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Summary and conclusions

The present study investigated the genetic and environmental contributions to individual differences in three indices of the central nervous system (CNS); EEG power, P300 amplitude and EEG coherence. EEG power and coherence were obtained during quiet resting, and P300 amplitude was measured during a simple oddball task. In the experiment 213 twin pairs (aged 16 years) participated twice, with one and half year in between. The thesis dealt with the genetic analyses of the data obtained on the first occasion, and the descriptive statistics of both measurements.

Behavior genetics is interested in the problem whether and to what degree individual differences of human behavior are influenced by genetic factors. For most behaviors genetic influences are substantial. They contribute to cognitive abilities, most personality traits, but also to psychopathological behaviors like schizophrenia, alcoholism and psychoses (Plomin et al., 1990). Because behavior is in continuous interaction with the environment, the mechanism of genetic involvement in human behavior is a complex issue to study. Genetic influences on behavior are most likely to be expressed via the brain. Therefore, by studying human brain functioning it may be possible to find an explanation for the genetically determined influences on behavior. For behavioral traits such as cognition, several studies indicate that slight genetic effects early in the development are magnified as development proceeds, creating increased genetic variance while genetic covariance remains high. It may be that genetic differences in neuroanatomy or neurophysiology early in life could cascade into increasingly larger behavioral differences among children as life goes on (Plomin, 1986, p. 328). Until now, there is not much information about the heritability of neurophysiological indices.

Chapter 2 presented a review of the twin and family studies of normal variation in the human EEG and ERPs. The discussion of chapter 2 listed several issues that deserved more attention in the quest for the genetically mediated differences in the CNS functioning. An issue that deserved attention is, that most of genetic studies of EEG/ERP have employed comparisons of correlations between relatives as their main method of genetic analysis. Almost no twin study of EEG/ERPs parameters applied the more powerful method of genetic model fitting, although this approach

provides many advantages (Eaves et al., 1978). Model fitting methods yield formal tests of the significance of genetic factors, tests of significance of sex differences in genetic architecture, and a relatively straightforward extension to multivariate analysis.

The present study has taken these issues into account. Most important, a large number, 213, twin pairs participated in the study. Furthermore by including female and male MZ and DZ twin pairs and DZ opposite-sex twin pairs, it was possible to test for sex differences in genetic architecture. Brain activity was measured on 14 brain locations and multivariate analyses have been applied to the EEG alpha band and P300 amplitude, allowing more insight into the underlying genetic factors of the covariances among brain areas.

In the next paragraph the results of the genetic analyses of EEG power, P300 and coherence of the first measurement are discussed with respect to the following issues: heritabilities in the different brain areas, multivariate genetic analysis, and sex differences. In the next paragraph methodological issues are considered. In third paragraph the stability of phenotypic values of the EEG/ERP parameters and the genetic stability are discussed.

Heritability estimates of measurement on time 1

The neural indices obtained in the 16-year old subjects showed a high heritability. A large part of individual differences in brain activity could be explained by genetic factors. The averaged heritability of the EEG power is above 80%, of the EEG coherence around the 60%. The brain response to a stimulus, obtained during the oddball task showed, however, a less clear result. Though clear familial resemblance for the P300 amplitude was found, no distinction could be made between the resemblance attributable to genetical or to shared environmental factors. For the P300 latency no heritable influences were found.

The environmental influences on the individual differences of the EEG power and EEG coherence consisted of influences nonshared within twin pairs. The mode of the inheritance of EEG power and coherence was additive. For some brain areas of the beta frequency the heritability consisted of additive genetic factors and genetic dominance.

Heritabilities of neural indices measured in different brain areas
EEG power varies as function of state of the subject and of the area of the brain over which the signals are measured. Figure 3.3 in chapter 3 presented the distribution of the EEG signals over the different brain areas, obtained during rest. It clearly shows the different distribution of frequencies and the larger amplitudes of the alpha frequency at the posterior positions and almost no EEG power for the

beta frequency band. The various brain areas seem to differ in anatomy and function. Also the function of the hemispheres seems to differ (Gur & Gur, 1987). If there are structural/functional differences among brain areas, different heritabilities could be expected. However, for EEG power the heritability is large in all brain areas and for all frequencies. The lowest heritabilities were found in the frontal areas of the lower frequency bands. It may be that eye-movements interfere with the frontal EEG activity and reduce the reliability and as a consequence lower the heritability estimates.

