GENETIC EFFECTS IN THE CORTICAL AUDITORY EVOKED POTENTIAL: A TWIN STUDY[1]

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Little is known about the significance of the cortical evoked potential. However, our understanding of it may be helped by knowledge of the extent to which it is inherited or alternatively is due to environmental factors This information can be obtained from observations of differences between the evoked potentials of twins.

There is some evidence in the literature on this. Dustman and Beck (1965) looked at the averaged cortical evoked potential of 12 monozygotic and 11 dizygotic young twin pairs. Visual stimuli were presented and within pair correlations for the response outline were calculated These were found to be 0 81 for monozygotic and 0.54 for dizygotic pairs. They also looked at the correlations between unrelated children matched for age and found this to be 0 61 indicating a strong relationship between evoked potential and age in their sample. When this is taken into account, a genetic contribution is still suggested by the difference in size of the correlations between the two types of twin This, however, is probably not significant as the sample size is small

Young *et al* (1972) looked at the auditory evoked potential to click stimuli of 17 monozygotic and 15 dizygotic adult twin pairs They looked separately at the latency and amplitude components and found a significant difference between the intrapair correlations of monozygotic and dizygotic twins in the P2, N2 and P3 latency components and in the P3–N3 amplitude

component, thus indicating some genetic influence on these components. This was not found in the P3 latency or in the P2–N2 and N2–P3 amplitude components However, as Young himself says, the amplitude scores were not very reliable so that not much importance can be attached to this failure to find a significant genetic effect for these variables.

The literature thus suggests that a genetic influence on the cortical evoked potential does exist, but, because of the small sample sizes involved, we have no real information on the size of this effect

In the present study a somewhat larger sample (40 twin pairs) was used, together with a more sophisticated analysis technique developed in biometrical genetics

METHOD

Subjects

Subjects were all male and were taken from a twin register compiled at the Institute of Psychiatry Eighty subjects were tested in all Twenty of the pairs were monozygotic while the rest were dizygotic Zygosity was tested by a battery of blood tests The mean age was 24.2 years, ranging between 17 and 44 years

Apparatus

The EEG was measured using a Mingograf EEG polygraph and recorded on a tape recorder for subsequent averaging. Tonal stimuli were generated by an audio oscillator and were presented binaurally through stereophonic headphones

Procedure

The subject was seated in a soundproofed, dark room He was informed of the stimulus conditions and was asked to keep his eyes closed during testing The subject then received 31 stimuli, each of 1 sec duration, at a regular inter-stimulus interval of 33 sec. All stimuli were sinusoidal, at a frequency of 1000 c/sec and at an intensity of 95 dB (re 0002 dyne/cm^2) One channel of EEG was measured from bipolar electrode placement to the Cz and T3 scalp positions A time constant of 0.3 sec and a frequency filter at 70 c/sec were used.

Averaging

A Linc-8 computer was used to average the data from 500 msec after each stimulus For each subject averaging was carried out over three successive blocks of ten stimuli (data from the response to the first stimulus were not included) There were thus three averages per subject, from which estimates of the reliability could be obtained. For scoring, the large negative deflection at about 100 msec and the large positive deflection at about 200 msec were defined as N2 and P3 respectively The largest positive deflection prior to N2 was then defined as P2, and the largest negative deflection after P3, as N3. The scores used were the latencies in milliseconds and the amplitudes in microvolts of these maximum and minimum points. In all cases amplitudes were converted into differences between successive positive and negative points There were thus seven variables for each average, these

being P2, N2, P3 and N3 latencies and P2–N2, N2–P3 and P3–N3 amplitudes.

ANALYSIS AND RESULTS

The means, standard deviations and between subjects correlations for the seven evoked potential variables and for age are given in Table I The scores from the three averages from each subject have been averaged together. It can be seen that age correlates only with N2 latency The three amplitude measures seem to form a common factor, being highly correlated with each other. The latency measures show a larger degree of independence, both from amplitude and from each other, although the P2 and N2 latencies have a sizable correlation

Jinks and Fulker (1970) suggest a test for genotype–environment interaction in which they correlate the monozygotic twin pair sums with the absolute differences. As it is inconvenient to fit models including interaction terms it would be better if such terms did not occur However, when they do occur we can in fact eliminate them by rescaling the data (Mather and Jinks 1971). The effectiveness of any rescaling can be tested by recalculating the correlation between the twin pair sums and absolute differences after transformation .

