Neuropsychopharmacology (2006) 31, 1335–1344

www.neuropsychopharmacology.org

A Ser9Gly Polymorphism in the Dopamine D3 Receptor Gene (DRD3) and Event-Related P300 Potentials

Christoph Mulert*[,1], Georg Juckel[1], Ina Giegling[1], Oliver Pogarell[1], Gregor Leicht[1], Susanne Karch[1], Paraskevi Mavrogiorgou[1], Hans-Jürgen Möller[1], Ulrich Hegerl[1] and Dan Rujescu[1]

[1]*Department of Psychiatry, University of Munich, LMU, Munich, Germany*

An important reason for the interest in P300 event-related potentials are findings in patients with psychiatric disorders like schizophrenia or alcoholism in which attenuations of the P300 amplitude are common findings. The P300 wave has been suggested to be a promising endophenotype for genetic research since attenuations of the amplitude and latency can be observed not only in patients but also in relatives. In parallel, the search for genes involved in the pathogenesis of psychiatric disorders has revealed for both, schizophrenia and alcoholism an association with a DRD3 Ser9Gly polymorphism in a number of studies. In the present study, we have investigated 124 unrelated healthy subjects of German descent and have found diminished parietal and increased frontal P300 amplitudes in Gly9 homozygotes in comparison to Ser9 carriers. This finding suggests a possible role of the DRD3 receptor gene in the interindividual variation of P300 amplitudes. Further studies should address the direct role of the DRD3 Ser9Gly polymorphism in attenuated P300 amplitudes in psychiatric disorders like schizophrenia or alcoholism.
Neuropsychopharmacology (2006) **31,** 1335–1344. doi:10.1038/sj.npp.1300984; published online 4 January 2006

Keywords: P300; Dopamine D3 receptor; schizophrenia; alcoholism; evoked potential; LORETA

INTRODUCTION

A number of neuroelectric measurements are highly heritable (van Beijsterveldt and van Baal, 2002). This is especially true for the amplitude of the event-related P300 potential: the heritability was estimated to be between 0.61 (Wright *et al*, 2001) and 0.79 (Katsanis *et al*, 1997) in twin studies. In addition, biologically related family members demonstrate significant inter-family correlations for P300 measures (Eischen and Polich, 1994). This fact and the finding of reduced P300-amplitudes in disorders like schizophrenia (Hegerl *et al*, 1995; Roth *et al*, 1981; Strik *et al*, 1994) or alcoholism (Polich *et al*, 1994) makes the P300 potential as a strong candidate for an endophenotype approach; although schizophrenia is a hereditable disease, the identification of involved genes is probably limited by the biological heterogenity behind the psychiatric diagnosis.

In a number of family studies, an attenuated P300-potential at parietal locations could be detected not only in patients with schizophrenia but also in unaffected siblings (Winterer *et al*, 2003). Recently, a meta-analysis of articles between 1983 and 2003 including 472 relatives of schizophrenic patients and 513 controls demonstrated significant reductions of the P300 amplitude reductions and latency delays in relatives (Bramon *et al*, 2005). The authors concluded that the P300 amplitude and the P300 latency are promising alternative phenotypes for genetic research into schizophrenia. Intermediate phenotypes or 'endophenotypes' as biological measures provide a means for identifying the 'downstream' traits or facets of clinical phenotypes, as well as the 'upstream' consequences of genes (Gottesman and Gould, 2003). Event-related potentials are easily obtained from large subjects samples, are stable quantitative measures and therefore interesting for genotype–phenotype association studies.

The search for genes associated with both, the diagnosis of schizophrenia and the P300-amplitude so far revealed a relationship to the Catechol-*O*-methytransferase (COMT) gene (Gallinat *et al*, 2003; Glatt *et al*, 2003) which is involved in dopamine catabolism.