Little differentiation of the heritability was also found for the P300 amplitude and EEG coherence. For the P300 the genetic analysis was employed only to the central, parietal and occipital electrode positions. The results did not show large differences among these brain areas. EEG coherence was estimated for electrode-combinations along the anterior-posterior axis within a hemisphere for four frequency bands. Again, little differentiation of the heritabilities was found for various combinations of electrode pairs in the four frequency bands.

Thus, in spite of the anatomical and functional differences of brain areas, no differences in heritability in these brain areas were found for the three EEG/ERP parameters. Although it has been suggested that the variability in brain morphology is larger (and heritability lower) in the younger, frontal brain regions (Meshkova & Ravich-Shcherbo, 1982; Markowitsh, 1988), this is not reflected in the genetics of EEG/ERP measures.

Multivariate genetic analyses

Multivariate genetic analysis provides information on the causes of covariance among various brain regions, i.e. whether the covariance between two or more measures arises because they are influenced by the same genes or by the same shared environmental factors. Multivariate analyses were applied to EEG power and P300 amplitude.

The phenotypic correlations of the EEG power between the left and right hemisphere and among the various brain areas were very high. Therefore, it was investigated in chapter 3, whether this covariation is due to the same underlying genetic factors. From the results it appears that, for all frequency bands, the covariances between the left and right hemisphere seemed primarily determined by the same genetic factors. Within each hemisphere, the covariance among the various brain areas for the alpha frequency seems primarily determined by the same genetic factors. A high phenotypic correlation among brain areas within a hemisphere is also seen for the other frequency bands, most probably due to the same genetic influences. Since neurophysiological mechanisms of alpha rhythm generation are complex and involve different brain structures with a complex interaction between them (Steriade et al., 1990), the mechanisms of genotypic

influence on brain electric activity may be rather complex and may involve intermediate levels, such as, a morphological and a biochemical level.

For the amplitude of the P300 the multivariate analyses were used to test whether the same genes/shared environmental factors were expressed over the different brain areas. The genetic analysis was carried out for the frequently occurring stimuli, the nontargets, and the infrequently occurring stimuli, the targets. The results showed that individual differences in the P300 amplitude of both targets and nontargets were influenced by the same genetic/shared environmental influences. In addition, a likely second factor influenced the P300 amplitude in the occipital positions. If this second genetic factor for the targets is significant, then it could be used as a possible indicator of more than one neural generator of the P300. The multivariate genetic analysis was extended to include both kinds of stimuli in the analysis, to investigate whether the same structure of genetic/shared environmental factors influences the targets and nontargets. The results showed one factor underlying both targets and nontargets, but also a second factor with a higher loading for nontargets. This implies an interesting interaction between a psychological test condition and familial resemblances: the familial resemblances depended on information processing demands.

Sex differences

In the human brain structural and functional sex differences are suggested (Gur et al., 1995; Kimura, 1987). For example, using in vivo magnetic resonance morphology Steinmetz et al. (1995) found sex differences in size and morphology of the corpus callosum. Sex differences in hemisphere specialization of function have also been found (McGlone, 1980; Gur et al., 1982). In spite of these differences, brain activity obtained with EEG/ERP measures showed no consistent results regarding sex differences in means. In the study reported in this thesis, no sex differences in mean EEG powers were found. For the P300 a significant interaction between sex and condition was found: females had slightly larger amplitudes for the infrequent target stimuli than males. Males had slightly larger amplitudes for the nontarget P300. The EEG coherence showed significant differences between males and females. The sex differences were significant for all four frequency bands. For most combinations of electrode pairs the coherence was larger for females than for males.

This is the first study that explicitly tested sex differences in the genetic architecture of individual differences. Models were used to test for sex differences in the magnitude of genetic influences and to test whether the same genes were expressed in males and females. For EEG power and EEG coherence, the analysis of individual differences in brain activity did not show sex differences in genetic architecture. Except, in some brain areas the heritability of EEG power was lower in females than in males. The differences in heritabilities, however, were very

small. No indication was found that different genes were expressed in males and females. For the EEG coherence no sex differences in genetic architecture were found.

The relative contribution of the genetic and/or environmental factors to the P300 amplitude differed in males and females. Although the nearby zero correlations of the opposite twin pairs suggested a different source for the familial resemblance in males and females, model fitting did not find any evidence for this hypothesis. An interpretation of these sex differences in terms of genetic and/or environmental factors is not possible. Although clear familial resemblance for the P300 amplitude was found, was it not possible to determine whether these familial resemblances are due to genetic or due to shared environmental factors. The problem of resolving whether the familial resemblance for the P300 amplitude is genetic or not, probably lies in statistical power, this topic is discussed in the next paragraph.