Significant correlations were found only for the amplitude components (0 50, 0 46 and 0 42 for the P2–N2, N2–P3 and P3–N3 components respectively). These were eliminated by carrying out log transformations on P2–N2 and N2–P3,

TABLE I

Means, standard deviations and between subject correlations for the evoked potential variables and for age in months

	Age	P2L	N2L	P3L	N3L	P2–N2	N2–P3	P3–N3
Mean	290 1	72 14	113 7	223 8	409 8	21 28	32 77	25.18
S D	75 87	66 46	15 82	38.46	37 17	8 820	12 59	12 32
Correlations								
P2L	0 02							
N2L	0 26**	0 66†						
P3L	0 04	0 07	0 18					
N3L	0 23	−0 04	0 09	0.25*				
P2–N2	−0 04	−0 04	−0 05	−0 29***	−0 22			
N2–P3	−0 06	−0 02	−0 06	−0 25*	−0 14	0 86†		
P3–N3	−0 16	0 13	0 03	−0 21	−0 06	0 36†	0 58†	

* $P < 0\ 05$, ** $P < 0\ 02$, *** $P < 0\ 01$, † $P < 0\ 001$ Latencies are in milliseconds and amplitudes in microvolts

and a square root transformation on P3–N3

Although the monozygotic twin pair sum–difference correlation has been interpreted as indicating a genotype–environment interaction it can also result from other types of interaction Generally in the genetic analysis of twin data no account is taken of the reliability of the data, so that unreliability is included in the within family environment variance. The test suggested by Jinks and Fulker is testing for an interaction of this variance term with the genotype. This correlation may therefore result from an interaction of genotype with either environment or reliability

As in the present study separate information was available on the reliability it was possible to separate any unreliability from the within families environmental effect For the interactions this was done by comparing the twin pair sum–difference correlation with the correlation between the mean and standard deviation scores of each subject calculated from the three averages Again no significant correlation was found for the latencies With the P2–N2 and N2–P3 amplitude components correlations of 0.47 and 0.36 were found. These are similar for the two types of correlation, suggesting a genotype–reliability interaction For the P3–N3 amplitude, however, the correlation of the mean with the standard deviation was only 0.10 The discrepancy between this and the twin pair sum–difference correlation of 0.42 suggests a true genotype–environment interaction here.

The mean scores for the monozygotic and dizygotic twins on the seven variables were compared using a t test No significant differences were found, indicating freedom from sample bias on this point

Analysis of variance was then carried out on the data to find the mean square estimates between families, within families and within subjects for both monozygotic and dizygotic twin pairs The within family mean square estimates were divided by three and the between family estimates by six to make them equivalent to the $\overline{V}_F$ and $V_{\overline{F}}$ of Cattell (1960). These six observational parameters were then fitted to various models using the biometrical genetical technique (Mather and Jinks 1971) This is a generalized technique developed in genetics, of which Cat-

tell's MAVA is a special case The F ratio of Vandenberg (1961) and the HR statistic of Nichols (1965) can also be deduced from the general model, but all these alternatives give less information than the full analysis

The number of possible parameters which can be fitted to twin data is large. Many esoteric but possible influences have been suggested by the various parties in the dispute over genetic influences on intelligence Many of these effects are confounded or highly correlated on twin samples, so that sample sizes of 1000 or more are often needed to separate them In an uncontroversial area such as the consideration of genetic influences on EEG, where little evidence on the subject is available at all, it is essential to fit very simple models as a starting point.

The most basic genetical model would allow for a single additive genetic component (D_R) and simple environmental effects with no interactions. If we fit such a model we are in effect assuming random breeding, no linkage, no non-allelic interactions, no dominance and no genotype–environment interactions. The effects of deviation from this model can be estimated, but with a small sample it would not anyway be possible to discriminate such effects.

For the environmental variables it is necessary to assume both a between families environmental effect (E_2) and a within families environmental effect (E_1) as these two parameters behave differently within the model With an experimental design containing only twins reared together Eaves (1972) has shown that under normal circumstances it will take a sample size of about 500 to discriminate D_R from E_2 However, the biometrical genetical approach does give us a chi-square test of goodness of fit of our models, so that we are able to test for D_R and E_2 separately (assuming the absence of the other effect) and find out which model gives the better fit

With the present experiment an additional unreliability parameter (S) was included. This was done separately for the monozygotic and for the dizygotic twins The full model is given in Table II.

