal, 2002). To illustrate this effect, we tested the heritability of alpha power based on a 2second epoch (~20 cycles) as opposed to the original ~180 s. This decreased the heritability for alpha power from an average across leads of 83% to 71%, which is not significantly different from DFA heritability.

Conspicuous genetic influences on the temporal structure of oscillations

Small yet significant differences in DFA exponents are known to be difficult to recognize upon visual inspection (Havlin et al., 1999; Linkenkaer-Hansen et al., 2005; Monto et al., 2007). In our data, however, subjects spanned a broad range of DFA values, and clear differences in the temporal structure of amplitude fluctuations could be identified when comparing subjects with small vs. high DFA exponents (Figure 4). Moreover, irrespective of whether the ongoing oscillations were characterized by low or high power, or small or large DFA exponents, monozygotic twin pairs clearly had qualitatively similar temporal fluctuation patterns (Figure 4). This finding provides additional support for the large genetic influence on the stability and variability of ongoing oscillations.

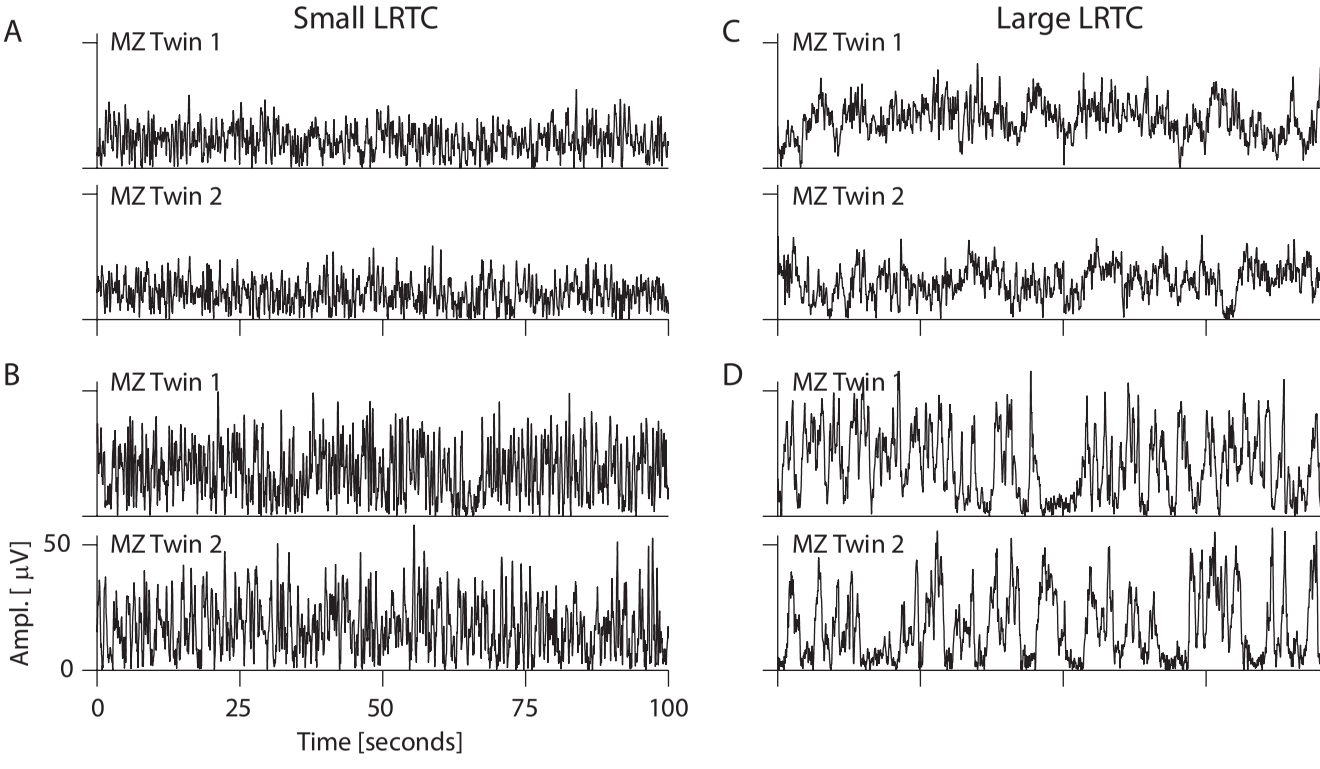

Figure 4. Monozygotic twins have similar temporal structure of amplitude fluctuations in ongoing oscillations. The amplitude envelopes of alpha oscillations at O2 are plotted for two representative twin pairs with small (A, B) and two twin pairs with large (C, D) DFA exponents. The large DFA exponents in C and D can be recognized qualitatively as longer contiguous periods of elevated activity and less erratic fluctuations on short time scales compared with the subjects in A and B.

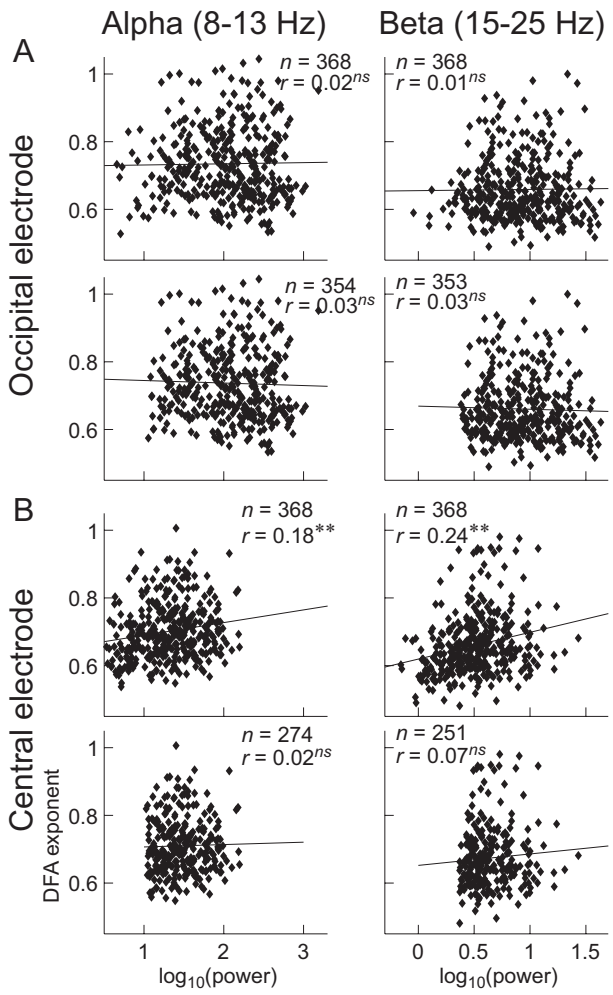

Figure 5. Genetic variances of DFA and power in ongoing oscillations are independent. There are no correlations between DFA exponents and power of ongoing alpha and beta oscillations at occipital electrode O2 (A), either before (upper plots) or after (lower plots) removing the subjects with a low SNR. (B) At the central electrode C3, one may get the impression that DFA and oscillation power are correlated (upper plots). However, after removing 94 and 117 subjects with a low SNR in the alpha and beta band, respectively, we recover the non-significant zero correlation, which was observed also in occipital and parietal leads with a high SNR. The number of subjects included in each scatter plot is indicated with 'n'. Significance levels of Pearson's coefficients of correlation: ns > 0.05; ** < 0.001.

DFA and power are uncorrelated

To determine whether the DFA exponents and the power of oscillations reflect overlapping aspects of brain function, we correlated the two measures across subjects for each channel. Channels with a high signal-to-noise ratio (SNR) in the alpha- and beta-frequency band, e.g., the occipital and parietal leads, had correlations between DFA exponents and power that were close to zero and nonsignificant (Figure 5A). In central and frontal scalp regions, a weak and positive correlation was observed between DFA exponents and power (Figure 5B, Table 1). Except for beta oscillations at lead F4, these correlations were, however, not robust against exclusion of those channels in each subject that did not meet

the SNR criterion (Figure 5B, Table 1, cf. Materials and Methods). These results suggest that the variances in LRTC and power of alpha and beta oscillations are in general truly independent.

DISCUSSION

We investigated genetic contributions to long-range temporal correlations in the amplitude fluctuations of ongoing EEG oscillations in the alpha and beta frequency bands during eyes closed rest in monozygotic and dizygotic twins. The LRTC as characterized by DFA were heritable at all scalp locations in the range of 33–60%, whereas oscillation power had heritabilities of 42–86%. Importantly, the variances in DFA exponents and power were uncorrelated and the difference between DZ and MZ twin correlations was relatively larger for DFA than for oscillation power. The results establish that LRTC have a firm biological basis. Furthermore, LRTC and oscillation power have a different genetic architecture and, thus, may be governed by distinct biophysical mechanisms and serve different functions in the brain.

Genetic influences on oscillation power and LRTC

In some subjects and at some scalp locations, non-invasively recorded ongoing oscillations approach the level of the background noise in the laboratory. In those cases, the DFA exponent will be biased towards the uncorrelated temporal structure of bandpass filtered electromagnetic noise (Linkenkaer-Hansen et al., 2001). We therefore tested the robustness of all analyses against the exclusion of channels in individual frequency bands and subjects that did not have a high SNR (see Materials and Methods). In the case of genetically determined LRTC that is independent of power, one would expect the estimated heritability to be influenced only marginally by subject selection based on amplitude. Moreover, phenotypic correlations between power and DFA are expected to disappear when the SNR bias of DFA is removed through the subject selection. This was indeed observed (Table 1), indicating that the DFA exponent may capture complementary effects to those revealed by spectral analysis of ongoing oscillations.

It has been shown previously that LRTC, as defined by a slow power-law decay of temporal autocorrelations, may also be identified with the classical autocorrelation function or power spectral density applied to the amplitude envelope of the oscillations (Linkenkaer-Hansen et al., 2001; Linkenkaer-Hansen et al., 2004). We confirmed this at the group level (Fig. 2); however, the 3-minute recordings were insufficient for a stable estimation of the power-law exponent of the ACF and PSD in individual subjects and channels. Therefore, the heritability analysis was restricted to the index of LRTC obtained with the DFA, which is

well-known to provide a robust estimate of correlations in finite data (Rangarajan and Ding, 2000; Gao et al., 2006). In fact, whereas previous reports on LRTC have relied on 15-minute recordings or more (Linkenkaer-Hansen et al., 2001; Leopold et al., 2003; Linkenkaer-Hansen et al., 2004; Nikulin & Brismar, 2004; Parish et al., 2004; Linkenkaer-Hansen et al., 2005; Nikulin & Brismar, 2005; Monto et al., 2007), the current study clearly documented that an informative analysis of temporal correlations up to 20 seconds can be performed on the basis of 3-minute EEG recordings, which are often used in clinical studies.

Heritability estimates of oscillation power in our study were in close agreement with previous reports (van Beijsterveldt & van Baal, 2002; Smit et al., 2005; Smit et al., 2006) and very high (42–86%). The heritability of the DFA exponents (33–60%) were comparable to that of biomarkers of human brain function such as the P300 component (van Beijsterveldt & van Baal, 2002) or complexity measures derived from nonlinear dynamic systems theory (Anokhin et al., 2006; Posthuma et al., 2005). The heritability of power was significantly higher than that of DFA exponents; however, this may in part be related to finite data effects and the inherently different amounts of data required for the two analyses (~180 s of data for temporal correlation analysis up to 20 s vs. ~1 s of data for analyzing the power of a 10 Hz oscillation). Indeed, computing alpha power based on epochs of 2 seconds, led to a significant drop of ~12 percentage-points in the heritability of alpha power, which is in line with previous reports showing that unreliability of measuring alpha power rather than unique environmental factors accounts for the nongenetic variance (Smit et al., 2006). We predict that heritability of DFA exponents will increase when longer recordings are used.

DFA as a tool for understanding the mechanisms and functions of neuronal oscillations

From a theoretical perspective, oscillation power may vary independently of LRTC across subjects (Leopold et al., 2003). From a physiological perspective, however, overall power and the temporal modulation of neuronal oscillations could be coupled if shared genes were underlying the mechanisms that govern the formation of synchronous cell assemblies. Our results indicate, however, that LRTC and power are independent and have a different genetic architecture, suggesting that LRTC and power may carry complementary functions.

This is supported by recent applications of DFA analysis to ongoing oscillations in disease. In epileptic patients, intracranially recorded oscillations in the interictal period are characterized by pathologically strong LRTC around the seizure locus (Parish et al., 2004; Stead, 2005; Monto et al., 2007), whereas mean oscillation amplitudes were not useful as indicators of the epileptic focus (Monto et al., 2007). Interestingly, Monto et al. (2007) also observed that lorazepam, a widely used antiepileptic drug, suppresses LRTC of beta oscillations close to the seizure

initiation zone, whereas in healthy cortical tissue, lorazepam strengthens LRTC. In major depressive disorder, the LRTC of non-invasively recorded theta oscillations have been reported to break down almost entirely despite the lack of an effect on the oscillation amplitude (Linkenkaer-Hansen et al., 2005). These results are in line with growing evidence indicating that physiologic systems in a healthy state generate activity fluctuations on many time scales, whereas disease states are associated with too strongly correlated or too disordered dynamics (Goldberger et al., 2002; Chialvo, 2007).

At the level of cognition, sustained increase in the amplitude of oscillations in different frequency bands and brain regions have been identified during mnemonic and attentional tasks lasting up to 10 seconds (Raghavachari et al., 2001; Jensen & Tesche, 2002; Jensen & Lisman, 2005). We have proposed previously that temporal correlations in oscillatory activity on time scales up to tens of seconds may be important for successful performance on tasks that require coordination of neuronal activity across many time scales (Linkenkaer-Hansen et al., 2005). In line with this idea, parietal oscillations in Alzheimer's patients exhibit weaker LRTC than those in age-matched controls subjects (T.Montez, B.Jones, I.Manshanden, J.P.A.Verbunt, B.W.van Dijk, C.J.Stam, P.Scheltens, K.Linkenkaer-Hansen, unpublished observations).

The observation that genetic factors have a major influence on the temporal structure of ongoing oscillations increases the likelihood of finding key mechanisms that regulate the stability of the oscillations, which will also provide a better understanding of what may go wrong in disorders associated with abnormal LRTC (Parish et al., 2004; Linkenkaer-Hansen et al., 2005; Stead, 2005; Monto et al., 2007). Moreover, we believe that the DFA could provide a valuable complement to frequency and power analysis in characterizing the dynamics of network oscillations in computational models (Kopell et al., 2000; Jensen et al., 2005).