A relationship between the DRD3 receptor gene and schizophrenia has been described in a number of studies (Crocq *et al*, 1992; Jonsson *et al*, 2003), while others did not find an association (Ioannidis *et al*, 2001; Sabate *et al*, 1994). DRD3 mRNA is predominantly expressed in limbic areas of the brain and may mediate the therapeutic actions of antipsychotic drugs (Suzuki *et al*, 1998). In a recent PET study with the D3-receptor agonist pramipexol a robust decrease was detected in the bilateral orbitofrontal cortex, thalamus, operculum, posterior and anterior cingulate

*Correspondence: Dr C Mulert, Department of Psychiatry, Univeristy of Munich, Nußbaumstraße 7, 80336 München, Germany, Tel: +49 89 5160 3392, Fax: +49 89 5160 5542,
E-mail: cmulert@psy.med.uni-muenchen.de
Received 7 October 2004; revised 28 September 2005; accepted 13 October 2005
Online publication: 26 October 2005 at http://www.acnp.org/citations/Npp102605040479/default.pdf

cortex and insula. The authors speculate that dopamine's effects on these regions via D3R may mediate some of the known psychiatric complications of dopamine deficiency or excess (Black *et al*, 2002). In a recent meta-analysis on the DRD3 Ser9Gly polymorphism in schizophrenia based on 11 066 subjects derived from 44 samples, there was a significant association between DRD3 Ser9Gly homozygosity and schizophrenia in the European subsample (based on 14 samples) with an odds ratio of 1.14 but not in the Asian subsample (based on 10 samples).

However, it has been suggested that the published positive findings might reflect a true association in a subgroup of patients who were nonresponder to traditional antipsychotic drugs. In addition, a number of studies suggest a relationship to tardive dyskinesia, sex, and the age of onset. Especially the relationship to tardive dyskinesia was shown nicely in a number of studies (Steen *et al*, 1997; Basile *et al*, 1999; Segman *et al*, 1999; Woo *et al*, 2002). Since there have been also negative findings (Rietschel *et al*, 2000) it is important to note that in a recent study investigating a large pooled sample of 780 patients drawn from six research centers, a significant association of the DRD3 genotype with tardive dyskinesia was described (Lerer *et al*, 2002). The authors also performed a meta-analysis including three other published studies and could again demonstrate a significant relationship between tardive dyskinesia and the DRD3 gly allele carrier status.

Interestingly, beside the fact that a reduced P300-amplitude is the typical finding in schizophrenia our own earlier work and studies of other groups suggests that pronounced P300-amplitude reduction is seen in a subgroup of patients with early onset, bad response to neuroleptics and increased risk for tardive dykinesia (Hegerl *et al*, 1995; Olichney *et al*, 1998). Exactly these characteristics have been recently again described to be associated with the DRD3 receptor: 'combining the previous studies, we therefore suppose that the gly allele not only contributes to the pathogenesis of schizophrenia and increases the occurrence of extrapyramidal symptoms including tardive dyskinesia but also associates with a less favorable therapeutic outcome in acutely treated psychotic patients' (Reynolds *et al*, 2005).

In the abovementioned DRD3 meta-analysis, comorbidity with alcohol abuse, which was described in earlier studies (Krebs *et al*, 1998) was not detected. However, in families with alcohol abuse, similar to the findings in schizophrenia, diminished P300-amplitudes have been described and a genetic background discussed (Almasy *et al*, 1999; Blackwood, 2000). Animal studies also suggest a relationship between the D3 receptor and motivational effects of ethanol (Boyce and Risinger, 2002), and a major role concerning the addictive properties of alcohol has recently been suggested (Heidbreder *et al*, 2004).

A relationship of the P300 potential to the dopaminergic system was already suggested by findings that the amplitude of the P300 of healthy subjects changes after the application of sulpiride, a dopamine antagonist (Takeshita and Ogura, 1994). Moreover, in Parkinson's Disease (PD), a disease with a reduction of dopaminergic neurons, a prolonged P300 latency has been described. The P300 latency in PD can be at least in parts be influenced by the application of dopaminergic drugs (Stanzione *et al*, 1990, 1991). For an overview about neurochemical substrates of the P300 see (Frodl-Bauch *et al*, 1999).