The model was fitted using the approximate maximum likelihood technique (Hayman 1960, Nelder 1960) The model equation is given by

$$g = (M'V^{-1}M)^{-1}M'V^{-1}v$$

TABLE II

The full model D_R is the additive genetic variance, E_2 the between families environmental variance, E_1 the within family environmental variance and S the unreliability estimated separately for monozygotic and dizygotic twins

Source	Parameter				
	D_R	E_2	E_1	S_{MZ}	S_{DZ}
Between MZ	1/2	1	1/2	1/6	0
Within MZ	0	0	1	1/3	0
Within MZ S's	0	0	0	1	0
Between DZ	3/8	1	1/2	0	1/6
Within DZ	1/4	0	1	0	1/3
Within DZ S's	0	0	0	0	1

TABLE III

Reliability of the data Reliabilities of the measures were calculated by both the Spearman and the approximate maximum likelihood methods For the latter the standard deviation of the squared reliability is also given as is the percentage of the total variance due to unreliability

Variable	r(Spearman)	r(ML)	r^2	SD(r^2)	$^o/_o$ var
P2L	0 70	0 65	0 42	0 094	57
N2L	0 82	0 75	0 57	0 082	42
P3L	0 92	0 86	0 75	0 054	25
N3L	0 78	0 76	0 58	0 084	42
P2-N2	0 88	0 80	0 64	0 074	36
N2-P3	0 93	0 88	0 77	0 052	23
P3-N3	0 84	0 82	0 67	0 070	33

where g is the vector of estimated parameters, v is the vector of observed statistics, derived in this case from the mean square estimates, M is the model matrix given in Table II and V is a diagonal matrix of weights obtained from the expected variances of our observed statistics. It is possible to obtain such weights as our observed statistics are in fact variances, and therefore have a chi-square distribution, the variance of which is given by $2V^2/N$ The maximum likelihood solution is approximated by an iterative process in which, following each solution to the equation, the expected value of the observed statistic under the model is recalculated $(E(v)=M'g)$ $E(v)$ is then used to give a new weight matrix (V). The iteration converges to a minimum chi-square solution.

The chi-square test of goodness of fit of the model is given by

$$X^2_{k-j} = (v - E(v))'V^{-1}(v - E(v))$$

where k is the number of observed statistics, j is the number of estimates and k −j are the degrees of freedom

The matrix $(M'V^{-1}M)^{-1}$ is a covariance matrix for the estimates. We can obtain the variances of the estimates from the diagonals of this matrix and can therefore test whether our estimates differ significantly from zero. With a maximum likelihood solution the estimates will have a chi-square distribution This increasingly approximates a normal distribution as the sample size (N) increases, so that if N is large enough a standard normal deviate test can be used. With smaller N's this will give an approximate signif-

icance level The correlations between the estimates can also be calculated from this matrix

A measure of reliability can be obtained from the between subjects correlations between the three averages. This is given by the Spearman formula

$$r_{nn} = nr_{ii}/(1 + (n-1)r_{ii})$$

where n is the number of averages, r_{ii} is the mean of the correlations between the averages and r_{nn} is the reliability of the mean

Reliabilities can also be calculated from the ratios of the maximum likelihood estimates

$$r^2_{nn2} = (D_R + E_2 + E_1)/(D_R + E_2 + E_1 + S)$$

As we have the variances and covariances of these estimates we are also able to calculate the expected variances of our reliability estimates Reliabilities calculated in both these ways are given in Table III. It can be seen that the two analyses show good agreement

The most reliable components are clearly N2 and P3 as we would expect The N2-P3 amplitude component has a reliability of about 0.9 The least reliable component is P2 latency which, with a reliability of about 0 7, has more than half its variance accounted for by unreliability. Our genetic and environmental estimates for this variable will only come from the remaining 50% and are therefore less likely to show significant effects

The results of the maximum likelihood analysis for the seven variables are given in Table IV. It was found that for the full model $(D_R.E_2:E_1 S)$

TABLE IV

Biometrical genetical analysis of evoked potential variables The narrow heredltabihty is shown in column 1, followed in column 2 by the significance level of the test that $D_R = 0$ The chi-square tests of goodness of fit for the genetical model $(D_R \, E_1 \, S)$ and the environmental model $(E_2 \, E_1 \, S)$ are in the third and fourth columns respectively The significance level of the test $E_1 = 0$ is in the last column Chi-square statistics are all with 2 degrees of freedom