Methodological issues

Statistical power of the twin study
The statistical power of a twin study depends on a number of factors, these include the number of twin pairs and the size of effect of the heritability or shared environmental factors in the population being studied (Neale and Cardon, 1992). Genetic analysis of the P300 amplitude showed clear familial resemblance, nonetheless with the current data set it was not possible to distinguish whether this resemblance was attributable to genetic or shared environmental factors. Probably no optimum statistical power was achieved to detect the presumably smaller genetic and/or shared environmental effects. In Table 6.1 the number of subjects is depicted that is required to reject the false model with a statistical power of 80%. The heritabilities of 80%, 60%, and 40% are values based on the heritabilities obtained for EEG power, P300 amplitude, EEG coherence, respectively. A CE model (=model with as latent factors shared and nonshared environmental factors) without sex differences was fitted to data that were simulated with an actual AE model (model with additive genetic factors and nonshared environmental factors).

Table 6.1 Number of subjects required to reject, at the .05 level and 80% power, a CE model when the true model is AE and the ratio MZ to DZ pairs is 1:1. h^2 refers to true heritability

$h^2 = 80\%$	52
$h^2 = 60\%$	180
$h^2 = 40\%$	597

With 52 twin pairs a statistical power of 80% is obtained to detect a trait which is largely determined by (h^2=.80) genetic influences. To detect a smaller heritability of about 40%, a larger set of twin pairs is required. To make a distinction between genetic and shared environmental factors for the P300 amplitude a larger sample thus will be needed. Alternatively statistical power can be increased by considering measurement error in the phenotype. For 60% and 80% heritability the sample size used in this study was clearly sufficient, however to detect a 40% heritability it was too small.

Implications of reliability for the heritability
Split-half correlations are informative for the heritability. The reliability of a trait sets an upper limit to its heritability (Falconer, 1981). Heritability is calculated as the variance accounted for genetic factors divided by the total variance (Va/(Va+Vc+Ve)). Because the Ve part includes both the nonshared environment and the measurement error, a high measurement error could give a biased picture of the heritability. In the review of the twin and family studies about the EEG and ERP it was already noticed that the reliability of the ERP is lower than that of the background EEG.

In this thesis the heritability of the EEG power, P300 amplitude and EEG coherence was estimated with traditional genetic models. In the traditional method the nonshared environmental factor includes the measurement error. Inclusion of reliability data would allow for explicit representation of assumptions about measurement error. For the EEG power the proportion of total variance accounted by measurement error is very small, so no large effect of separately estimating the measurement error is expected on the heritability. However, as the split-half correlations indicate, the effect of measurement error on the P300 amplitude is larger. If the heritability would be estimated from the proportion of reliable variance, the heritability would be enlarged by only analyzing the reliable part of the variance in the genetic model. Likely, the nonshared environmental part of the EEG coherence is also inflated by the measurement error. The split-half correlations of the combinations of electrode pairs over longer distances of the delta and theta frequency bands were lower than of the alpha and beta band. For these bands the heritability, corrected for measurement error, is probably higher than it was estimated in chapter 5.

Stability

Stability of phenotypes values

For EEG power, high test-retest correlations for all electrode positions of the four frequency bands were found, especially for alpha frequency. For each electrode position of the alpha frequency the test-retest correlation was above .8. Lower test-retest correlations wer found for the frontal electrode positions of the delta frequency. Possible contamination of eye-movements, which are in the same range of the delta frequency, could account for these low test-retest correlations. However, the period between the two measurements concurs with a period of maturation in the frontal lobes (Thatcher et al., 1987; Hudspeth & Pribram, 1992; Buchsbaum et al., 1992). Different rates of development changes could account for these lower test-retest correlations (additional variability).

In contrast with the EEG power, the test-retest correlations obtained for the P300 amplitude are lower (see appendix). For target P300 the test-retest correlation was, averaged over all leads, .6. For the nontarget amplitude similar correlations were found. These correlations agree with those obtained by Segalowitz and Barnes (1993). In the same age group (15 years) and with a comparable time-interval (2 years) they found for the target P300 amplitude a correlation of .62. Polich (1986) and Fabiani et al. (1987), however, reported higher test-retest correlations. The stability is smaller than obtained for the EEG power, which is probably due to the lower reliability, as the split-half correlations were also lower than for the EEG power.