Interestingly, concerning psychiatric diseases with P300 attenuations like schizophrenia and alcoholism (Blackwood, 2000) central dopaminergic dysfunction has been discussed to show analogies in both diseases in the dysfunction of the ventral striatum (nucleus accumbens), which is involved not only in the reinforcing effects of drug intake but also in the pathogenesis of positive schizophrenic symptoms (Heinz, 2002). The ventral striatum may be referred to as dopaminergic reward system and high levels of dopamine D3 receptor mRNA have been described in the nucleus accumbens (Gurevich and Joyce, 1999; Landwehrmeyer *et al*, 1993). Increased D3 receptor levels in the limbic striatum of patients with schizophrenia have been described in post-mortem investigations (Gurevich *et al*, 1997) with an influence of the drug history being less likely (Joyce, 2001). Similar, increased D3 receptor levels and D3 receptor mRNA have been described in brains of cocaine overdose victims (Mash and Staley, 1999; Meador-Woodruff *et al*, 1995; Staley and Mash, 1996), while studies investigating D3-receptor levels in the ventral striatum in alcohol consumption are still required. In addition, a common genetic background for schizophrenia and alcoholism has been suggested (Schuckit *et al*, 2003).

The P300 potential is usually evoked with an oddball paradigm. Stimuli (for example tones), are presented that way that one stimulus is rare and relevant (for example a button must be pressed) and another stimulus is often presented but irrelevant. The P300 potential is then recorded widely across the scalp some 300 ms after the rare stimulus. P300-analysis has been limited due to the restrictions of source analysis and the fact that multiple electrical generators in the brain are involved, including the temporo-parietal junction (TPJ) the supplementary motor-cortex (SMA) and the anterior cingulate cortex (ACC), the bilateral middle frontal gyrus, the inferior frontal gyrus and the insula. In a recent simultaneous fMRI-EEG-study, we could demonstrate a high degree of accordance in the fMRI-based localizations and ERP-based localizations as described by a current source density approach (Mulert *et al*, 2004a).

In the present study, we were interested in the relationship of the DRD3 Ser9Gly polymorphism and the P300-potential, both in the conventional analysis of the scalp-amplitudes and using the tomographic current source-density approach LORETA (Mulert *et al*, 2004b; Pascual-Marqui *et al*, 1994; Pascual-Marqui *et al*, 1999). We hypothesized that subjects with the Gly/Gly genotype would show reduced parietal P300 amplitudes in comparison to subjects with the Ser/Ser genotype and that the tomographic comparison would show maximal differences between the groups in the temporo-parietal junction.

MATERIALS AND METHODS

Subjects

Unrelated healthy volunteers of German descent were randomly selected from the general population of Munich, Germany, and contacted by mail. In order to exclude subjects with neuropsychiatric disorders or subjects who

had first-degree relatives with neuropsychiatric disorders, we conducted further screenings before the volunteers were enrolled in the study. First, subjects who responded were initially screened by phone. Their detailed medical and psychiatric histories, and those of their first-degree relatives were assessed using systematic forms. Only German volunteers (ie both their parents were German) were included. Second, they were invited to a comprehensive interview including the Structured Clinical Interview for DSM-IV, SCID I/II (First *et al*, 1997; Wittchen *et al*, 1997) to evaluate their lifetime Axis I and II disorder. Psychiatric diagnoses among their first-degree relatives were also assessed using the Family History Assessment Module (Rice *et al*, 1995). Subjects with relevant somatic diseases or a lifetime history of any Axis I or II psychiatric disorders were excluded. Exclusion criteria for the subjects were also head injury in the history or any medication with influence on the central nervous system (eg, like cortisol, tranquilizers) in the last 3 months. Subjects who had first-degree relatives with a lifetime history of a mental disorder were also excluded. In all, 124 healthy volunteers without a history of neurological or psychiatric disorders and without recent drug consumption were studied (mean age $= 45.0 \pm 15$ years; range 19–72 years). After complete description of the study to the subjects, written informed consent was obtained. Subjects were 71 women and 53 men. Auditory dysfunction was excluded by auditory testing of hearing threshold with a Philips audiometer.