Variable	Heredltabihty		Chi-square		E_1
	$^n_0 \tfrac{1}{2} D_R$	Sig	D_R	E_2	
P2L	81 81	0 005	0 56	1 21	ns
N2L	58 44	0 01	1 21	0 06	0 02
P3L	71 56	0 001	5 80	4 26	0 05
N3L	35 17	0 1	0 67	1 40	0 02
P2–N2	86 44	0 001	1 68	6 79	ns
N2–P3	88 53	0 001	2 47	9 89	ns
P3–N3	81 96	0 001	1 27	5 11	ns

ns, non-significant

D_R and E_2 show a model correlation of about $-0\ 9$ If D_R is left out E_2 and E_1 correlate at about -0.3, while without E_2 the $D_R \, E_1 \, S$ model gives an E_2, E_1 correlation of about $-0\ 60$ It is thus clear that with a small sample size we should not expect a clear discrimination between D_R and E_2 except under extreme circumstances. The chi-square tests of goodness of fit for the $D_R \, E_1 \, S$ and the $E_2 \, E_1 .S$ models are given in columns 3 and 4 respectively of Table IV It can be seen that the genetic model gives a better fit to the data for all variables except the N2 and P3 latencies The advantage of the genetic over the environmental model is clearest for the amplitude components where the effect is so extreme that the E_2 model fails, even on the present small sample

As we have been able to partition what is normally called E_1 variance into a true within families environmental effect (E_1) and unreliability (S), we are in a position to test for the significance of this environmental component. The significance levels for this test appear in column 5 of Table IV. It can be seen that we have clear evidence of such an effect in three of the latency components while it appears to be absent from the amplitudes It thus seems that where we have evidence for the operation of a between families environmental effect (E_2) there

is also evidence for an environmental effect within families (E_1) This makes intuitive sense

The narrow heredltabihty as a percentage is given in column 1 of Table IV This is calculated as $100 \times \tfrac{1}{2} D_R / (\tfrac{1}{2} D_R + E_1)$ from the $D_R \, E_1 \, S$ model. It will only be a reliable estimate of the heredltabihty if we can assume no E_2, an assumption which is justified for the amplitude components and perhaps for P2 and N3 latencies The significance levels for the test of the difference of the D_R effect from zero are also given (column 2, Table IV) These are significant for all variables with the exception perhaps of N3 latency which is only marginally significant

DISCUSSION

It is clear that in the present study we have very strong evidence for a genetic influence on the amplitude of the cortical auditory evoked potential There is no evidence at all for an environmental effect in these components For the latency measures the position is less clear It seems that these are affected by the within families environment. Also there is a source of variation which is neither E_1 nor unreliability, but which could be accounted for either by the between families environment (E_2) or by additive genetic variation (D_R), the former being the more likely source. Larger samples will be needed to discriminate these effects and both will probably be operating

Strictly speaking, these results will of course only apply to auditory evoked potentials on adult subjects from the Cz–T3 scalp positions with a 95 dB 1000 c/sec stimulus at an inter-stimulus interval of 33 sec The extent to which the results will generalize along the suggested dimensions is a matter for further experimentation. The Dustman and Beck (1965) results suggest a genetic component for response outline with the visual evoked potential in children Young et al (1972) using click stimuli on adult subjects found a genetic component in the latencies rather than in the amplitudes

What sort of interpretation can be made from this type of result? One approach would be to look at the paradigm within a Skinnerian framework, seeking to identify the predictor variables for evoked potential variation These may be

divided into several sources, the most important of which will be the stimulus parameters such as intensity and modality Electrode placement might also be included among these Secondly, there will be common organismic variables such as expectancy or habituation where the response will be dependent on the subject's previous history but where, given common history, the response effects will be common across subjects Finally there will be the individual difference variables where the evoked responses from different subjects differ in terms of concomitant differences between the subjects in other variables Within this framework we can seek to make predictions about the evoked potential parameters of a particular subject, given a particular set of stimulus parameters, an experimental set, and any individual difference variables which may have been found to be relevant

Any genetical analysis will have relevance mainly for identifying the sources of individual variation If we find a genetic component we should look for concomitant variables which are also likely to be genetically determined. If we find no genetic effect we should look to the environment for possible causes of variation This distinction should not be strictly held as it is possible for variables which at first might seem to be environmental, such as for example growth rate, to be genetically determined