Outlook

For decades, fundamental and clinical research has characterized ongoing oscillations in terms of their frequency and power. Until recently, there was no reliable measure for quantifying the complicated temporal structure of spontaneously waxing and waning oscillations. The present findings of a firm genetic basis of long range temporal correlations and the independence of DFA exponents and oscillation power, provide a mechanistic rationale as to how it is possible that disease states are increasingly being linked to abnormal dynamics of ongoing oscillations. Knowing the neuronal mechanisms that shape the temporal structure of ongoing oscillations could prove an invaluable step towards the development of treatment strategies aimed at normalizing LRTC in neuronal oscillations and their associated neuronal or cognitive functions.

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PHENOTYPIC AND GENETIC CORRELATIONS BETWEEN THE SLOW CORTICAL POTENTIAL, UPPER ALPHA SYNCHRONIZATION, AND THETA DESYNCHRONIZATION DURING THE RESPONSE ANTICIPATION PERIOD OF A DELAYED RESPONSE TASK

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ABSTRACT

This study investigated three different EEG phenomena observed during response anticipation: the slow cortical potential, upper alpha synchronization, and theta desynchronization. Response anticipation was created with a spatial working memory delayed response task with a four second interval between the spatial cue onset (target) left or right of the fixation mark, and a movement cue (imperative stimulus). Working memory load was manipulated by leaving the target on screen (low memory load) or requiring active rehearsal of the target location (high memory load). By studying family members of varying degrees of genetic relatedness (including monozygotic twins) we first determined the heritability of the three ERP/EEG measures. Theta desynchronization and upper alpha synchronization showed significant heritability across the scalp in both low (θ: 18% - 49%; α: 35% - 60%) and high (θ: 31% to 46%; α: 35% to 65%) memory load conditions, the latter yielding the highest estimates. SCP showed low to moderate heritability at the midline, occipital, and left parietal electrodes, with estimates again be higher in the high (25% to 43%) than in the low (21% to 37%) load condition. During the high load condition, theta desynchronization and upper alpha synchronization showed a significant correlation ($r \sim 43\%$), which was partly genetic (~50% of the correlation). No phenotypic or genetic association was found between the SCP and theta or upper alpha power. Spatial working memory activity induced significant changes in all three measures but these changes were not heritable and did not show any association across measures. The results suggest that the SCP, theta desynchronization, and upper alpha synchronization, although evoked by the same antecedent conditions, reflect different neural substrates.

INTRODUCTION

The Contingent Negative Variation (CNV) is as a shift in Direct Current (DC) potential that is elicited by a warning stimulus preceding a later 'imperative' stimulus (Walter et al., 1964; Altenmüller & Gerlof, 1999; Rockstroh et al, 1989). Highly related to this phenomenon is the Slow Cortical Potential (SCP) elicited in spatial and non-spatial delayed response tasks (DRTs). The SCP is elicited only if the imperative stimulus requires a response, and if its timing is predictable (Rockstroh et al., 1989). On this basis, it is concluded, that the SCP indexes *response anticipation* (e.g., Fan et al., 2007). As such, the SCP is often compared to the *Bereitschaftspotenzial* (BP, readiness potential), a negative DC shift that is seen in anticipation of voluntary movement (Kornhuber & Deecke, 1965). Working memory load strongly enhances the magnitude of the SCP (Ruchkin et al., 1995), as do motivational aspects including positive (reward level) and negative (shock avoidance) motivators (Birbaumer et al., 1990).

DRT tasks of the type that elicit the SCP have also been reported to produce a small but consistent upper alpha synchronization (Klimesch, Pfurtscheller, et al., 1999; Jensen, Gelfand, et al., 2002; Bastiaansen et al., 2002; Sauseng et al., 2005). It seems plausible to hypothesize that EEG phenomena sharing antecedent conditions may also – at least in part – share neural substrates. The thalamo-cortical connections may be such a substrate for the SCP and alpha oscillatory activity. Thalamic activity has been shown to be correlated to the SCP (Birbaumer et al., 1990; Strehl, 2006) and thalamo-cortical connections are essential in the formation of alpha oscillatory activity (Steriade, 2000). In addition, the SCP has been shown to correlate on a trial-by-trial basis with fMRI BOLD signal in the thalamus (Nagai et al., 2004) and lateral thalamic metabolic rate has also been found to correlate highly with alpha power (Danos et al., 2001; Goldman et al., 2002; Schreckenberger et al., 2004). Alternatively, the reticular formation (RF) may be the primary source since the RF is known to modulate thalamic activity and to affect both slow cortical potentials and oscillatory activity (Birbaumer et al., 1990; Rohstock, 1989). In addition, the link between the SCP and alpha synchronization may be direct and both phenomena may be different sides of the same coin. It has been argued that ERPs may (partially) arise from changes in ongoing oscillatory activity through phase locking (Sauseng et al., 2007; Klimesch et al., 2007b; Min et al., 2007) or through desynchronization of oscillations with a non-zero mean (Nikulin et al., 2007).

Apart from the SCP and alpha synchronization, a DRT task was also seen to generate theta desynchronization during the interval between the warning and imperative stimulus (Bastiaansen et al., 2002). Just as the SCP, this theta synchronization showed sensitivity to increases in working memory load. Currently, a

joint neural substrate for SCP and theta synchronization is less clear. Changes in theta activity are related to changes in activity in cortico-hippocampal loops (Bastiaansen & Hagoort, 2001) but no studies have linked the medial temporal lobe to the SCP. By virtue of sharing the same antecedent conditions, however, theta desynchronization may by hypothesized to partly reflect the same neural substrate as the SCP and alpha synchronization.

Large individual differences are apparent for SCP, upper alpha synchronization, and theta synchronization, and various studies have linked these differences to variation in cognitive ablities (e.g., Basile et al, 2007; Hansell et al., 2005; Perez-Edgar et al., 2006; Klimesch, 1999; Dopplmayr, Klimesch, Sauseng et al., 2005; Dopplmayr, Klimesch, Hödelmoser, et el., 2005; Jausovec & Jausovec, 2004). If these ERP/EEG measures indeed reflect the same neural substrate, we expect that individual differences in SCP, upper alpha synchronization, and theta desynchronization are highly correlated. Furthermore, we expect that the enhancement of these measures by working memory load or motivational manipulation also shows a high cross-measure correlation, such that individuals that strongly increase SCP amplitude also show large changes in alpha synchronization and theta desynchronization. The current study tests these expectations by a behavioral genetic approach. First, it is tested whether individual variance in the three ERP/EEG measures during response anticipation are correlated. By studying family members of varying degrees of genetic relatedness (including monozygotic twins), it can de determined whether this correlation arises from an overlapping sets of genes. Next, we will establish whether the effects of working memory load on SCP amplitude, upper alpha synchronization, theta desynchronization are correlated and the extent to which this correlation is caused by genetic factors.

METHOD

Subjects

The EEG sample in this study was derived from an ongoing twin family study on cognition (e.g., Posthuma et al., 2001; Smit et al., 2005; Smit et al., 2007) in twins and family members from the Netherlands Twin Registry (Boomsma, Vink, et al., 2002). It consisted of 760 subjects from 309 families divided into two age cohorts based on the age of the twins: a younger cohort (M = 26.2 years, SD = 4.1) and a middle-aged cohort (M = 49.4 years, SD = 7.2). On average, 2.50 participants per family participated; family size ranged from one to seven siblings (including twins). Informed consent was obtained in writing for the EEG study. The study received approval from the VU university ethical committee.

Apparatus

Subjects were seated in a comfortable reclining chair in a dimly lit, sound attenuated, and electromagnetically shielded room. A touch-sensitive computer screen was placed 80 cm in front of the subjects. The chair was adjusted such that the center of the screen was at eye level. Task instruction was given on a written sheet. For responding, subjects used a rubber tipped pointer (5 mm diameter) to touch the screen. The pointer was held like a pen, in the preferred hand. Before the trial started, subjects placed their hand on a 5 x 5 cm^2 response pad placed centrally in front of them, 20 cm before the screen. Release of the response pad was used to indicate the end of the decision time and the start of the movement time. Screen touch with the pen constituted the end of the movement time.

The screen background was dark gray. A black hood with a 205 mm diameter hole in the middle was fastened to the monitor face to ensure that stimuli at all locations were at an equal distance from the edge of the screen.

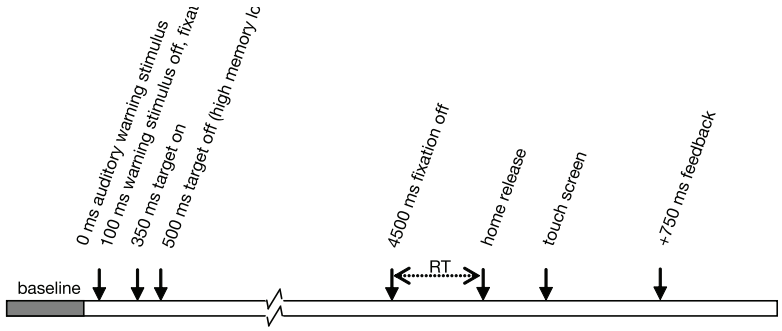

Figure 1. Trial stimulus presentation timeline.

DRT task

In Figure 1, the time course of a single trial in the DRT task is schematically depicted. Each trial started with an auditory beep (100 ms at 1000 Hz) followed at offset by the appearance of a black fixation square (width ca. 0.5 cm, visual angle 0.58) in the center of the screen. At 250 ms after onset of the fixation square, the target, a checkered black circle (diameter ca. 1.5 cm, visual angle 1.58) was presented anywhere on an annulus (9.25 cm, 9.26 radius) from the fixation square, except for 4 symmetrical 158 areas around the vertical and horizontal meridians. At the imperative stimulus, the offset of the central fixation square, subject had to lift their hand from the response pad and touch the screen as accurately and as fast as possible. In the low memory load condition (Low) the target remained visible until the onset of the imperative stimulus. In the high memory load condition (High) the target disappeared 150 ms after onset, so that the subjects had

to memorize the location of the target until the onset of the imperative stimulus Two types of delay intervals were used, in which the fixation square either disappeared 1150 ms after target onset (short delay) or 4150 ms after target onset (long delay).

Before the actual task was started, subjects engaged in a 10 min training session (data not used). The actual task consisted of a total of 240 trials split into two 120-trial blocks lasting about 14 min each. In 224 trials targets were presented in either left or right, top and bottom visual fields. (at 7.58 degree off the vertical and horizontal meridians). There were 16 trials in which the target was presented within the meridian areas. These 'catch trials' were included to increase the average spatial effort required, but were not used in the analyses. There were 96 trials in the low memory load condition and 132 trials in the high memory load condition. Half of the each of these trials had a short delay (1 s), while the others a long delay (4 s). In addition, in half of the trials a distracter, identical to the target, was presented in a random position in the annulus but not within a 1.58 degree radius of the target position. Distracters lasted 150 ms with an onset of 300–700 ms after target onset.

The order of presentation of the total set of the 240 possible trials was randomized once, and was of the same for each subject. For a trial to be correct at the behavioral level, the decision time needed to fall within an interval of 0.1 and 1.5 s after fixation offset, and the screen had to be touched within 1.5 and 3 s after fixation offset within a radius of 2 cm of the target center. Reaction time was computed as the sum of decision and movement time. A trial was correct if the screen was touched within a radius of 2 cm of the target center. Spatial accuracy was quantified by applying the following rules for earning or losing points: touching within the center target area (0.4 cm) earned 10 points, off target responses earned 8 (0.4–0.8 cm), 6 (0.8–1.2 cm), 4 (1.2–1.6 cm) or 2 (1.6–2 cm) points. Feedback was displayed 250 ms after touching the screen, in the center of the screen, for a period of 1500 ms. This included a running total of the winnings so far, and the amount of points won or lost at the preceding trial. Touching outside of the target area lost 5 points and a red error message INCORRECT was displayed. Lifting the hand before offset of the fixation spot caused TOO FAST to be displayed. If the maximal response time of 1500 ms expired, TOO SLOW was signaled. After feedback offset, an intertrial interval of 250– 750 ms was followed by onset of the next trial.

Behavioral accuracy was indexed with the number of points earned in the task as described above. However, all incorrect trials received a score of 0 in stead of the -5 indicated as feedback on the screen. Therefore, behavioral scores ranged from 0 to 10. Behavioral speed was index by the interval between fixation offset (the imperative stimulus) and the moment of the release of the home button in-

dicating the response initiation time.

EEG recording

EEG was registered for three minutes with 19 Ag/AgCl electrodes mounted in an electrocap. Signal registration was conducted using an AD amplifier developed by Twente Medical Systems (TMS; Enschede, The Netherlands) for 657 subjects (381 young, 380 middle-aged) and NeuroScan SynAmps 5083 amplifier for 103 subjects (24 young, 80 middle-aged). Signals were continuously represented online on a Nec multisync 17" computer screen using Poly 5.0 software or Neuroscan Acquire 4.2. Standard 10-20 positions were F7, F3, F1, Fz, F2, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1 and O2. The vertical electrooculogram (EOG) was recorded in bipolar derivation between two Ag/AgCl electrodes, affixed one cm below the right eye and one cm above the eyebrow of the right eye. The horizontal EOG was recorded bipolarly between two Ag/AgCl electrodes affixed one cm left from the left eye and one cm right from the right eye. An Ag/AgCl electrode placed on the forehead was used as a ground electrode. Impedances of all EEG electrodes were kept below 3 kΩ, and impedances of the EOG electrodes were kept below 10 kΩ. The EEG was amplified, digitized at 250 Hz and stored for offline processing. Amplifier filter settings for TMS were a single order FIR bandpass filter with cutoff frequencies of 0.05 Hz and 30.0 Hz. NeuroScan filter settings were a lowpass filter at 50.0 Hz.

EEG Data processing

The signals were recalculated with averaged earlobes (A1 and A2) as reference. All EEG was automatically and visually checked for bad channels such as absence of signal, hum, clipping, persistent muscle tone artifacts, and external noise. Files were epoched with a 0.5 s baseline before the warning stimulus to 7.5 s after the warning stimulus. For each subject, artifactual epochs were identified automatically using the EEGLAB (Delorme & Makeig, 2004) 'reject by threshold' and 'reject by spectra' option. Threshold settings for all leads was +/- 200 μV. The spectral analysis procedure identified deviant epochs by comparing each epoch's power spectrum to the spectrum averaged over all epochs. Epochs with more than 32 dB excess power within the frequency range below alpha (1.0 – 8.0 Hz) or above alpha (13.0 – 30.0 Hz) were marked artifacts. Visual inspection confirmed these epochs and corrections were made as necessary. If less than 29 trials were available for either condition due to either behavioral errors of EEG artefacts the particular lead was marked as missing for this subject.