Paradigm

An auditory oddball paradigm with 80% nontarget stimuli (540 tones, 500 Hz) and 20% target stimuli (135 tones, 1000 Hz) presented binaurally through headphones in a pseudo randomized order was used (80 dB SPL, 40 ms duration with 10 ms rise and fall time, interstimulus interval 1.5 s). Subjects were seated with their eyes closed in a reclining chair and had to press a button with their dominant hand after target stimuli.

ERP-Recording

Recording took place in a sound-attenuated and electrically shielded room adjacent to the recording apparatus (Neuroscan Synamps). Subjects were seated with closed eyes in a slightly reclined chair with a head rest. Evoked potentials were recorded with 33 electrodes referred to Cz (32 channels). The electrodes were positioned according to the International 10/20 system with the additional electrodes FC1, FC2, FC5, FC6, T1, T2, CP5, CP6, A1, A2, PO9, PO10. Fpz served as ground. Electrode impedance was <10 kohms. Data were collected with a sampling rate of 250 Hz and an analogous band pass filter (0.16–50 Hz). 200 ms prestimulus and 800 ms poststimulus periods were evaluated. For artifact suppression an amplitude criterion has been used ($\pm 70\,\mu V$) involving all EEG channels and EOG at any time point during the averaging period. Only wave-shapes, based on at least 50 averages were accepted. The P300 amplitude and latency was detected semi-automatically using the BrainVision Analyzer software (Munich) as the most positive value in the timeframe 250–500 ms poststimulus with a visual control afterwards at the electrode positions F3 (left frontal) F4 (right frontal) Fz (fronto-central), P3 (left parietal) P4 (right parietal) and Pz (centro-parietal) each linked to common average.

LORETA

LORETA assumes that the smoothest of all activity distributions is most plausible ('smoothness assumption') and therefore, a particular current density distribution is found. This fundamental assumption of LORETA directly relies on the neurophysiologic observation of coherent firing of neighboring cortical neurons during stimulus processing (Gray *et al*, 1989; Llinas, 1988; Silva *et al*, 1991) and therefore can be seen as a physiologically based constraint. The characteristic feature of the resulting solution is its relatively low spatial resolution, which is a direct consequence of the smoothness constraint. Specifically, the solution produces a 'blurred-localized' image of a point source, conserving the location of maximal activity, but with a certain degree of dispersion. The version of LORETA used in the present study used the digitized Talairach atlas (Talairach and Tournoux, 1988) available as digitized MRI from the Brain Imaging Centre, Montreal Neurologic Institute, estimating the current source density (microAmperes/mm^2) distribution for either single time points or epochs of brain electric activity on a dense grid of 2394 voxels at 7 mm spatial resolution (Pascual-Marqui *et al*, 1999). The solution space (the three-dimensional space where the inverse EEG problem is solved) was restricted to the gray matter and hippocampus in the Talairach atlas (anatomically based constraint). Localization with regard to spherical and realistic head geometry was done using EEG electrode coordinates reported by Towle *et al* (1993). A voxel was labeled as gray matter if it met the following three conditions: its probability of being gray matter was higher than that of being white matter, its probability of being gray matter was higher than that of being cerebrospinal fluid, and its probability of being gray matter was higher than 33% (Pascual-Marqui *et al*, 1999).

LORETA-analysis was performed in the time-frame 300–600 ms poststimulus after timeframe-wise normalization. LORETA has been widely used in the last years in order to localize electrical generators of scalp EEG data (Anderer

Table 1 Genotype and Allele Frequencies of the Ser/Gly Polymorphism in the Dopamine D3 Receptor Gene (DRD3)

		Ser/Ser	Ser/Gly	Gly/Gly	Ser	Gly
Healthy volunteers	($n = 124$)	53 (42.7%)	60 (48.4%)	11 (8.9%)	166 (66.9%)	82 (33.1%)

Hardy-weinberg equilibrium: $\chi^2 = 0.467$, df $= 2$, $p = 0.792$.

et al, 2003; Fallgatter *et al*, 2003; Gallinat *et al*, 2002; Mulert *et al*, 2001) and a good correspondence between fMRI-based localizations and LORETA-maxima has recently been described (Mulert *et al*, 2004a, 2005).