The present study therefore indicates that we should look to variables which are themselves inherited as possible causative influences on evoked potential amplitude. It might be thought that brain structural variables are the most likely candidates here. This does seem probable, although it should be stressed that such a conclusion is not strictly justified as we do not know the point of selection by which evoked potentials are genetically determined It does, however, fit in with the well known structural similarities between identical twins

For the evoked potential latency components where an environmental influence was found we should look for likely concomitant sources of environmental variation The author has no firm suggestions to make on this point at this stage of research For the future it is hoped to test more twins while varying the stimulus parameters, so that data can be provided for a multi-

variate genetic analysis The genetical contribution can then be examined against a background of variation in these other parameters It is also hoped that testing the same subjects in different situations will enable the gross environmental effect to be partitioned further It is felt that the inclusion of reliability data in the model, a procedure little used in the past, has proved exceptionally useful, and will be essential for future work where more precision is to be aimed at

SUMMARY

An experiment was carried out on 40 pairs of adult male twins to investigate the extent of genetical determination in the cortical auditory evoked potential. Tonal stimuli of 1 sec duration, at an intensity of 95 dB and a frequency of 1000 c/sec, were used. The inter-stimulus interval was 33 sec and the bipolar evoked potential was measured between the Cz and T3 scalp positions The reliability of the seven latency and amplitude measures was also calculated and this was taken into account in the subsequent genetic analysis The biometrical genetical approach, which gives maximal information particularly on small samples, was used to analyse the data. A strong genetic influence was found on all the amplitude scores The environment made no significant contribution to these For the latencies there was some evidence for a genetic effect, however, this was not very strong and can well be interpreted as being due to between family environmental effects

RESUME

EFFETS GENETIQUES SUR LE POTENTIEL EVOQUE CORTICAL AUDITIF

Une expérience portant sur 40 paires de jumeaux mâles a été effectuée en vue d'examiner l'influence de facteurs génétiques sur le potentiel évoqué cortical auditif. Les stimulus étaient des sons de 1 sec, 1000 c/sec, 95 dB, espacés de 33 sec, et le potentiel évoqué était recueilli en montage bipolaire Cz–T3 sur la calotte crânienne La fiabilité des sept mesures effectuées sur les amplitudes et les latences a été calculée et utilisée dans l'analyse Une approche biométrique génétique a été utilisée donnant l'information maxi-

male en particulier dans le cas de petits échantillons Une forte influence génétique a été trouvée pour toutes les variables d'amplitude, sans aucune contribution significative de l'environnement Pour les variables de latence, quelques indications se sont dessinées en faveur d'un effet génétique, encore que peu importantes, et qui pourraient bien être interprétées comme dûes à des effets de l'environnement familial

REFERENCES

CATTELL, R B The multiple abstract variance analysis equations and the solutions for nature-nurture research on continuous variables *Psychol Rev*, **1960**, *67* 353–372

DUSTMAN, R E and BECK, E C The visually evoked potential in twins *Electroenceph clin Neurophysiol*, **1965**, *19* 570–575

EAVES, L J Computer simulation of sample size and experimental design in human psychogenetics *Psych Bull*, **1972**, *77* 144–152

HAYMAN, B I Maximum likelihood estimation of genetic components *Biometrics*, **1960**, *16* 369–381

JINKS, J L and FULKER, D W Comparison of the biometrical, genetical, MAVA and classical approaches to the analysis of human behaviour *Psychol Bull*, **1970**, *73* 311–349

MATHER, K and JINKS, J L *Biometrical genetics* Chapman, and Hall, London, **1971**.

NELDER, J A Estimation of variance components in certain types of experiment in quantitative genetics In O KEMPTHORNE (Ed), *Biometrical genetics* Pergamon Press, London, **1960**.

NICHOLS, R C The national merit twin study In S G VANDENBERG (Ed), *Methods and goals in human behaviour genetics* Academic Press, New York, **1965**.

VANDENBERG, S G Psychophysiological reactions of twins hereditary factors in GSR, heart rate and breathing rates *Eugen Quart*, **1961**, *12(1)* 7–10

YOUNG, J P R, LADER, M H and FENTON, G W A twin study of the genetic influences on the EEG *J med Genet*, **1972**, *9* 13–16