Next, EEGLAB was used to identify eye movement and blink sources of activation using ICA decomposition based on the infomax algorithm (Makeig et al, 1997). After ICA analysis on both EEG and EOG data, components were

identified that were related to artifactual sources and were removed (Delorme and Makeig, 2004). Eye movement and blink artifacts can be identified by frontal scalp distribution (lateralized for horizontal eye movements), high correlation with EOG signals, and a match in timing for clear blinks and/or saccades. 97% of the subjects revealed a first vertical EOG related component, and 91% a second, horizontal EOG related component as independent component number 2 -10. A small subset (13%) revealed a third component that seemed to reflect separate aspects of EOG movement and blink activity.

ERPs and EEG frequency measures were derived by averaging across all correct trials. Only the trials with a long delay interval (96 low and 132 high memory load trials) will be considered in this paper. Because EEG/ERP data were not sufficiently different in distracter and non-distracter trials these two trial types were collapsed to increase the total number of trials in the low and high memory load conditions. Time-frequency analysis used event related spectral perturbation as implemented in EEGLAB (Makeig, 1993; Delorme & Makeig, 2004). Sine and cosine wavelets of a minimum of 2 oscillations were used to estimate power in the 0.5 s baseline period and the 7 s period after warning stimulus onset. The SCP was scored as the average potential in the interval of 1800 to 4500 ms after warning stimulus onset.

Power was estimated from 2 Hz to 49.8 Hz in 50 frequencies 0.98 Hz apart. Wavelets were maximally 1140 ms wide with a Hanning envelope, and were applied at 200 time points windows from 70 ms to 6925 ms after the warning stimulus onset. Theta syn-/desynchronization was scored in the same interval as the SCP (1800 to 4500 ms after warning stimulus onset) by averaging frequency bins 4.9 and 5.9 Hz. Alpha synch-/desynchronization was scored by averaging frequency bins of 9.8 and 10.8 Hz in the same interval.

Genetic analyses

We first established the heritability of SCP, upper alpha synchronization, and theta desynchronization using the extended twin design (Posthuma et al., 2003). Resemblance (covariances or correlations) between cotwins and siblings in these three traits derives from genetic relatedness or shared environmental influences (Falconer & Mackay, 1996; Boomsma, Bushjan et al., 2002). If the SCP correlation between DZ twins or siblings, who share on average 50% of their segregating genes, is half the correlation between MZ twins, who are genetically identical, this is seen as evidence for additive genetic influences (A) on SCP variation. If the SCP correlation between DZ twins or siblings is less than half the correlation between MZ twins this is seen as evidence for dominant (non-additive) genetic influences (D) on SCP variation. If the correlations between MZ and DZ twins/siblings are comparable and nonzero this is evidence for common environmental

influences (C) shared by family members.. If the correlation between MZ twins is not unity this is evidence for environmental effects on the SCP that are unique to each individual (E) and which include, for example, measurement error. By using structural equation modeling, we obtained maximum likelihood estimates of the relative contributions of each of these factors to the total variance in the SCP, upper alpha synchronization and theta synchronization. Since no information is available to estimate the effects of both C and D simultaneously in a twin-sibling design, we used a model with A+D+E if the DZ/sibling correlation was less than half the MZ correlation and a model with A+C+E if it was more than half the MZ correlation. Heritability of the ERP/EEG measures was defined under the best fitting model as the proportional contribution of additive (and, if applicable, dominant) genetic effects to the total variance.

Second, we estimated the correlation (covariance as a proportion of the total variance) between SCP, upper alpha synchronization and theta synchronization. We also estimated the genetic correlation (the genetic covariance as a proportion of the total variance). The information in this bivariate genetic modeling comes from the differences in cross-twin-cross-trait correlations between MZ and DZ twins and siblings. The cross-twin-cross-trait correlations allow a similar decomposition of the correlations between the ERP/EEG measures into their A, D, C, and E parts. For more information on multivariate genetic modeling and the estimation of the genetic correlation and the proportional contribution of genetic factors to phenotypic correlations we refer to Posthuma et al. (2003).

Finally, we tested heritability of the changes in SCP, upper alpha synchronization and theta synchronization induced by the manipulation of memory load. For this analysis we computed the High-Low difference scores for each of the measures. We then tested whether memory-load induced changes in SCP, upper alpha synchronization and theta synchronization were correlated. If so, we again tested to what extent these correlations were attributable to genetic factors.

All genetic analyses were performed using Structural Equation Modeling implemented in the program Mx (Neale, Boker, Xie, and Maes, 2006). An extended twin design as used here provides data from families of variable size. Mx handles such unbalanced datasets via full information maximum likelihood, which uses the observed, raw data.. To evaluate how well the specified model fits the observed data, the raw data option in Mx calculates the negative Log-Likelihood (-LL) of the raw data for each family (Lange, Westlake, & Spence, 1976), as:

$$-LL = -k \cdot \log(2\pi) + \log|\Sigma| + (y_i - \mu_i)^T \Sigma\sigma^1 (y_i - \mu_i),$$

where k (k = 1, …, p) denotes the total number of observed variables within a family (and can vary over families), σ(pxp) is the expected covariance matrix of family members, y_i (for i = 1,… p) is the vector of observed scores, μ_i is the

column vector of the expected values of the variables, and $|\Sigma|$ and Σ^{-1} are the determinant and inverse of matrix Σ, respectively.

Twice the difference between the likelihood of two nested models ($-2\{LL_{full}$ model $- LL_{nested}$ model $\}$) is asymptotically distributed as χ^2. A high χ^2 against a low gain of degrees of freedom (Δdf) denotes a worse fit of the second, more restrictive model relative to the first model. By stepwise restricting the number of parameters, the most parsimonious model for the dataset can be found. Each nested model is compared to the previous one. Additionally, a linear regression model was employed to include effects of age cohort and sex on the observed scores. In addition, a covariate was added to regress out the effect of equipment to cancel out possible effects of equipment (Neuroscan or Poly). When significant, covariates were also used to scale the variance of one group (e.g., males) to equal those of the other (e.g., females). This procedure corrects for possible differences in scaling between groups.

All significance was tested against an alpha level of 0.01.

RESULTS

Performance data

Table 1 shows the average RT and accuracy in the low and high memory load conditions, separated for distractor and non-distractor trials. Reaction time was barely affected by the difficulty level, but the accuracy data confirmed that the manipulation was effective. On average, the high memory load condition de-

Table 1. Effects of memory load and the presence of distractors on response speed and spatial accuracy

	Low		High		memory load effect (High - Low)		
	M	**SD**	**M**	**SD**	**M**	**SD**	**significance**
Speed (ms)							
Distractor							
No	408	80.2	398	72.5	-9.4	37.8	***
Yes	395	71.9	397	67.7	1.5	28.2	ns
Accuracy (points earned)							
Distractor							
No	8.15	0.92	4.60	1.31	-3.55	1.40	***
Yes	8.09	0.95	3.70	1.32	-4.40	1.42	***

Significance was tested with Structural Equation Modeling using Mx accounting for the within-family dependency of the data.

creased spatial accuracy, indicated by the points earned, by 3.55 to 4.4 units compared to the low memory load condition (χ^2=499.6, $p<10^{-70}$). We estimated the effect size of this effect by virtue of the fact that the χ^2 distribution is equal to the F distribution with a numerator degrees of freedom of 1 and infinite denominator degrees of freedom. The effect size defined as percent reduction in error variance, or η^2) corresponding with an $F(1, \infty) = 499.6$ was an estimated η^2=48.5%). The distractor interacted with memory load so that it reduced performance in the high but not in the low memory load condition (χ^2=71.6, $p<10^{-15}$, estimated η^2=11.8%). Because the effect size of the load condition was much larger than that of the distractors, further analyses were collapsed across distractor and no-distractor trials to increase signal to noise ratio.

SCP

Figure 2 shows the grand average ERP waves. Note that the early ERP components are visually compressed due to the long time interval plotted. The ERP for channel Pz was expanded for illustration purposes. As can be seen, the warning stimulus which included an auditory beep produced a clear N1 which was maximal at Cz, consistent with many previous findings (e.g., Altenmüller & Gerloff, 1999). After that, two positive complexes developed related to the warning stimulus/fixation on event. Next a small positive complex developed related to the target onset developed which overlapped with the initial rise of the SCP. The SCP started to develop at around 400 ms after the warning stimulus and reached a maximum level about 1.7 s after trial onset. The large negativity following the imperative stimulus (ca. 5000 – 5500 ms) revealed in the more centrally located areas is the Post-Imperative Negative Variation (PINV) related to expectation to the feedback stimulus (Birbaumer et al., 1990)

Voltages during the SCP were significantly below baseline for all leads tested in both memory load conditions (Table 2, left panel). On most leads, the SCP appears to slowly decay as it usually does in these paradigms, but this reflects in part the effect of the high pass filtering. SCPs in the low and high memory load conditions where very similar in shape, but significant lower voltages were found during the high memory load condition on all leads. Largest SCPs were found along the midline. The effects of memory load was largest in central and parietal leads. The middle aged adults had significantly smaller SCPs at the mid-parietal leads (C4: 1.34, T8: 0.81, P3: 1.24, Pz: 1.85, P4: 1.84, and P8: 0.92). No systematic effects of sex interactions were found on the SCP.

Time-frequency analysis

Figures 3 and 4 show the results for the time-frequency analysis in both conditions averaged across all subjects. The plot colors are scaled in dB—i.e.,

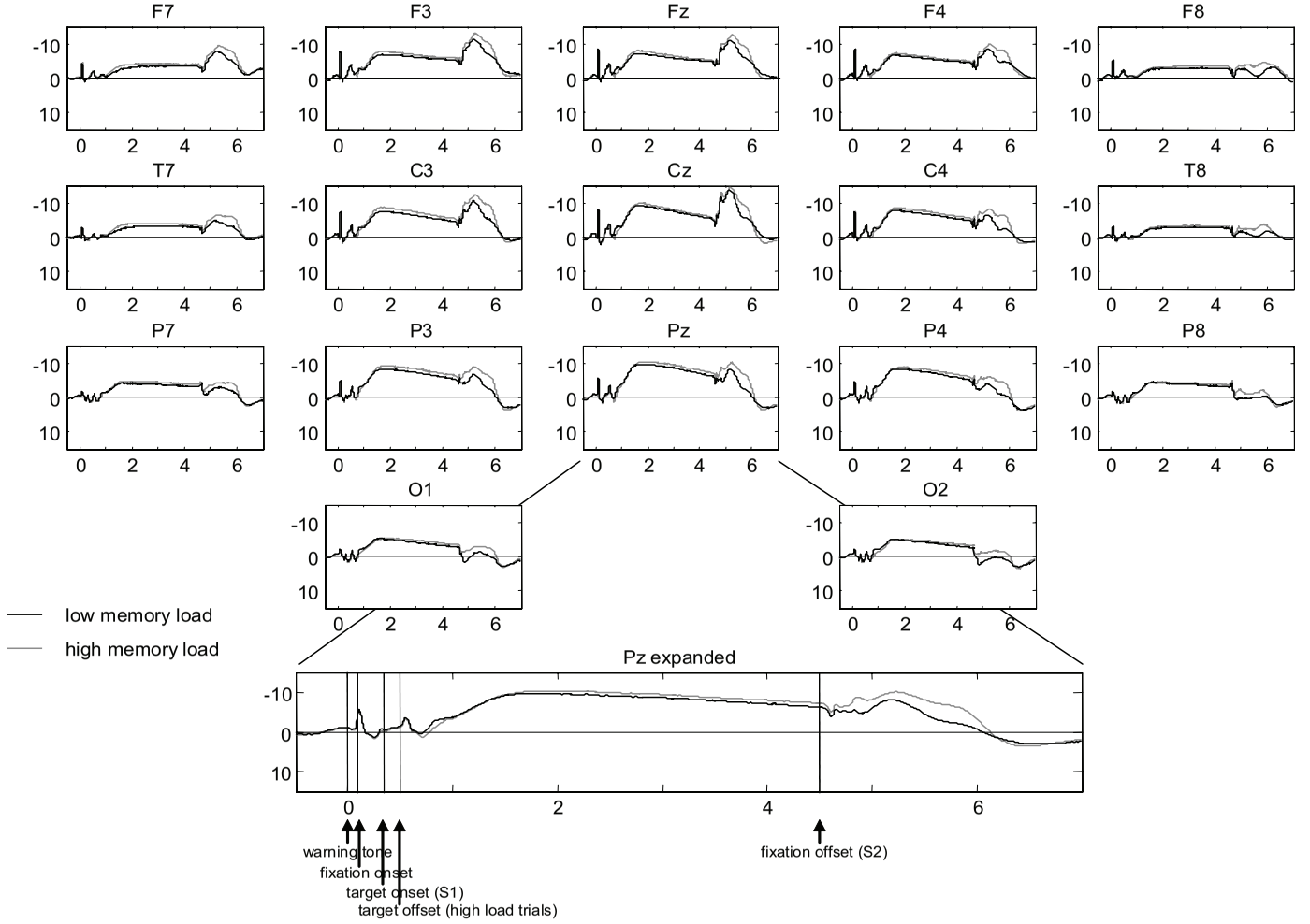

Figure 2. Slow cortical potentials in the response anticipation interval during the low (solid) and high (dashed) memory load conditions.

$10 \cdot \log_{10}(\mu V^2)$—compared to baseline power. There was a clear pattern of alpha and beta synchronization directly after stimulus presentation that corresponds to the ERP generation due warning tone and fixation onset (A). Slightly after target presentation, *theta desynchronization* occurred (B) that is likely to reflect the late positive waves related to the target. Both A and B showed inter-trial coherence (data not shown) indicating that the oscillatory activity is phase-locked to the stimulus event, and will therefore also appear in the ERP. Within the delay interval before the imperative stimulus a clear theta desynchronization was seen compared to baseline (C). In addition, the same interval showed *alpha synchronization* on practically all leads and in both conditions in the delay interval (D). This upper alpha synchronization showed maximum power in both the 9.8 Hz and 10.8 Hz frequency bins. The weighted average frequency of these bins was 10.3 Hz. Since the average peak frequency for this sample is 9.9 Hz, the alpha synchronization could indeed be considered upper alpha synchronization. This finding is consistent with the previous finding of Bastiaansen et al. (2002) in a subset of the current sample.