Genotyping

Genomic DNA was prepared from 10 ml blood using the Quiagen Maxi DNA Extraction Kit (Hilden, Germany). The distribution of the Ser9Gly polymorphism in the dopamine DRD3 receptor was determined by PCR and RFLP analysis. Amplification was carried out in 50 µl reactions using 50 ng of genomic DNA, 1 U of Taq Polymerase (Life Technologies, Karsruhe, Germany) in presence of 60 mM TrisHCl (pH 9), 2 mM $MgCl_2$, 15 mM ammonium sulfate, 0.1 µM of each primer and 0.05 mM of each dNTP. A 462 bp fragment was amplified using the sense 5′-gctctatctccaactctcaca-3′ and antisense 5′-aagtctactcacctccaggta-3′ primer pair. Following an initial denaturation step at 94.0°C for 5 min, the DNA was amplified in 39 cycles (94°C for 30 s, 61°C for 30 s, and

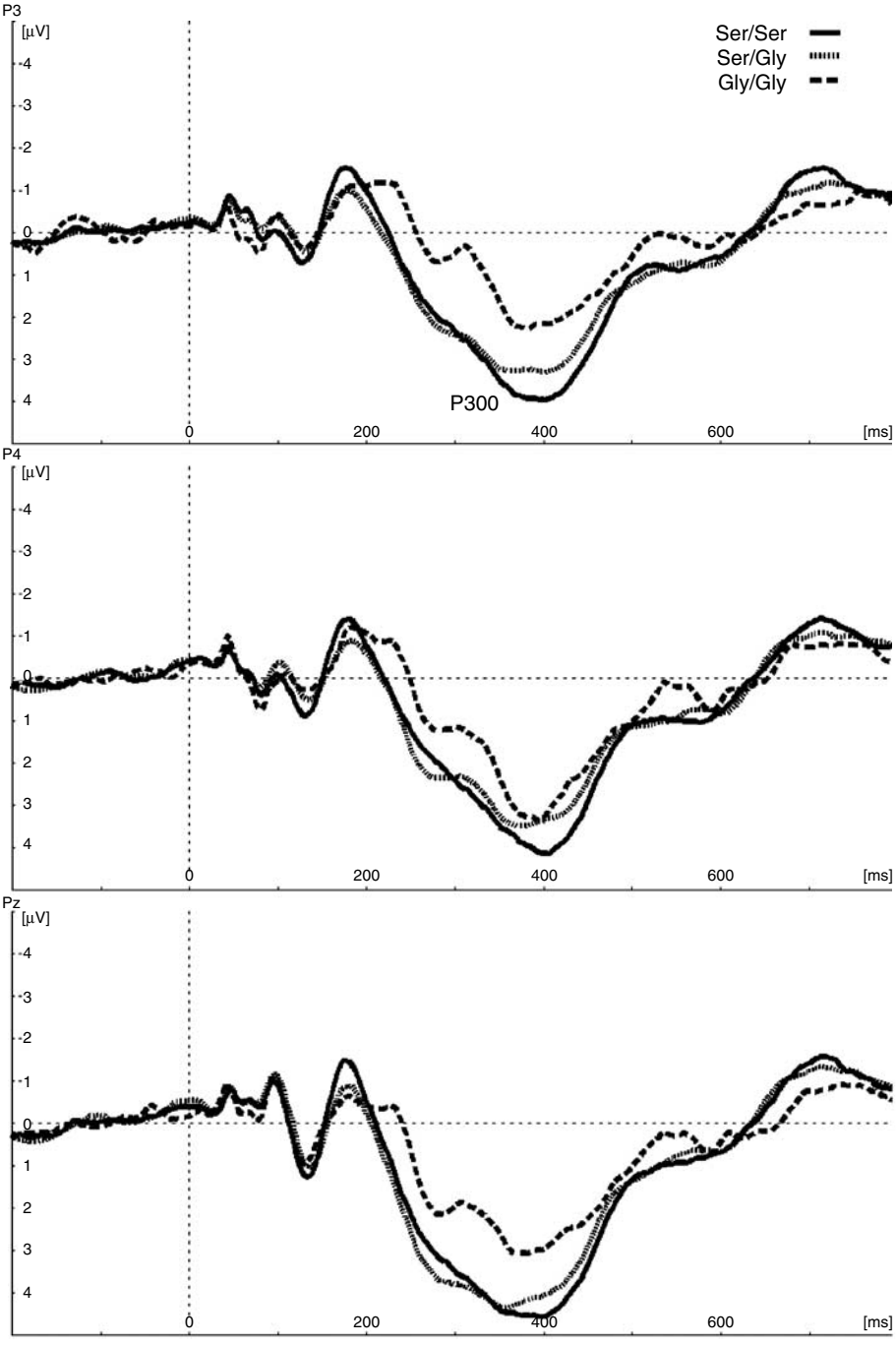

Figure 1 Grand averages of the P300 potentials at P3, P4 and Pz related to the target tone for the three genotype groups. The attenuated P300 amplitude of the Gly/Gly group is most pronounced on the left side (P3).

72°C for 30 s). The final extention step was 72°C for 5 min. The reaction was digested with 12 U MscI (New England Biolabs, Frankfurt, Germany), analyzed by gel electrophoresis in a 2% agarose gel containing ethidium bromide and visualized under UV light. Cleavage with MscI in the two nonpolymorphic sites gave fragments of size 111 and 47 bp. Depending on the absence or presence of the *Msc*I polymorphic restriction site either a fragment of 304 bp (Ser) or two fragments of 206 and 98 bp (Gly) were produced.

Statistical Analyses

Statistics were performed using the SPSS 12.5. Software (Statistical Package for Social Sciences, SPSS Inc, Chicago). Frequency data were analyzed by χ^2 tests. ANOVA or χ^2 tests were performed to test for differences concerning sociodemographic variables. For P300 amplitudes, we performed repeated measurement MANCOVAs assessing the main and interaction effects of the within-subjects factors brain region (frontal, parietal) and hemisphere (left,