Concerning the magnitude of the test-retest correlations, EEG coherence occupy a midposition between EEG power and P300 amplitude. As far as we know, only three earlier studies mentioned the test-retest correlations. However, the test-retest correlations were calculated in very different subject samples (children, patients and normal adults) with different methods to estimate the EEG coherence. The values for the test-retest correlations fluctuate from .4 to .8. (Gasser et al., 1987; Dunkin et al., 1994; Harmony et al., 1993). In the present study the test-retest correlations vary per frequency band and electrode combination. For alpha reasonable correlations have been found, varying between .7 and .8. Stability was low for the combinations of electrode pairs over longer distances of the delta frequency, the split-half correlations were also low for these combinations of electrode pairs. Again, the interval between the two sessions was 1.5 years and the possibility exists that true changes are reflected in the size of the test-retest correlations. Preliminary, it can be concluded that the EEG parameters, obtained during quiet rest, are very stable characteristics, and that P300 amplitude is less stable, which most likely is due to its lower reliability.

Stability of genetic effects
Heritability is not a fixed parameter, but may change during life. That is, the relative contribution of genetic factors could increase as people grow older (as seems the case for IQ) or decrease (as seems the case for blood pressure). A change

in heritability need not mean a change of the molecular mechanism. For example, the heritability could increase even if the same genes were involved because the environmental influences decrease or because the effects of genetic factors are amplified. Conversely, the heritability can remain similar but different genes can affect the phenotype. A longitudinal design has the potential to study if the same genes contribute to the observed trait on different time-points. In this thesis the discussion on changes in heritability is limited to a comparison the twin correlations at both occasions and thus only gives a first impression of the stability of the size of the genetic influences. As we have already mentioned in the introduction looking only at twin correlations has limitations. Therefore it is only meant as first indication of the stability in heritability of the neural indices used in this study. In the appendix the twin correlations for the neural indices are given for the measurement at time 1 and time 2.

On first sight the twin correlations of the three neural indices used in the current study showed no large changes between the measurement for the 16-year-old and 17.5-year-old subjects. For P300, the male and female MZ correlations remain predominantly similar for the target and nontarget P300 amplitude. Only the female MZ correlations for the targets increase slightly. For EEG power, the only differences that are suggested are for delta and theta frequency bands and mainly in the prefrontal and lateral frontal electrode positions. The male MZ correlations decreased, suggesting lower influence of genetic factors on these electrode positions. The twin correlations of the other frequency band remained the same. Twin correlations for the EEG coherence in the alpha and beta frequency band did not differ between the two measurements. Differences occurred in the lower frequency bands, delta and theta. The MZ and DZ correlations became smaller at the second measurement, suggesting a lower genetic influence, especially those estimated from the prefrontal positions. Changes of the twin correlations may reflect real changes in heritability. However, in the lower frequency bands the reliability of these electrode-combinations was also low. This is a difficult problem, because in the age between 16 and 18, developmental changes are primarily expected in the frontal part of the brain, while in these brain areas it is more difficult to obtain reliable EEG parameters. On basis of these data it could be concluded that the heritability for the EEG/ERP parameters remains rather stable. If there are any changes in the heritability, these are rather small.

Final remarks

This thesis has focused on the genetic analysis of EEG/ERP parameters obtained in sixteen year-old twins. The results indicated that the individual differences in EEG parameters are mainly influenced by genetic factors. Especially the EEG power is a

highly genetic characteristic. With a heritability of over 80%, EEG power is one of the most heritable human traits. Of course, this does not tell us what the genes are that underlay brain function. To identify these genes linkage studies have to be carried out, that use both the recent developments in molecular genetics and DNA technology as well as the recent advantages in statistical methodology for genetic linkage between DNA markers and complex traits. In general, large numbers of sibling pairs (DZ twins) are needed to detect linkage between a quantitative trait locus (QTL) and a DNA marker, especially if the heritability of the trait is small. For EEG power the total heritability is large, and this is a favorable condition to start looking for linkage (Risch & Zhang, 1995). Our strategy to increase power to detect linkage is to analyze multivariate phenotypes or estimated individual genotypic values (Boomsma, 1996). This study has employed multivariate measures and together with the high heritabilities of these measures therefore offers promising possibilities to start looking for genes that influence brain activity.

This is the first longitudinal study of the genetics of neural indices of brain functioning, but the data of the second measurement are still to be analyzed. Therefore, some questions remain unanswered at his moment about the genetic influence on the development of the brain. However, the data are available to apply model fitting methods to test stability of the genetic and environmental factors. Also, it will be possible to investigate the genetic and environmental influences on the growth process of the brain themselves.