Like the SCP, upper alpha synchronization showed a distinct topographic pattern (Table 2, middle panel). Whereas frontal leads showed no significant change over baseline, all central and parietal and occipital leads did. Most leads did not show sensitivity to memory load, with exception of the right frontal leads (Fz, F4 and F8), the left and right temporal region (T7, T8), the central region (Cz and C4) and the right parietal region (P4). Small sex differences emerged such that males had higher upper alpha synchronization than females (F4: 0.15 dB; F8: 0.17 dB; T8: 0.15 dB; P4 & P8: 0.20 dB). Upper alpha synchronization did not differ in the two age cohorts at any lead.

Significant theta desynchronization was found on all leads in both memory load conditions (Table 2, right panel). In contrast to the SCP and upper alpha synchronization, little topographic differentiation in the theta desynchronization was found, and the effects of memory load were very comparable cross the entire scalp. No significant effects involving age cohort or sex were found for theta desynchronization.

Genetic analyses

No evidence for shared rearing environment (C) or non-additive genetic effects (D) were found on either of the three ERP/EEG measures. Additive genetic effects were significant on all leads for upper alpha synchronization and theta desynchronization. For these measures, and AE model did not significantly reduce the fit of the model than either an ADE or ACE model. We therefore proceeded with an AE model for these two measures. For SCP, however, those leads that showed a significant familial effect (A + C), significance testing could not decide

Table 2. SCP, Upper Alpha Synchronization, and Theta Desynchronization in the low and high memory load conditions.

	SCP (mV)			Upper Alpha Synch. (dB)			Theta Desynch. (dB)		
lead	High	Low	memory load effect (high - low)	High	Low	memory load effect (high - low)	High	Low	memory load effect (high - low)
F7	-4.03***	-3.23***	-0.80*	0.01	-0.04	0.05	-0.79***	-0.92***	0.13***
F3	-6.34***	-5.76***	-0.58	0.10	0.05	0.05	-0.55***	-0.68***	0.13***
Fz	-6.33***	-5.67***	-0.65	0.13	0.07	0.06*	-0.50***	-0.63***	0.13***
F4	-5.77***	-5.30***	-0.47	0.09	0.04	0.05*	-0.56***	-0.69***	0.13***
F8	-3.39***	-2.81***	-0.58	0.00	-0.05	0.05*	-0.82***	-0.91***	0.10***
T7	-3.69***	-3.02***	-0.67**	0.11*	0.04	0.07**	-0.48***	-0.58***	0.10***
C3	-7.06***	-5.97***	-1.08***	0.18***	0.14*	0.04	-0.53***	-0.64***	0.10***
Cz	-7.77***	-7.27***	-0.50	0.22***	0.15**	0.07***	-0.47***	-0.59***	0.11***
C4	-7.00***	-6.16***	-0.84**	0.24***	0.16*	0.08***	-0.56***	-0.67***	0.11***
T8	-3.08***	-2.70***	-0.38	0.18**	0.11	0.07*	-0.55***	-0.62***	0.07***
P7	-4.21***	-3.54***	-0.67**	0.27***	0.23***	0.05	-0.60***	-0.72***	0.11***
P3	-8.01***	-6.96***	-1.05***	0.31***	0.28***	0.04	-0.60***	-0.71***	0.11***
Pz	-9.07***	-8.20***	-0.87***	0.33***	0.27***	0.06	-0.55***	-0.66***	0.11***
P4	-7.63***	-6.86***	-0.77***	0.40***	0.33***	0.07*	-0.63***	-0.75***	0.12***
P8	-4.06***	-3.73***	-0.33	0.42***	0.36***	0.06	-0.67***	-0.79***	0.12***
O1	-4.49***	-3.96***	-0.54**	0.29***	0.29***	0.01	-0.92***	-1.03***	0.11***
O2	-4.07***	-3.66***	-0.41*	0.32***	0.31***	0.01	-0.92***	-1.05***	0.13***

*p<0.01; **p<0.01; ***p<0.0001

between models AE and CE. However, in all but two cases (O1 and Pz in the low memory load condition) the AE model provided the best fit. Therefore—consistent with Hansell et al. (2001)—we proceeded with an AE model for the SCP as we did for upper alpha synchronization and theta Desynchronization. Table 3 shows the heritabilities derived from these models.

Heritability for the SCP in the low load condition did not reach significance on all leads, but significant contribution of genetic factors was found in the right frontal, left parietal-central, and occipital areas. A similar pattern was obtained for the high load condition, with heritability being slightly but systematically higher at many leads. Upper alpha synchronization was heritable across the entire scalp in both conditions (35% to 60% in the low load condition and 35% to 65% in the high load condition). Theta desynchronization also showed heritability across the scalp, but showed reduced heritabilities in the low load condition (18% to 49%) compared to the high load condition (31% to 50%) particularly on the anterior leads.

To test for an overlap in individual differences in the three ERP/EEG measures we decided to focus on the high load condition, because it had shown highest univariate heritability for all three measures. Table 4 shows the phenotypic correlation between the measures, followed by the genetic correlation. For instance, for lead P3 the phenotypic correlation between SCP and upper alpha synchronization was 0.20 with a genetic correlation of 0.19. As is clear from the table, the overlap between individual differences in SCP amplitude and upper alpha synchronization is very small and limited to Cz, C4, P7, P3, and Pz. A significant genetic contribution to these correlations could be established only for P3 and P4. Between SCP and theta desynchronization no significant correlation was found on any lead. In contrast, upper alpha synchronization and theta desynchronization showed significant positive correlation across the entire scalp (r from 0.38 to 0.50). Many of the leads showed a significant genetic correlation and the proportion of the phenotypic correlation that was accounted for by genes was on average 50%.

Genetic analyses of the High-Low difference scores showed very little contribution of genetic factors to the changes in SCP, upper alpha synchronization and theta synchronization that were induced by the manipulation of memory load (see columns 4, 7 and 9 of table 3). In addition, as shown in Table 5, phenotypic correlations between SCP and upper alpha synchronization or theta desynchronization were all non-significant. Modest correlation between memory effects on upper alpha synchronization and theta desynchronization were found, but none derived from a shared genetic source.

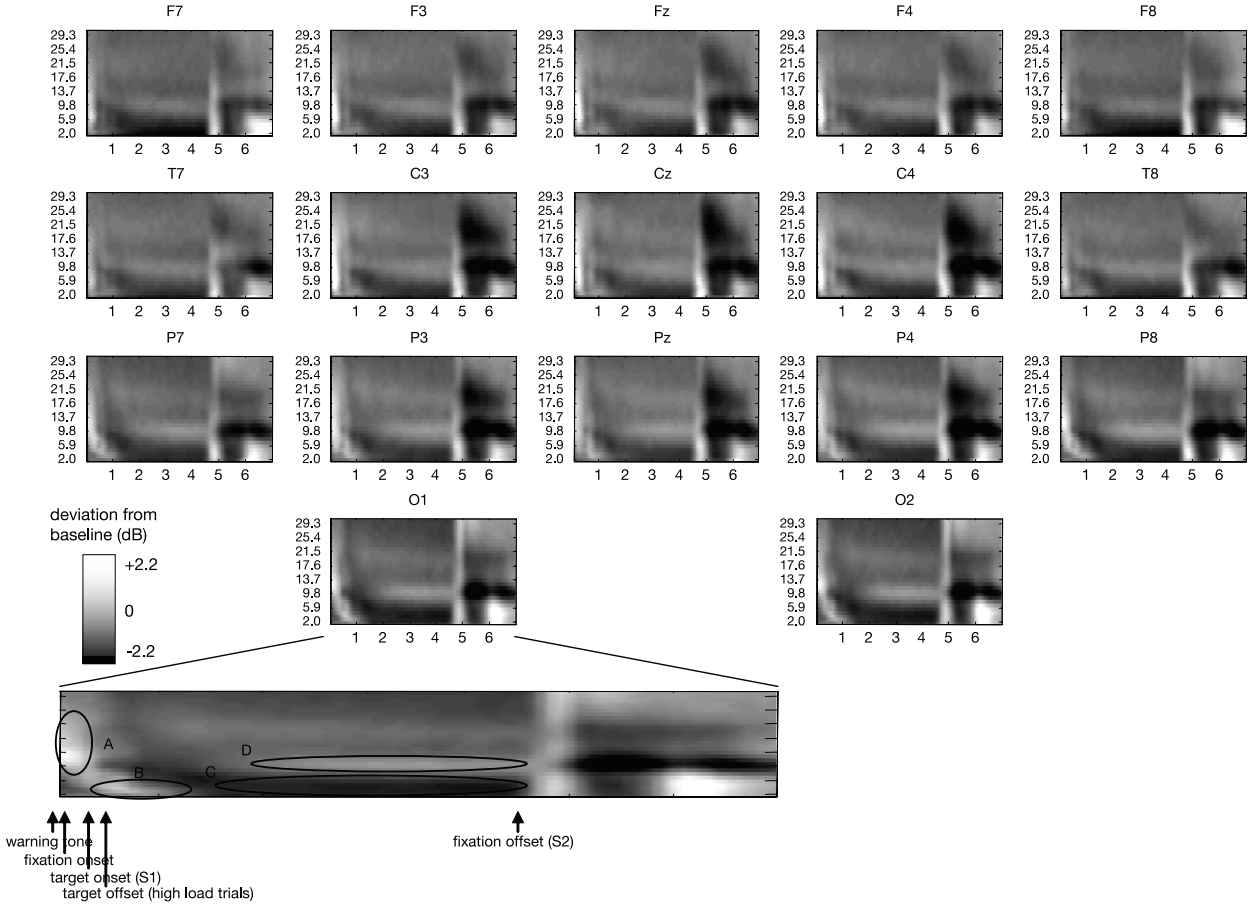

Figure 3. Time-frequency plot in the entire trial interval for the low memory load condition.

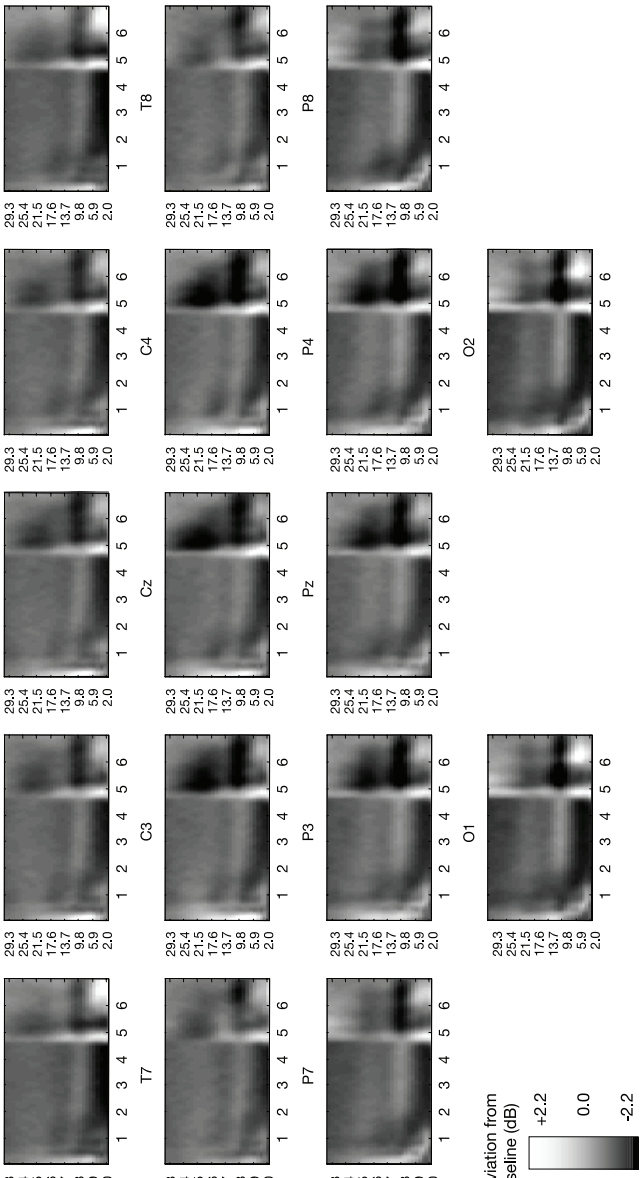

Figure 4. Time-frequency plot in the entire trial interval for the high memory load condition.

Table 3. Heritability of SCP, Upper Alpha Synchronization, and Theta Desynchronization.

	SCP			Upper Alpha Sync.			Theta Desync		
lead	**High**	**Low**	**Memory load effect (High - Low)**	**High**	**Low**	**Memory load effect (High - Low)**	**High**	**Low**	**Memory load effect (High - Low)**
F7	0.00	0.07	*0.12*	0.43***	0.40***	*0.07*	0.32***	0.19	*0.00*
F3	0.16	0.14	*0.11*	0.44***	0.39***	*0.15*	0.40***	0.18	*0.00*
Fz	0.25*	0.22	*0.00*	0.48***	0.41***	*0.00*	0.50***	0.28*	*0.00*
F4	0.29**	0.21*	*0.00*	0.42***	0.40***	*0.01*	0.39***	0.25*	*0.01*
F8	0.19	0.12	*0.00*	0.45***	0.39***	*0.00*	0.34***	0.23*	*0.01*
T7	0.04	0.07	*0.01*	0.45***	0.35**	*0.20*	0.41***	0.22*	*0.00*
C3	0.30**	0.26*	*0.00*	0.35***	0.41***	*0.00*	0.41***	0.29**	*0.00*
Cz	0.30**	0.19	*0.04*	0.38***	0.39***	*0.00*	0.48***	0.34***	*0.00*
C4	0.17	0.22*	*0.04*	0.43***	0.40***	*0.00*	0.40***	0.36***	*0.00*
T8	0.08	0.07	*0.06*	0.50***	0.35**	*0.00*	0.38***	0.22*	*0.08*
P7	0.35***	0.36***	*0.00*	0.48***	0.51***	*0.17*	0.31**	0.18	*0.00*
P3	0.41***	0.34***	*0.07*	0.45***	0.51***	*0.10*	0.37***	0.31**	*0.00*
Pz	0.35***	0.29**	*0.00*	0.53***	0.52***	*0.00*	0.46***	0.45***	*0.00*
P4	0.20	0.16	*0.00*	0.55***	0.50***	*0.02*	0.43***	0.49***	*0.04*
P8	0.15	0.19	*0.00*	0.55***	0.43***	*0.17*	0.32**	0.34***	*0.17*
O1	0.43***	0.37***	*0.00*	0.64***	0.60***	*0.06*	0.35***	0.44***	*0.06*
O2	0.33**	0.27*	*0.00*	0.65***	0.60***	*0.22*	0.36***	0.43***	*0.23*

**<p<0.01; **p<0.001; ***p<0.0001*

DISCUSSION

Consistent with studies using the same or comparable delayed response or memory retention designs, the current data showed a clear SCP and upper alpha synchronization in the response anticipation interval between a warning stimulus and an imperative stimulus (SCP: e.g., Filipovic et al., 2001; Hansell et al., 2001; upper alpha synchronization: e.g., Bastiaansen, 2001; Klimesch et al., 1999; Klimesch et al., 2007; Krause et al., 1996; Filipovic et al., 2001). We also replicated the theta desynchonisation in this interval previously observed in a subset of these subjects by Bastiaansen et al. (2001). In keeping with previous studies we confirm that

Table 4. Phenotypic and genetic correlation between SCP, Upper Alpha Desynchronization, and Theta Synchronization in the high memory load condition.