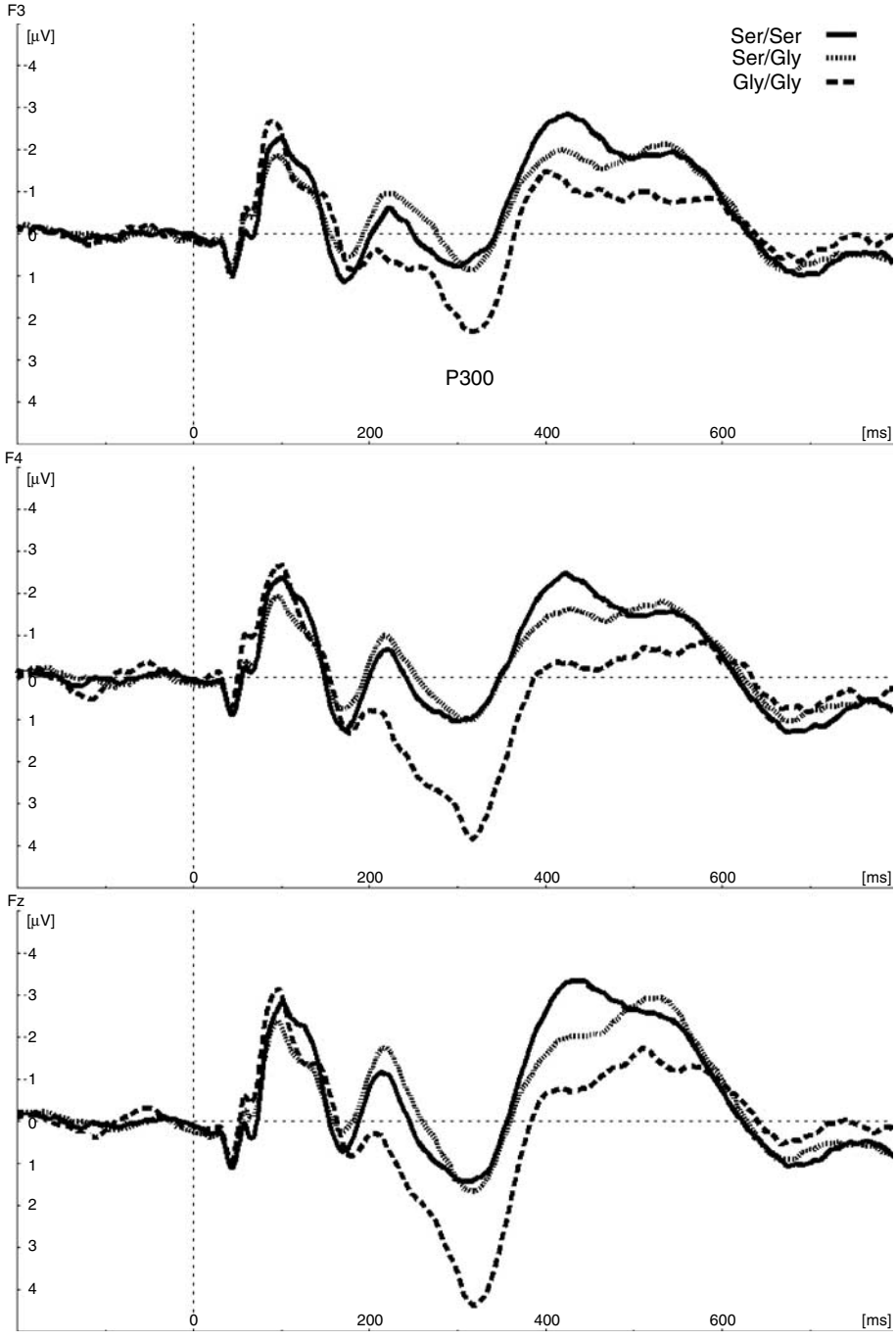

Figure 2 Grand averages of the P300 potentials at F3, F4 and Fz related to the target tone for the genotype groups. The increased P300 amplitude of the Gly/Gly group is most pronounced on the right side (F4).

right), and the between-subjects genotype (Ser/Ser, Ser/Gly, Gly/Gly, or Allele, respectively). The covariates sex and age were added to the analysis. All analyses used two-tailed estimation of significance. The significance level applied to the data was set at $p<0.05$. The repeated measurements were followed by multivariate analyses for brain regions (frontal, parietal, and central) and genotype (allele respectively) controlled for sex and age.

LORETA comparisons between the Ser/Ser group and the Gly/Gly group was carried out with the implemented statistical nonparametric mapping (SnPM).

RESULTS

Genotype counts did not significantly deviate from those expected from the Hardy–Weinberg equilibrium ($\chi^2=0.47$, $df=2$, $p=0.79$). Demographic data are presented in Table 1 for the different genotypes. There was no significant difference between the groups with regard to age ($F=1.36$; $df=2$, $p=0.26$).

Grand averages of auditory P300 referenced to common average are shown for the parietal electrodes (P3, P4, Pz) in Figure 1 and for the frontal electrodes (F3, F4, Fz) in Figure 2. The mean amplitude of the P300 peak was $5.96\pm2.86\,\mu V$ in the Ser/Ser group, $5.68\pm2.52\,\mu V$ in the Ser/Gly group, and $4.20\pm1.96\,\mu V$ in the Gly/Gly group as measured at Pz. At Fz, the mean amplitude of the P300 peak was $2.61\pm2.09\,\mu V$ in the Ser/Ser group, $2.72\pm2.00\,\mu V$ in the Ser/Gly group, and $4.95\pm2.61\,\mu V$ in the Gly/Gly group.