	SCP & Upper Alpha Sync.		SCP & Theta Desync.		Upper Alpha Sync. & Theta Desync.	
lead	**phenotypic correlation**	**genetic correlation**	**phenotypic correlation**	**genetic correlation**	**phenotypic correlation**	**genetic correlation**
F7	0.03	0.00	0.02	0.00	0.50***	0.17
F3	0.03	-0.02	0.01	-0.02	0.47***	0.22*
Fz	0.06	-0.02	0.03	-0.03	0.41***	0.21*
F4	0.09	-0.03	0.03	-0.03	0.44***	0.19*
F8	0.04	0.01	0.05	0.07	0.46***	0.19*
T7	0.07	0.00	-0.01	0.00	0.49***	0.25***
C3	0.12	0.10	-0.05	-0.04	0.49***	0.25**
Cz	0.13*	0.00	0.02	-0.02	0.39***	0.17
C4	0.15*	0.14	0.04	-0.01	0.42***	0.17
T8	0.02	0.03	0.02	-0.01	0.47***	0.15
P7	0.14*	0.10	0.01	0.01	0.45***	0.21*
P3	0.20**	0.19*	0.07	0.02	0.44***	0.18
Pz	0.17*	0.15	0.04	0.02	0.39***	0.15
P4	0.14	0.19*	0.01	0.06	0.43***	0.25**
P8	0.07	0.04	0.00	0.03	0.44***	0.19*
O1	0.09	0.13	-0.05	-0.04	0.43***	0.25**
O2	0.03	0.03	0.04	0.00	0.38***	0.21*

Note. The proportion of the phenotypic correlation that is due to genetics can be computed as phenotypic R divided by genotypic R.
**<p<0.01; **p<0.001; ***p<0.0001*

the amplitude of the SCP is modulated by working memory load (Geffen et al., 1997; Cameron et al., 2003) and that the upper alpha synchronization and theta desynchronization seen during response anticipation also significantly increase with higher spatial working memory load.

Large individual differences were present in all three ERP/EEG measures and we tested the relative contribution of genetic influences to these measures by comparing trait resemblance in siblings of varying degree of genetic relatedness (i.e., MZ and DZ twins and non-twin siblings). Significant heritability was found for SCP, upper alpha synchronization and theta desynchronization. In keeping with the idea that challenges to the system tend to increase genetic variance (de Geus et al., 2007), heritability was generally higher in the high memory load condition than in the low memory load condition. During high memory load, significant heritability for SCP varied from 25% to 43%, for upper alpha synchronization from 35% to 65%, and for theta desynchronization from 31% to 50%. Genetic contribution to SCP was localized mainly in the right frontal, left parietal-central, and occipital areas, but for upper alpha synchronization and theta desynchronization no clear topographic pattern in heritability could be distinguished. Instead, as in resting state EEG measures, heritability was of similar magnitude across the entire scalp (Smit et al., 2006).

Although memory load tended to increase the genetic contribution to variation in the three ERP/EEG measures, the memory load induced changes in SCP, upper alpha synchronization, and theta desynchronization were not heritable at all. This means that the individual differences in brain activity during response anticipation are heritable, that there is significant modulation of this activity by working memory load, but that individual differences in this modulation are completely driven by environmental effects and may even be entirely state-dependent.

From the literature the antecedent conditions evoking the SCP seem to be threefold: cued expectancy of a salient stimulus, an actual motor response, and motivational salience of the response. That is, the SCP only develops after a cue or warning stimulus and it is strongly reduced in amplitude when no overt response is required. A stronger negative potential is obtained when a feedback stimulus is an aversive tone or a shock (Rockstroh et al., 1989). The interpretation of the SCP has been manifold (Birbaumer et al., 1990) but most sources consider it to reflect active inhibition of some and facilitation of other areas. This was already defined in 1976 by Deecke et al. (Deecke, 1976 quoted by Rockstroh et al., 1989 p. 168) as "a general facilitation process, preactivating those brain regions which will be needed under the special experimental condition". A modern definition restates this as "the allocation of attentional resources for action" (Filipovic, 2001;

Rockstroh et al., 1989) or "attentive effort" (Brunia & van Boxtel, 2001).

The interpretation of alpha oscillations has witnessed changes in recent years. From some of the earliest human scalp recorded EEG investigations it had been proposed that alpha oscillations desynchronize upon activation of the cortical area under scrutiny (Adrian & Mathews, 1934). Therefore, alpha rhythms (and related rhythms such as mu) were thought to appear only in states of inactivity, that is, during cortical 'idling'. Increasingly, however, alpha synchronization has been ascribed a more active role as an index of top-down inhibition (Basar, 1997; Jensen et al., 2002; Sauseng et al, 2005; Klimesch et al., 2006, 2007; Joskisch, 2007; Neuper, Wortz, & Pfurtscheller, 2006; Hummel et al., 2002) or alertness

Table 5. Phenotypic and genetic correlations between memory-induced changes in SCP, Upper Alpha Synchronization, and Theta Desynchronization.

	SCP &Upper Alpha Sync.		SCP &Theta Desync.		Upper Alpha Sync. &Theta Desync.	
lead	phenotypic correlation	genetic correlation	phenotypic correlation	genetic correlation	phenotypic correlation	genetic correlation
F7	0.00	0.09	0.05	0.00	0.28***	0.00
F3	0.00	0.03	0.02	0.01	0.22***	0.02
Fz	0.00	0.00	0.00	0.00	0.19***	0.00
F4	-0.06	0.04	0.00	0.01	0.23***	0.02
F8	-0.08	0.01	0.02	0.05	0.25***	0.01
T7	-0.02	0.00	-0.02	0.00	0.25***	0.01
C3	-0.05	0.05	0.02	0.01	0.24***	0.07
Cz	-0.06	0.00	-0.03	0.03	0.15**	0.01
C4	0.00	0.00	-0.04	0.05	0.12*	0.01
T8	-0.01	0.03	-0.02	0.00	0.13*	0.03
P7	-0.08	0.00	0.01	0.01	0.18***	0.04
P3	0.00	-0.01	0.00	0.05	0.12*	0.04
Pz	-0.03	0.00	-0.02	0.01	0.17***	0.00
P4	-0.04	0.00	-0.03	0.06	0.15**	0.11
P8	0.00	0.04	0.00	0.09	0.10	0.12
O1	-0.03	0.00	0.01	0.02	0.09	0.07
O2	-0.01	-0.01	-0.01	0.07	0.09	0.07

*p<0.01; **p<0.001; ***p<0.0001

(Knyazev et al., 2006), rather than as a measure of 'cortical idling'.

Since the SCP and alpha activity share antecedent conditions they may perhaps be closely related neural phenomena. This idea is reinforced by the potential sharing of thalamocortical loops as the most likely source of slow cortical potentials such as the SCP as well as alpha generation (Birbaumer et al., 1990; Rockstroh et al., 1989; Nagai, 2004; Danos et al., 2001; Goldman et al., 2002; Schreckenberger et al., 2004). It is even possible that the SCP is in part directly generated by a change in alpha synchronization (Sauseng et al., 2007; Klimesch et al., 2007; Min et al., 2007; Nikulin et al., 2007). Based on the idea that SCP and alpha power may share a neural substrate we hypothesized that individual differences in both measures would be correlated and influenced by a common set of genes. Multivariate analysis strongly refuted this hypothesis. SCP and upper alpha synchronization were uncorrelated and, although both traits were heritable, the genetic factors underlying the heritability of SCP did not overlap with those underlying the heritability of upper alpha synchronization. In addition, working memory load induced increases in SCP and upper alpha synchronization were uncorrelated.

Some previous studies, using a comparable design, had already alluded to this outcome, although they did so on the basis of very small sample sizes. For example, Filipovic et al. used a go-no go task that evoked a small alpha synchronization in a three-second interval between a cue and imperative stimulus. Observing no condition effect for AS while SCP showed a clear go/no-go difference, they concluded AS and SCP reflected different aspects of cognitive processing. Pfurtscheller and Aranibar (1977) reached the same conclusion on the basis of different scalp distribution for alpha synchronization (sensory areas) and SCP (motor areas). Fan (2007) also reported no correlation between alpha activity of several dipoles with the SCP in a 2.5-second interval between a cue and imperative stimulus. Taken together, the bulk of the evidence suggest that the SCP and alpha synchronization reflect unique aspects of response anticipation.

SCP and alpha synchronization were accompanied by a significant theta desynchronization throughout the interval between warning stimulus and response stimulus. On top of this overall decrease in theta power, an increase in working memory load caused a relative increase of theta power. This is consistent with previous studies reporting theta synchronization during episodic memory processing (e.g., 1998; Gevins et al., 1997; Klimesch, 1999;), a 2-back task (Krause et al., 2000), and a spatial memory task (Jensen & Tesche, 2002). In keeping with Bastiaansen et al (2001) we interpret the theta desynchronization to have a functional role in enhancing the signal-to-noise ratio of the activity in the hippocampal-cortical loops. We do not, however, replicate the topography observed by them. Instead, theta desynchronization was found across the entire scalp as was

the memory-induced attenuation of this effect. During both memory load conditions, individual differences in theta desynchronization showed consistent overlap with differences in upper alpha synchronization (r from ca. 0.40 to 0.50), and about 50% of this correlation was due to shared genes. This correlation between alpha synchronization and theta desynchronization is a novel finding. Taken the substantial heritability of both, it suggests that the alpha and theta responses to this types of task reflects a stable bivariate characteristic of individuals that could be a useful "endophenotype" in genetic research of brain function.

Some limitations to this study should be noted. The basic approach in this study is a hybrid of a universal processes design and an individual differences design. In the universal processes approach we assume that the same antecedent conditions will produce an SCP, upper alpha synchronization and theta desynchronization in all subjects. To test whether these EEG/ERP phenomena derive from the same neural substrate we then switched to an individual differences approach. This means that we tested whether the amplitude of the SCP, and the extent of upper alpha synchronization and theta desynchronization were correlated across individuals. This, as has been shown above, did not appear to be the case. However, one may argue that within a single individual, these measures might still be correlated. To test this, a parametric approach would be needed that manipulates the amplitude of the SCP in a within-subject repeated measures. This could be done by using multiple memory loads as well as multiple levels of motivational salience of the task by adding larger incentives like threat of shock, or tones on errors. Here, we used only two task conditions (low and high memory load) which did not allow computation of within-subject correlations of SCP, alpha synchronization and theta desynchronization. It is hard to envision how the SCP could correlate with upper alpha synchronization and theta desynchronization within each subject and yet show no correlation at the between subjects level. Still, this possibility cannot be ruled by the current design.

To summarize, response anticipation evokes an SCP together with significant upper alpha synchronization and theta desynchronization. Each of these traits showed significant heritability classifying them as viable endophenotypes for genetic research on basic brain functions. Genetic effects on the SCP are specific to this measure, whereas alpha synchronization and theta desynchronization have some genes in common. In the average subject, increasing working memory load induced marked changes in all three measures but these effects of memory load are not viable markers of genetic variability between individuals.

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9

SUMMARY AND DISCUSSION

The core mission of this dissertation was to examine the genetic architecture of selected EEG and ERP measures that may index important individual differences in brain structure and function. To do so, data were used from an extensive 2.5-hour EEG recording session in a sample of 263 MZ, 303 DZ twins and 195 of their singleton siblings from two age cohorts, one with an average age of about 25 yrs, one of about 50 yrs.

HERITABILITY OF EEG/ERP TRAITS

Table 1 gives a summary of the heritability of all EEG/ERP measures tested in this dissertation separately for age cohorts and sex where appropriate. If a measure was assessed at multiple leads, the two leads are provided that showed highest or lowest magnitude on the respective EEG/ERP measure. For example, for alpha power heritability is shown for the leads with the highest and lowest alpha power (O2 and T8, respectively). Additionally, the mean heritabilities across all leads are given. Frontal asymmetry, graph theoretical measures, and the anterior and posterior N1 do not provide topographic information and only the overall heritability is reported for these measures. All heritabilities provided in table 1 are uncorrected for measurement error.

The table is ranked by the magnitude of average/overall heritability, i.e. the most heritable traits are listed first. Only for the last five rows - frontal asymmetry in the middle-aged adults and working memory induced changes in SCP, alpha synchronization and theta desynchronization - no evidence of significant genetic contribution was found. For all other measures a significant heritability was found ranging from moderate to very high. This includes the new EEG measures based on graph theoretical analysis and detrended fluctuation analysis which this dissertation has, for the first time, established as heritable traits.

Age did not have a large impact on the heritability estimates. For the majority of the parameters, no systematic differences in MZ and DZ correlations across age cohorts were found, with the clear exception of frontal asymmetry, where heritability was only found in the young adult cohort. Likewise, no systematic sex differences in twin correlations were encountered in the bulk of our tests, even when significantly different means were found for males and females. Although power to detect subtle effects was low given the sample size, these results suggest that the heritability of many EEG/ERP variables are not subject to much change over the adult years, and that they do not differ between the sexes.

The main conclusion from Table 1 must be that brain activity recorded from the scalp can be reliably used to index stable genetic variation in adult brain function. Below, I will summarize the main findings of this dissertation in the order of the chapters.

Resting EEG Power

As mentioned in the introduction of chapter 2, overall power from background EEG recordings has been related to psychopathology. Beta power has consistently been shown to be decreased in children with ADHD (e.g., Barry et al., 2003a, b; Lazzaro et al., 1998; Chabot & Serfontein, 1996; Satterfield et al., 1972). This beta power decrease is generally found with concurrent theta power increase, although different subtypes may exist (Clarke et al., 2001). Studies of alcoholism have shown deviant resting EEG for several frequencies. For example, increased beta power has been reported in alcoholics compared to controls (e.g., Gabrielli et al., 1982; Rangaswamy et al., 2002, 2004). The successful use of beta power as an endophenotype for alcoholism has been demonstrated in a linkage/association study (Porjesz et al., 2002; Edenberg et al., 2004; Dick et al., 2006), implying the dependence of both beta power and alcoholism on gaba-ergic 'neural excitability' (Rangaswamy et al., 2004; Porjesz et al., 2005). Alterations in other frequency bands have been reported in alcoholism too (Porjesz et al., 2005).

In adult subjects we find very high heritability of EEG power. Power in the alpha band is almost about as heritable as what could well be the most heritable quatitative trait in humans: body height (Silventoinen et al., 2006). Heritabilities of theta and beta power were also high and only delta power showed moderate heritability. Our findings in adults are highly consistent with many previous findings in childhood and adolescence (see van Beijsterveldt & van Baal, 2002). Heritability was significantly lower in middle-aged adulthood, but the difference was very modest (for example, 90% and 85% respectively for alpha power averaged over leads). Although these were not formally tested, topographic differences were also very minor, with comparable heritability of EEG power across the scalp.

To verify whether the classical frequency bands where recapitulated in 'heritability bands', we plotted heritability across the power spectrum in narrow 1 Hz frequency bins. This yielded a fairly continuous heritability spectrum, with gradually lower heritability for frequencies under 6 Hz (mainly frontal leads) and above 13 Hz (leads T7 and T8). Based on the proposed differences between different frequency bands with respect to their cognitive function or relation to psychopathology (theta, lower alpha 1 and 2, upper alpha: Klimesch, 1999; beta band in alcoholism: Rangaswamy et al., 2002; beta band in ADHD: Clarke et al., 2001) we tested the hypothesis that the heritability of EEG in different bands reflected different genetic factors. The data presented did not support this since at least 55%, and typically 60% - 75% of the genetic variation overlaps between the bands. This converges with findings by Anokhin et al. (2004) who also reported a substantial genetic covariation between the frequency bands delta, theta, alpha, and beta. We concluded, therefore, that a single genetic factor accounts for most

Table 1. Summary of heritabilities rank ordered by magnitude

Analysis	variable/frequency	subjects	@ max effect lead	@ max effect h^2	@ min effect lead	@ min effect h^2	mean/overall effect
Power	α	Young Adult	O1	0.85	T8	0.9	0.88
		Middle-aged		0.83		0.79	
	θ	Young Adult	Cz	0.87	T8	0.84	0.80
		Middle-aged		0.77		0.75	
	β	Young Adult	Pz	0.80	F7	0.73	0.77
		Middle-aged		0.82		0.66	
	δ	Young Adult	Fz	0.56	T8	0.63	0.57
		Middle-aged		0.46		0.5	
Graph theoretical analysis	Path length, θ	All	*n/a*		*n/a*		0.63
	Path length, β2	All	*n/a*		*n/a*		0.60
	Path length, α2	All	*n/a*		*n/a*		0.59
	Path length, β1	All	*n/a*		*n/a*		0.46
	Path length, α1	All	*n/a*		*n/a*		0.35
Visual oddball amplitude and latency	P300 amplitude	All	Pz	0.5	Fz	0.56	0.52
	Posterior N1 amplitude	All	*n/a*		*n/a*		0.50
	Anterior N1 latency	All	*n/a*		*n/a*		0.45
	P300 latency	All	Pz	0.45	Fz	0.42	0.44
	Posterior N1 latency	All	*n/a*		*n/a*		0.43
	Anterior N1 amplitude	All	*n/a*		*n/a*		0.22
Long range temporal correlations	DFA α	All	P7	0.46	Fp2	0.58	0.47
	DFA β	All	C3	0.5	F8	0.37	0.42
Delayed response task (response anticipation)	DRT upper α	All	P8	0.55	F8	0.45	0.48
	DRT θ	All	O2	0.32	Cz	0.48	0.40
	DRT SCP	All	Pz	0.35	T8	0.08	0.23
Graph theoretical parameters	Clustering Coeff, β2	All	*n/a*		*n/a*		0.33
	Clustering Coeff, β1	All	*n/a*		*n/a*		0.32
	Clustering Coeff, θ	All	*n/a*		*n/a*		0.24
	Clustering Coeff, α1	All	*n/a*		*n/a*		0.20
	Clustering Coeff, α2	All	*n/a*		*n/a*		0.20

Frontal EEG asmmetry	FA	Young females	*n/a*		*n/a*		0.37
	FA	Young males	*n/a*		*n/a*		0.32
	FA	Middle-aged females	*n/a*		*n/a*		0.11
	FA	Middle-aged males	*n/a*		*n/a*		0.01
Delayed response task (working memory effect)	DRT α memory effect	All	C4	0	O1	0.06	0.07
	DRT SCP memory effect	All	C3	0.04	P8	0	0.03
	DRT θ memory effect	All	O2	0.23	T8	0.08	0.03

n/a=not applicable; h^2=heritability

of the genetic variation of EEG power across the entire 1 to 25 Hz frequency range.

P300

The P300 is the positive deflection that begins 300 ms after the presentation of an infrequent target stimulus. In the field of cognition, it is thought to represent working memory processes called context closure (Verleger, 1988), context updating (Donchin & Coles, 1988), or event categorization (Kok, 2001). In the field of psychopathology, P300 amplitude has been systematically related to Alcoholism (Almasy et al., 1999; Polich et al., 1994) in alcoholics and family members of alcoholics. Also, reduced temporal P300 amplitude has been shown in Schizophrenia (Levit et al., 1973; Verleger & Cohen, 1978).

Adult P300 amplitude (50%) and latency (45%) in a visual oddball task showed substantial heritability. These results, too, seemed consistent with previous findings of twin and family studies in childhood and adolescence (e.g., Wright et al., 2001; Begleiter et al., 1998; see also van Beijsterveldt & van Baal, 2002).

The P300 is now widely believed to reflect multiple constituent components (Falkenstein et al., 1994; Dien et al., 2004) that may reflect stimulus evaluation, novel stimulus processing, and response selection processes respectively. This suggest that different genetic factors might influence the early, middle and late part of the P300. We therefore tested whether the P300 development over time (from 100 ms before the peak to 100 ms after) reflected the expression of different genes. Within 120 ms around the P300 peak at least 90% of the variation attributed to genetic influence on the signal amplitude were found to be overlapping. Within a 200 ms range (the full 100 before and after) at least 75% of the genetic variation was shared. We concluded that the subcomponents that constitute the full P300 wave, are influenced by the same genetic factor. From an individual differences perspective there is little evidence to suggest that differ-

ent parts of the P300 reflect stimulus evaluation, novel stimulus processing, and response selection processes, unless all these processes are influenced by the same genetic factor.

Recently, a central triggering mechanism for the P300 waves across the scalp was proposed by Nieuwenhuis et al. (2005) which is localized in the Locus Coeruleus. Individual differences in P300 latency at frontal, central and occipital leads could be explained by a single genetic factor, which, as argued in chapter four, may be consistent with a central trigger. In contrast, different genetic factors seem to influence the P300 amplitude at different scalp locations, suggesting local modulation of the P3 once triggered.

N1

The visual oddball task showed a clear occipito-temporal N1, and an earlier anterior N1 (Vogel and Luck, 2000). The N1 reflects early attentional resource allocation (Luck, 1995; Altenmüller & Gerlof, 1999). We showed that for the visual N1 a refined peak picking strategy is needed that uses separate windows for the anterior and posterior N1.The two previous twin studies on the visual N1 had not assessed the anterior and posterior components separately resulting in low twin correlations for anterior N1 amplitude and all latency scores (Almasy et al., 1999; Katsanis et al., 1997). In contrast, our study showed that posterior amplitude (50%) and latency (43%) both have substantial heritability, with estimates of comparable magnitude as the P300. Only anterior N1 amplitude showed a low heritability, but heritability of latency was again of comparable magnitude as that of the posterior N1 and P300 latency. From these results we concluded that the N1 deserves the same level of attention by geneticists that has now been reserved exclusively for the P300.

Frontal EEG Asymmetry

Frontal EEG asymmetry has been related, amongst others, to depression, anxiety, and affective style (approach vs. avoidance behavior) (e.g., Coan & Allen, 2004; Coan et al., 2006). Genetic analysis showed that in our sample, heritability was significant only in the young adult group (30% for males, 37% for young females). This is consistent with earlier studies by Coan (2003) in young adult males and females, and by Anokhin et al. (2006) in young adult females. No significant heritability was found in the middle-aged adults.

It is noteworthy that even in the young adults, heritability of frontal asymmetry is a far cry from the heritabilities of its constituent variables, F3 and F4 alpha power (89% and 90%).

Graph theoretical parameters

Graph theoretical ('Small-world') parameters clustering coefficient C and average path length L (Watts & Strogatz, 1998) define spatial patterning in connectivity between brain areas. Patterns with high clustering and low average path length are called 'small-world', and characterize an efficient functional brain network (Achard & Bullmore, 2007). Micheloyannis et al. (2005) reported the loss small world efficiency in a group of schizophrenics who had had a single psychotic episode. The same loss of 'small-worldness' was reported for a group of Alzheimer's patients by Stam et al. (2007). Ponten et al. (2007) reported that the small world parameters C and L show an increased ordered state (higher C but also higher L) in functional connectivity from intracranial recordings in epileptic patients.

Chapter 6 showed that in adults L has substantial heritability in the different frequency bands (35% to 63%). C was less heritable (20% to 35%) which may reflect larger measurement error as evident in the low epoch-to-epoch reliability coefficients. Taken together we concluded that the constituents of 'small-worldness' of the brain are heritable traits.

Long range temporal correlations

Detrended Fluctuation Analysis (DFA) provides a measure of temporal patterning as opposed to the spatial patterning described in the graph theoretical approach. Generally, oscillatory activity in the EEG signal, such as alpha, shows clear time-based structure in the amplitude of these oscillations. Previous amplitude levels in the system tend to determine the level at later time-points, and the strength of this auto-correlation decays following a power law. A power law decrease over time in the auto-correlation is a property of self-organized critical systems (Linkenkaer-Hansen, 2001).

Recently, Linkenkaer-Hansen et al. (2005) showed that long-range temporal correlations in EEG theta activity show a breakdown in the depressed patients compared to healthy controls. Monto et al. (2006) reported that DFA exponent of beta activity in subdurally recorded EEG is increased near the ictal focus in epileptic patients. Non-epileptic brain areas showed normal DFA exponents. In addition, they reported a deviation of the power law scaling of amplitude in the lower beta band (around ca. 13 Hz) which was larger near to the ictal focus.

As the first study to investigate family resemblance in long range temporal correlations, chapter 7 revealed a clear genetic basis to individual differences in the DFA exponent (heritability around 50%).

Delayed Response Task

Chapter 8 investigated the relationships between the Slow Cortical Potential (SCP) and upper alpha synchronization, and theta desynchronization that are

all seen to emerge in the response anticipation period of the DRT task, and are similarly responsive to an increae in working memory load during this interval. Theta desynchronization and upper alpha synchronization showed significant heritability across the scalp in both low (alpha: 18% - 49%; theta: 35% - 60%) and high (alpha: 31% to 46%; theta: 35% to 65%) memory load conditions, the latter yielding the highest estimates. SCP showed low to moderate heritability at the midline, occipital, and left parietal electrodes, with estimates again being larger in magnitude in the high (25% to 43%) than in the low (21% to 37%) load condition. The slow cortical potential showed a specific heritability distribution which was mostly posterior, whereas upper alpha synchronization and theta desynchronization showed scalp-wide heritability. Trivariate analysis of SCP, upper alpha synchronization, and theta desynchronization showed that the these parameters were largely influenced by *different* genetic factors, although some of the variation in upper alpha synchronization and ThD could be attributed to shared genes (ca. 20-25%). We concluded that these measures, although they share antecedent conditions—namely, the response anticipation in a delayed response task—do not reflect the same neural substrate.

Interestingly, the effect of memory load effect on these three parameters, although highly significant, were not heritable at all, which disqualifies them as endophenotypes of spatial working memory capacity.

Ranking of EEG/ERP measures

Why do the heritabilities of EEG/ERP parameters have the ranking they have? An obvious explanation is that they differ in the amount of measurement error. Since measurement error is attributed to the unique environmental component of the parameter, the relative contribution of the genetic component is reduced. It is reasonable to suggest that measurement issues are larger for Event Related Potentials (ERP), Event Related Spectral Perturbations (ERSP), and spatial or temporal patterningmeasures based on 4 to 6 relatively short (16 - 20 sec) epochs than for traits extracted from continuous recordings over a few minutes (Power, Frontal Asymmetry). N1 peaks, for example, are based on many trials that are averaged, but only on short periods within in those trials are used to determine the anterior N1 (88 – 168 ms) or posterior N1 (132 – 220 ms). Continuous recordings may have the advantage of all (or most) of the data providing information for the parameter in question.

To estimate measurement error—and its counterpart reliability—one can take the approach as taken in chapters four and six. In chapter four measurement error was estimated by taking the split half of trials, where odd and even trials were used to create two estimates for N1 amplitude and latency. The proportion overlap in variance between the two measures represents the amount of reli-

able variation, the rest was assumed to represent unstable variance. Posterior N1 amplitude and latency could be measured reliably (ca. 0.90). Here, adjusting the heritability estimates resulted in a neglible increase. Reliability was estimated to be much lower for Anterior N1 amplitude and latency (ca. 0.60). Adjusting for the unreliable variance increased these estimates from 22% to 35% for amplitude and from 45% to 56% for latency. These increases are not trivial.

In chapter six, a similar approach was used to incorporate measurement error into the genetic models, based on four repititions of 16 sec epochs. This led to a large increase in the heritability of C and L. Heritability of uncorrected C ranged from 20% to 33% and increased to 37% to 62% after correction for measurement error. Heritability of uncorrected L ranged from 35% to 68% and increased to 46% to 89% after correction. Again, these increases are not trivial.

In conclusion, differential amounts of measurement error account for part of the low heritability found in some of the EEG/ERP measures. Clearly higher heritability estimates are obtained if reliability of the EEG/ERP measures is statistically taken into account. In addition, signal-to-noise ratio may be improved experimentally by increasing the number of trials and the length and/or the number of epochs.

ENDOPHENOTYPES

An overarching idea driving our genetic dissection of ERP/EEG measures is the idea that they may be useful as endophenotypes. The obvious pathway to link genetic variation to variation in complex behavior is through the brain, i.e. allelic variation causes variation at the cellular level in the brain that in turn influences its network properties and complex output. To 'fill the gap' between genotype and complex behavior, the concept of endophenotype has been introduced (Gottesman & Shields, 1972; Gottesman & Gould, 2003; de Geus, 2002) to represent this intermediate brain level in the pathway from gene to its expression. Due to their simpler genetic structure, endophenotypes can (1) help localize parts of the genome that harbor genes for complex traits or diseases and, once candidate genes have been identified, (2) help explain how these genes exert their effects on brain and behavior.

As an example of the first, Williams (1999) could pinpoint the genomic region that codes for alcohol dehydrogenase on chromosome 4q through the use of the P300. Previous research had indicated that the amplitude of the P300 wave is reduced in alcoholics and family members of alcoholics. By using this endophenotype in a bivariate linkage analysis of alcohol problems and P300 amplitude, Williams et al. were able to detect a linkage peak on chromosome 4q, and a smaller peak near the GABA receptor gene area. Fine mapping of these areas resulted in

the identification of GABRA2 and ADH4, amongst others (Dick et al., 2005; Edenberg et al., 2004; Edenberg & Faroud, 2006).

Imaging genetics provides an example of how endophenotypes can be used to unravel the effects of an established candidate gene on brain activation (Hariri and Weinberger, 2003; Hariri et al., 2002; Hariri, et al., 2006; Meyer-Lindenberg et al., 2006). A functional variant in the serotonin transporter gene 5-HTT had been associated with neuroticism, particularly in combination with major life stress (Lesch et al., 1996; Caspi & Moffitt, 2006). Using fMRI, carriers of the risk allele were shown to have heightened activation of the amygdala in response to emotional stimuli. These results imply that amygdala activation assessed by fMRI is an endophenotype for effects of the 5-HTT gene on emotional processing, and perhaps anxiety differences in humans.

The results summarized in Table 1 bode well for EEG/ERP measures as potential endophenotypes. With a few exceptions, the ERP/EEG measures are heritable indices of brain function, fulfilling the second requirement listed on page 6 in the introductory chapter. Because they directly reflect brain activity they also seem to fulfill the fifth requirement of being meaningfully intermediate between genes and behavior. In keeping with the third requirement, these measures have shown significant association with disease states like depression, alcoholism, ADHD, schizophrenia, epilepsy, and Alzheimer in clinical populations (e.g., Allen et al., 1993; Almasy et al., 1999; Barry et al., 2003a, b; Begleiter et al., 1984; Blackwood, 2000; Bruder et al., 2001; Chabot & Serfontein, 1996; Clarke et al., 2001; Davidson et al., 1992; Debener et al., 2000; Ehlers & Schuckit, 1990, 1991; Elmasian et al., 1982; Field et al., 2000; Gabrielli et al., 1982; Gotlib et al., 1998; Henriques & Davidson, 1991; Lazzaro et al., 1998; Levit et al., 1973; Nitschke et al., 1999; Polich et al., 1994; Porjesz & Begleiter, 1990; Propping, 1977; Rangaswamy et al., 2002, 2004; Reid et al., 1993; Satterfield et al., 1972; Schaffer et al., 1983; Silva et al., 2002; Turetsky et al., 2000; Van Sweden & Niedermeyer, 1999; Verleger & Cohen, 1978; Vogel, 2000; Wiedemann et al., 1999).

We tried to replicate some of these associations in our non-clinical population-based sample. This met with little success. Frontal asymmetry did not show the expected relation to the risk for anxiety and depression. The small-world parameters C and L were not found to be related to cognitive performance (WAIS IQ). Similarly, individual differences in the DFA exponent did not predict Raven's IQ score. One potential explanation for the lack of these correlations is that frontal asymmetry, C, L, and DFA were all based on resting EEG. The unchallenged brain may not reveal those aspects of brain function that are functionally interesting. For example, resting state during which we collected EEG may not be as standardized as may appear from a methods section (Linkenkaer-Hansen,

personal communication, June 2007). Studies investigating 'resting-state' activity with fMRI have found that specific brain areas become activated. These areas are then deactivated during task execution (Raichle et al., 2001; Greicius et al., 2003; Raichle, 2006). However, we do not know what thought processes the subjects are engaged in during a resting state, which could consist of reminiscing, working memory activation, or consist of a real deactivated state. Measuring EEG under relevant (i.e., evoking and challenging) circumstances could provide a better match with behavioral traits. For frontal EEG asymmetry, for example, anxiety provoking situations could be used such as watching positively and negatively valenced scenes (Reeves et al., 1989) or emotional facial expressions (Jones & Fox, 1992). Likewise, endophenotypes of IQ should preferably be measured while performing actual IQ tasks such as the Raven's.

By way of exploration we computed correlations between all of our EEG/ERP measures and four complex behavioral traits, namely anxious depression, attention problems, weekly alcohol use and full scale IQ (see appendix 1). Clearly, there is no simple one-to-one mapping between our measures and these specific traits. Future research must establish the true nature of the relation between the level of brain function and the level of complex behavior, and under which experimental conditions these measures start to capture variation in these types of behavioral traits.

FUTURE DIRECTIONS

The EEG/ERP measures in this dissertation represent complex aspects of brain activity. Measures like C, L, and DFA have a strong theoretical basis and are directly linked to spatial and temporal organization of neural activity. Finding genes for these measures could constitute a major step forward in understanding individual differences in brain function and, potentially, how these phenomena are generated in brain tissue. Likewise, the "simpler" measures like P3, N1, and EEG power would be well served by increased genetic understanding. Finding even a single gene for a longstanding, but still quite elusive phenomenon like alpha oscillations—now nearing 90 years in age—could provide a bottom-up approach to its explanation in neural terms.

An important next step is to perform whole genome searches (through both linkage and whole genome association approaches) on these measures to find the genes underlying the heritabilities presented in Table 1. To do so, large samples with EEG data and genetic markers are needed. Fortunately, such samples are increasingly becoming available through biobanking of genetic material in several psychophysiological labs. The first successful gene finding studies have already been performed by Steinlein et al. (1992), who showed significant link-

age for the low-voltage EEG phenotype (Vogel, 1959, 2000) on chromosome 20q. Also, significant linkage of the biomarkers for alcoholism—P300 and beta power—were reported by the COGA group (Begleiter et al., 1998; Porjesz et al., 2002). Finally, Hansell et al. (2005) provided suggestive linkage for the SCP during response anticipation.

Taken together, I conclude that future identification of the actual genes underlying the heritability of my electrophysiological measures is both valuable and feasible.

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APPENDIX

Correlations between EEG/ERP measures and behavioral phenotypes.
The following table shows correlations between the EEG and ERP measures and four behavioral phenotypes: the *factor score* of anxious depression, *attention problems, alcohol use,* and *intelligence*.. If a measure was assessed at multiple leads, we used the lead with the highest magnitude on the respective EEG/ERP measure. For example, for alpha power O1 was used because power was maximal at this lead.

The liability for anxiety and depression (the factor score) was a weighted score on selected scales of the Dutch versions of the Spielberger Anxiety Inventory (STAI; Spielberger et al., 1970) and the Young Adult Self Report scale (YASR; Achenbach, 1990). Neuroticism and somatic anxiety were assessed with the Amsterdamse Biografische Vragenlijst (ABV; Wilde, 1970). The item content of the ABV neuroticism scale is very similar to that of the Eysenck Personality Questionnaire. From these traits a factor score was calculated after weighing each trait to maximize heritability of the factor score. Attention problems were assessed using the corresponding items of the Achenbach YASR (see van den Berg et al., 2006). Alcohol use items included questions on frequency and amount of use. Alcohol use was determined with a question on weekly alcohol intake (in units). Intelligence was scored for this subject sample with the Dutch version of the WAIS.

Correlations between endophentypes and behavioral phenotypes.

Analysis	variable	subjects	factor score	attention problems	units per week	Full scale IQ
Power	α	Young	0.01	-0.03	-0.01	-0.08
		Middle-aged	-0.02	-0.03	0.01	0.12
	θ	Young	-0.03	-0.02	0.03	-0.11
		Middle-aged	-0.03	-0.04	0.08	0.01
	β	Young	0.04	0.04	0.00	0.01
		Middle-aged	0.04	0.01	0.05	0.02
	δ	Young	0.05	0.05	0.06	-0.14
		Middle-aged	-0.01	0.00	0.04	-0.06
Graph theoretical analysis	Path length, θ	All	-0.02	0.07	0.07	-0.03
	Path length, β2	All	0.06	0.09	-0.01	-0.03
	Path length, σ2	All	0.03	0.04	-0.02	-0.07
	Path length, β1	All	0.09	0.11	-0.01	-0.03
	Path length, σ1	All	0.03	0.01	0.05	0.03
Visual oddball amplitude and latency	P300 amplitude	All	-0.03	-0.03	-0.05	-0.01
	Posterior N1 amplitude	All	-0.01	0.04	-0.01	0.05
	Anterior N1 latency	All	0.10	0.10	-0.03	0.04
	P300 latency	All	0.06	0.02	0.04	-0.03
	Posterior N1 latency	All	0.03	0.03	0.02	-0.03
	Anterior N1 amplitude	All	0.03	0.01	0.03	0.00
Long range temporal correlations	DFA σ	All	0.07	-0.02	0.10	-0.05
	DFA β	All	0.10	0.06	0.06	0.01
Delayed response task	DRT upper σ	All	0.03	0.10	0.10	0.02
	DRT θ	All	-0.04	0.03	0.00	-0.01
	DRT SCP	All	0.10	0.10	0.02	0.05
Graph theoretical analysis	Clustering Coeff, β2	All	-0.01	0.02	0.05	0.04
	Clustering Coeff, β1	All	-0.03	0.00	0.00	0.02
	Clustering Coeff, θ	All	-0.01	0.04	**0.14**	0.04
	Clustering Coeff, σ1	All	0.07	0.05	0.07	0.06
	Clustering Coeff, σ2	All	-0.02	0.00	-0.03	-0.04
Frontal EEG asmmetry	FA	Young females	0.12	0.05	0.01	-0.16
		Young males	0.01	0.04	0.13	0.14
		Middle-aged females	0.01	0.07	-0.04	0.12
		Middle-aged males	0.02	0.01	-0.12	0.08
Delayed response task (memory effect)	DRT σmemory effect	All	0.00	0.00	0.01	-0.01
	DRT SCP memory effect	All	-0.04	0.02	0.02	0.00
	DRT θ memory effect	All	-0.05	-0.02	-0.01	0.01

Note. All correlations corrected for cohort and sex effects. Bold is significant at 0.05.

P

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SAMENVATTING

DE GENETICA VAN ELEKTROFYSIOLOGISCHE HERSENACTIVITEIT

Het doel van dit proefschrift was om de genetische architectuur te onderzoeken van hersenactiviteit gemeten met elektroencephalografische registraties (EEG). Hersenactiviteit kan in rusttoestand worden gemeten om een indruk te krijgen van de voor het brein kenmerkende ritmische activiteit van zenuwcellen in verschillende frequentiebanden. Uit dit zogenaamde rust-EEG kan worden afgeleid welke frequenties sterk vertegenwoordigd zijn in het brein van een individu en welke minder sterk. De ritmische activiteit van het brein vindt verspreid over het brein plaats en bepaalde ritmes kunnen met enige regelmaat verdwijnen en terugkeren in de tijd. Uit het optreden van de ritmes op verschillende plaatsen in het brein en op verschillende momenten in de tijd kunnen we iets opmaken over de mate waarin verschillende delen van het brein functioneel met elkaar verbonden zijn en de mate waarin hersenactiviteit stabiel blijft over de tijd. Hersenactiviteit kan ook in geactiveerde toestand worden gemeten. Daartoe wordt herhaaldelijk een bepaalde prikkel aan de proefpersoon aangeboden en wordt de gemiddelde hersenactiviteit in reactie op die prikkel berekend. Dit noemen we een Event Related Potential, of ERP. ERP's kunnen positieve of negatieve potentialen zijn, die op verschillende tijdstippen na de prikkel optreden en sterk verschillen in voltage. Verschillen tussen personen in het tijdstip en de hoogte van het gemeten potentiaalverschil worden gebruikt om vast te stellen hoe de informatieverwerking van de aangeboden prikkel precies verloopt.

Van zowel het rust-EEG als verschillende ERP maten is reeds lang bekend dat ze een grote mate van variatie vertonen tussen verschillende individuen. In dit onderzoek wilden we vaststellen of erfelijke factoren een rol speelden in deze individuele verschillen. Hiervoor waren EEG-registraties van 263 eeneiige en 303 twee-eiige tweelingbroers en -zussen en hun 195 eenlingbroers en -zussen beschikbaar.

Tabel 1 uit de engelse samenvatting geeft een overzicht van de erfelijkheid van alle EEG/ERP maten die in dit proefschrift worden onderzocht. De erfelijkheidsschattingen zijn gescheiden per cohort en/of sekse indien van toepassing. Sommige EEG/ERP maten zijn bepaald op meerdere plaatsen op de schedel. In dat geval zijn in de tabel de erfelijkheid van de plaats met het grootste en kleinste effect opgenomen. Bijvoorbeeld, de erfelijkheid van hersenactiviteit in de alfa-frequentieband (alpha power) wordt getoond voor de elektroden met de hoogste en laagste activiteit (O2 en T8, respectievelijk). Tevens toont de tabel de gemiddelde schatting van de erfelijkheid over alle elektroden. De belangrijkste conclusie uit Tabel 1 is dat elektrofysiologische hersenactiviteit zeer goed bruik-

baar is als een stabiele en vooral genetische maat voor het functioneren van het brein. In de volgende paragrafen zal ik elk van de gemeten EEG/ERP maten in meer detail bespreken.

Ritmische activiteit in verschillende frequentiebanden
De ritmische activiteit van het rust-EEG kan worden verdeeld in verschillende frequentiebanden. De klassieke banden zijn delta, van 1 tot 4 Hertz, theta van 4 to 8 Hertz, alfa van 8 tot 12 Hertz en bèta van 12 tot 25 Hertz. In hoofdstuk 2 wordt besproken hoe de ritmische activiteit in deze verschillende frequentiebanden samenhangt met verschillende vormen van psychopathologie, zoals ADHD (Barry et al., 2003a, b; Chabot en Serfontein, 1996; Lazzaro et al., 1998; Satterfield et al., 1972; Clarke et al., 2001) en alcoholisme (Gabrielli et al., 1982; Rangaswamy et al., 2002, 2004). In deze studies wordt als maat voor de ritmische activiteit in een bepaalde frequentie het 'vermogen' gebruikt, meestal aangeduid met de Engelse term power. Vanwege de samenhang tussen het vermogen in de bètafrequenties en alcoholisme is het EEG reeds succesvol gebruikt om de genen die een risico op alcoholisme geven op te sporen (Porjesz et al., 2002; Edenberg et al., 2004; Dick et al., 2006; Porjesz et al., 2005).

In dit proefschrift heb ik laten zien dat in volwassenen de EEG power in de verschillende frequentiebanden sterk erfelijk bepaald is. De erfelijkheid van alfapower is zelfs vergelijkbaar met de erfelijkheid van lichaamslengte, een van de meest erfelijke eigenschappen die mensen bezitten (Silventoinen et al., 2006). De erfelijkheid van power in de thèta- en bètabanden was tevens hoog, terwijl de delta-activiteit als enige een lage erfelijkheid vertoonde. Deze schattingen stemmen goed overeen met eerder gerapporteerde resultaten (van Beijsterveldt en van Baal, 2002). De erfelijkheid was significant lager op middelbare leeftijd vergeleken met jongvolwassen leeftijd, hoewel het verschil was bescheiden (respectievelijk 90% en 85% voor de power in de alfaband bijvoorbeeld). Topografische verschillen waren ook minimaal, dat wil zeggen dat de EEG activiteit op de verschillende plaatsen op het hoofd ongeveer even erfelijk was.

Tenslotte bleek er geen genetische grondslag te zijn voor het indelen van de ritmische hersenactiviteit in de klassieke frequentiebanden. Wanneer we de erfelijkheid berekenden in smalle bandjes die maar 1 Hertz breed waren leverde dit redelijk eenparige 'erfelijkheidsspectra'. Alleen onder de 6 Hertz (frontale electroden) en boven de 13 Hertz (electroden T7 en T8) zakten de erfelijkheidschattingen wat. Dit betekent dat de functionele verschillen die aan activiteit in de klassieke frequentiebanden wordt toegeschreven (Klimesch, 1999; Rangaswamy et al., 2002; Clarke te al., 2001) niet terugkomen in de genetische grondslag van deze banden. Tenminste 55%, maar meestal 60% tot 75% van de genetische variatie wordt gedeeld door alle frequentiebanden. Dit is in goede overeenstemming

met de resultaten van Anokhin et al (2004) die ook hoge genetische covariatie rapporteerden tussen de power in de delta, thèta, alfa, en bèta frequentiebanden. Geconcludeerd mag worden dat één enkele genetische factor het merendeel van de genetische variantie verklaart in het hele frequentiespectrum (1 tot 25 hertz) van het rust-EEG.

P300

De P300 is een positieve ERP in het EEG die ongeveer 300 ms na de presentatie van een stimulus optreedt die van belang is voor het god uitvoeren van de taak (target). In de zogenaamde visual oddball task is de taak bijvoorbeeld om plaatjes van katjes te tellen. De proefpersoon krijgt dan vooral hondjes te zien met af en toe daartussen een katje. In reactie op het katje is in het EEG een duidelijke P300 te zien. In het cognitieve onderzoeksveld wordt de P300 gebruikt om werkgeheugenprocessen in kaart te brengen zoals context closure (Donchin en Coles, 1988), context updating (Verleger, 1988), of event categorization (Kok, 2001). In de psychopathologie representeert een gereduceerde P300 amplitude een neiging tot alcoholisme (Almasy et al., 1999; Polich et al., 1994) bij alcoholici en hun familieleden. Gereduceerde P300 amplitude is ook aangetoond bij schizofrenie (Levit et al., 1973; Verleger en Cohen, 1978).

In dit proefschrift bleek de P300 bij volwassenen duidelijk te worden beïnvloed door erfelijke factoren (erfelijkheid amplitude was 50% en latentie 45%). Deze resultaten waren in overeenstemming met eerdere tweeling- en familiestudies bij kinderen en adolescenten (Wright et al., 2001; Begleiter et al, 1998; zie ook van Beijsterveldt en van Baal, 2002).

Er wordt over het algemeen aangenomen dat de P300 opgebouwd is uit meerdere componenten (Falkenstein et al., 1994; Dien et al., 2004) die cognitieve subprocessen reflecteren zoals stimulusverwerking en responsselectie. Dit kan suggereren dat verschillende genetische factoren een rol spelen in de vroege, middel, en late gedeeltes van de P300. Daarom hebben we onderzocht of de ontwikkeling van de P300 over tijd (van 100 ms voor tot 100 ms na de piek in amplitude) de expressie van dezelfde of verschillende genen reflecteert. Binnen 120 ms rond de piek (dus 60 ms ervoor en 60 ms erna) is er tenminste 90% overlap in genetische expressie. Binnen de volle 200 ms voor en na de piek bleek tenminste 75% van de individuele variantie een gedeelde genetische bron te hebben. Om deze redenen concluderen wij dat er weinig bewijs is voor verschillende genen voor de cognitieve subprocessen die ten grondslag liggen aan de P300.

Recentelijk is geopperd dat een centrale invloed van een bepaalde hersenstructuur op de P300 -- de locus coeruleus, en de neurale projecties vanuit deze kern naar de neocortex -- goed kan verklaren waarom de P300 op sommige plaatsten in het brein eerder valt waar te nemen dan op andere plaatsen (Nieuwenhuis et

al., 2005). In overeenstemming met deze theorie bleek dat ook bij onze data het geval. Daarbij konden individuele verschillen in P300 latentie op de frontale, centrale en pariëtale elektroden verklaard kunnen worden door één enkele genetische factor.

N100

De visual oddball task zoals gebruikt in de voorgaande paragraaf leverde naast een duidelijke P300, ook een duidelijke N100 op. Deze ERP was op twee plaatsen te onderscheiden, aan de voorkant van het brein, de anterior N1, en aan de achterkant van het brein, de posterior N1. De twee eerdere tweelingstudies die eenzelfde visuele taak hanteerden hebben geen afzonderlijke anterior en posterior N1 gebruikt en rapporteerden lage erfelijkheidscores voor de amplitude en latentie van de N1 (Almasy et al., 1999; Katsanis et al., 1997). We hebben aangetoond dat het met een strategie waarbij elke golf een eigen tijdspanne toegewezen krijgt wel mogelijk is om de anterior en posterior N1 apart te scoren. Met deze benaderingen vonden we wel een flinke erfelijkheid voor individuele verschillen in posterior N1 amplitude (50%) en latentie (43%), zeer vergelijkbaar met die voor de P300. De amplitude van de anterior N1 liet ook bij ons een lage erfelijkheid zien (20%), maar de erfelijkheid van de latentie was duidelijk hoger dan in voorgaand onderzoek (45%). De N1 wordt als een maat van vroege aandachtsprocessen gezien (Luck et al., 1995; Altenüller en Gerloff, 1999) die een belangrijke rol kunnen spelen bij allerlei cognitieve vaardigheden. Onze conclusie luidde dan ook dat de (correct gescoorde) N1 dezelfde aandacht verdient van gedragsgenetici die tot nu toe was voorbehouden aan de P300.

Frontale asymmetrie

De linker- en rechterhersenhelft vertonen vaak een asymmetrie in hersenactiviteit. Deze frontale EEG asymmetrie is herhaaldelijk in verband gebracht met de verwerking van emotie en verschillen tussen personen in emotionele stabiliteit (neuroticisme, angst en depressie) en affective style (ontwijkend en toenaderend gedrag) (bijv. Coan en Allen, 2004; Coan et al., 2006). Onze genetische analyse van frontale asymmetrie toonde aan dat in de door ons onderzochte groep erfelijkheid alleen een rol spelde in de frontale asymmetrie van de jongvolwassenen (30% voor mannen, 37% voor vrouwen). Dit is consistent met eerdere studies van Coan (2003) in jongvolwassen mannen en vrouwen, en Anokhin et al. (2005) bij jongvolwassen vrouwen. Bij volwassenen van middelbare leeftijd werd geen genetische invloed op frontale asymmetrie gevonden.

Graaftheoretische analyse

In hoofdstuk 6 van dit proefschrift werden twee graaftheoretische parameters ge-

introduceerd – clustercoeffiënt C en padlengte L, ook wel small-world parameters genoemd. Deze parameters beschrijven de belangrijkste patronen die in alle denkbare verbindingen tussen hersengebieden worden aangetroffen. Patronen met een hoge clustergraad en lage gemiddelde padlengte worden small-world genoemd, en karakteriseren een efficiënt netwerk van breinverbindingen (Achard en Bullmore, 2007). Micheloyannis et al. (2005) hebben aangetoond dat schizophreniepatiënten en verlaagde small-world-efficiëntie hebben. Eenzelfde verlies van efficiëntie is aangetoond bij de ziekte van Alzheimer door Stam et al. (2007). Ponten et al (2007) hebben aangetoond dat C en L een hogere ordening tonen (hogere C, maar ook hogere L) in de connectiviteit tussen hersengebieden gemeten met intracraniale elektroden bij patiënten met epilepsie. Al deze resultaten tonen een mogelijk klinisch belang van graaftheoretische parameters aan.

In hoofdstuk 7 hebben we de erfelijkheid van L en C bepaald in het rust-EEG. We toonden aan dat L in elk van de verschillende frequentiebanden redelijk erfelijk (35% tot 63%). C was minder erfelijk (20% tot 35%), maar dit kwam deels door een grote gevoeligheid van C voor meetfout.

Terugkerende ritmische hersenactiviteit over langere tijd (Long range temporal correlations)
Detrended Fluctuation Analysis (DFA) is een analysemethode die temporele patronen in het EEG kan ontdekken, dit in tegenstelling tot de spatiële patronen die we met C en L in kaart konden brengen. Over het algemeen toont ritmische hersenactiviteit (zoals alfa-oscillaties of alfa-activiteit) een duidelijke structuur in de tijd waarbij hetzelfde ritme met dezelfde amplitude steeds terugkeert. Dat eerdere niveaus van de amplitude van de alfa-oscillaties goede voorspellers voor de latere amplitudes zijn betekent in statistische zin dat een signaal over een langere periode met zichzelf correleert (Long Range Tenmporal Correlations). De sterkte van deze autocorrelatie neemt logaritmisch af met de tijd wat typisch is voor zelforganiserende systemen die zich in een zogeheten kritische overgangstoestand bevinden (Linkenkaer-Hansen et al., 2001).

De snelheid waarmee de autocorrelatie daalt heet de DFA-exponent. Recentelijk is aangetoond dat de DFA-exponent van theta-band oscillaties verlaagd is in depressieve patiënten (Linkenkaer-Hansen et al., 2005). Anderen hebben aangetoond dat de DFA-exponent juist verhoogd is nabij de focus van epileptische activiteit – gemeten met subdurale elektroden bij epileptische patiënten.

In dit proefschrift werd voor het eerst naar de erfelijkheid van de DFA component gekeken. Hoofdstuk 7 toonde glashelder aan dat genetische verschillen tussen mensen in belangrijke mate bijdragen aan de individuele verschillen in deze EEG maat (erfelijkheid = 50%). Tot onze verassing spelen heel andere genen een rol bij de power van de hersenritmes dan bij de temporele structuur in die ritmes.

Trage Potentialen

Hoofdstuk 8 onderzocht de relatie tussen de Slow Cortical Potential (SCP), Upper Alpha Synchronization (UAS), en Theta Desynchronization (ThD). Deze drie maten treden allen op in de periode tussen een waarschuwingsignaal en een responssignaal in een zogeheten 'uitgestelde responstaak'. Eerder onderzoek heeft aangetoond dat de mate waarin deze drie optreden is afhankelijk van de mate waarin het werkgeheugen wordt aangesproken. Hoofdstuk 8 onderzocht of SCP, ThD, en UAS erfelijke eigenschappen zijn in twee condities (de lage werkgeheugenconditie waarin geen ruimtelijk geheugen vereist was en de hoge werkgeheugenconditie waarin wel ruimtelijk geheugen vereist was.) Daarnaast werd gekeken of de verschilscore tussen beide condities erfelijk was.

ThD en UAS waren beide significant erfelijk in alle hersengebieden. Dit gold voor zowel de lage werkgeheugenconditie (Thd: 18% tot 49%, UAS: 35% tot 60%) als de hoge werkgeheugenconditie (Thd: 31% tot 46%, UAS: 35% tot 65%). SCP bleek laag tot gematigd maar significant erfelijk voor de elektroden op de middenlijn, op het achterhoofd en de linker zijkwab, met erfelijkheid hoger in de hoge werkgeheugenconditie (25% tot 43%) dan in de lage werkgeheugenconditie (21% tot 37%). De erfelijkheid van de ThD en UAS was over de hele schedel ongeveer gelijk. Deze drie parameters bleken voornamelijk door afzonderlijke genetische factoren te worden beïnvloed, hoewel 20% tot 25% van de variantie van ThD en UAS toch nog enige genetische overlap toonde.

Uit bovenstaande concludeerden wij dat de drie EEG/ERP maten weliswaar alle optreden in de periode tussen waarschuwingssignaal en responsesignaal, maar dat individuele verschillen erin niet gebaseerd zijn op dezelfde genen, en daarom wellicht niet op hetzelfde biologisch mechanisme teruggaan.

Endofenotypes

Een van de belangrijkste redenen om de genetische architectuur van de bovengenoemde EEG/ERP maten in kaart te brengen is dat deze maten mogelijke endofenotypen kunnen zijn voor belangrijke gedragskenmerken (of psychopathologie). Een endofenotype is een breinkenmerk dat ligt tussen genen en gedrag (Gottesman en Irving, 1972). Endofenotypen kunnen 1) helpen in het lokaliseren van die delen van het genoom die genen bevatten voor complexe eigenschappen, en 2) helpen verklaren hoe deze genen hun invloed uitoefenen op de individuele verschillen in de het gedrag of de psychopathologie. De eerste en cruciale vereiste aan een endofenotype is dat het een erfelijke eigenschap is. Voor de meeste van de EEG/ERP maten die in de voorgaande paragrafen behandeld werden, blijkt dat zeker te gelden. Een zoektocht naar de exacte genen die deze maten beïnvloeden lijkt dus verantwoord.

Zelfs zonder een directe link naar gedrag te maken verdienen de hier bestu-

deerde maten verder genetisch onderzoek. Ze representeren complexe aspecten van het functioneren van het brein. Maten afgeleid van graaftheoretische analyse of Detrended Fluctuation Analysis hebben een sterke theoretische basis en zijn direct gelinkt aan spatiële en temporele organisatie van neurale activiteit. Het zoeken naar genen die de individuele verschillen in deze maten veroorzaken zou een grote stap voorwaarts kunnen betekenen in ons begrip van het functioneren en disfunctioneren van het brein.

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Dit proefschrift is niet totstandgekomen door mijzelf alleen. Grote delen van de last werd door anderen gedragen. Hieronder volgt een kleine, niet uitputtende opsomming van namen die ik in het bijzonder zou willen noemen.

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