For P300 amplitudes, we observed an interaction between Region × Genotype ($F=4.109$, $df=2/117$ $p=0.019$); as well as Hemisphere × Genotype ($F=2.990$, $df=2/117$, $p=0.054$) after controlling for sex and age (Table 2). The genotype had a main effect frontal right ($F=4.657$, $df=2/117$, $p=0.011$) with a reduction in Ser-carriers and parietal left ($F=3.602$, $df=2/117$, $p=0.030$) with an increased amplitude in Ser-carriers. This effect was similar when looking at alleles or at Ser-carriers *vs* Gly-homocygotes.

In the statistical nonparametric LORETA comparison between the two homozygous groups (Ser/Ser *versus* Gly/Gly) differences were found in frontal and parietal regions as presented in Figure 3 and Table 3.

The mean latency of the P300 peak was 371 ± 63 ms in the Ser/Ser group, 351 ± 65 ms in the Ser/Gly group and 382 ± 83 ms in the Gly/Gly group as measured at Pz. At Fz, the mean latency of the P300 peak was 319 ± 68 ms in the Ser/Ser group, 331 ± 66 ms in the Ser/Gly group and 311 ± 15 ms in the Gly/Gly group. There were no significant differences between the three genotype groups or alleles.

DISCUSSION

Conventional Analysis

The main finding of this study were reduced left parietal P300 amplitudes and increased right frontal amplitudes in DRD3 9Gly homozygotes. In a previous study by Tsai *et al* (2003) investigating the relationship between the DRD3 9Gly polymorphism and the P300 amplitude, the parietal P300 amplitudes have been reported descriptively to be lowest in the Gly9Gly group, however, this effect was not significant, which may be attributed to the relative rare occurrence of Gly9Gly homzygotes ($n=7$) in this study. Analyses for frontal leads have not been mentioned in this paper. The strongest effects in our data set were present in the left parietal lead P3 and the right frontal lead F4, which

Table 2 Repeated Measurement MANCOVAs and Multivariate Analyses

	F	df	p		F	df	p
(a) Repeated measurement MANCOVAs							
G (Genotype)	1.188	2/117	0.309	A (Allele)	0.273	1/243	0.602
R (Region)	36.975	1/117	<0.001	R (Region)	110.847	1/243	<0.001
R × G[a]	4.109	2/117	0.019	RA	6.460	1/243	0.012
H (Hemisphere)	0.635	1/117	0.427	H (Hemisphere)	0.030	1/243	0.862
H × G[b]	2.990	2/117	0.054	H × A	1.351	1/243	0.246
R × H	0.002	1/117	0.961	R × H	0.038	1/243	0.846
R × H × G	0.289	2/117	0.749	R × H × A	0.153	1/243	0.696
(b) Multivariate analyses							
Frontal left	1.053	2/117	0.352	Frontal left	1.691	1/243	0.195
Frontal right[c]	4.657	2/117	0.011	Frontal right	4.443	1/243	0.036
Frontal central	2.247	2/117	0.110	Frontal central	3.395	1/243	0.067
Parietal left[d]	3.602	2/117	0.030	Parietal left	5.375	1/243	0.021
Parietal right	2.347	2/117	0.100	Parietal right	3.645	1/243	0.057
Parietal central	2.547	2/117	0.083	Parietal central	2.704	1/243	0.101

[a](Ser-Carrier *vs* Gly Homocygotes) $F=6.563$, $df=1/119$, $p=0.012$.
[b](Ser-Carrier *vs* Gly Homocygotes) $F=6.174$, $df=1/119$, $p=0.014$.
[c](Ser-Carrier *vs* Gly Homocygotes) $F=9.431$, $df=1/119$, $p=0.003$.
[d](Ser-Carrier *vs* Gly Homocygotes) $F=4.516$, $df=1/119$, $p=0.036$.

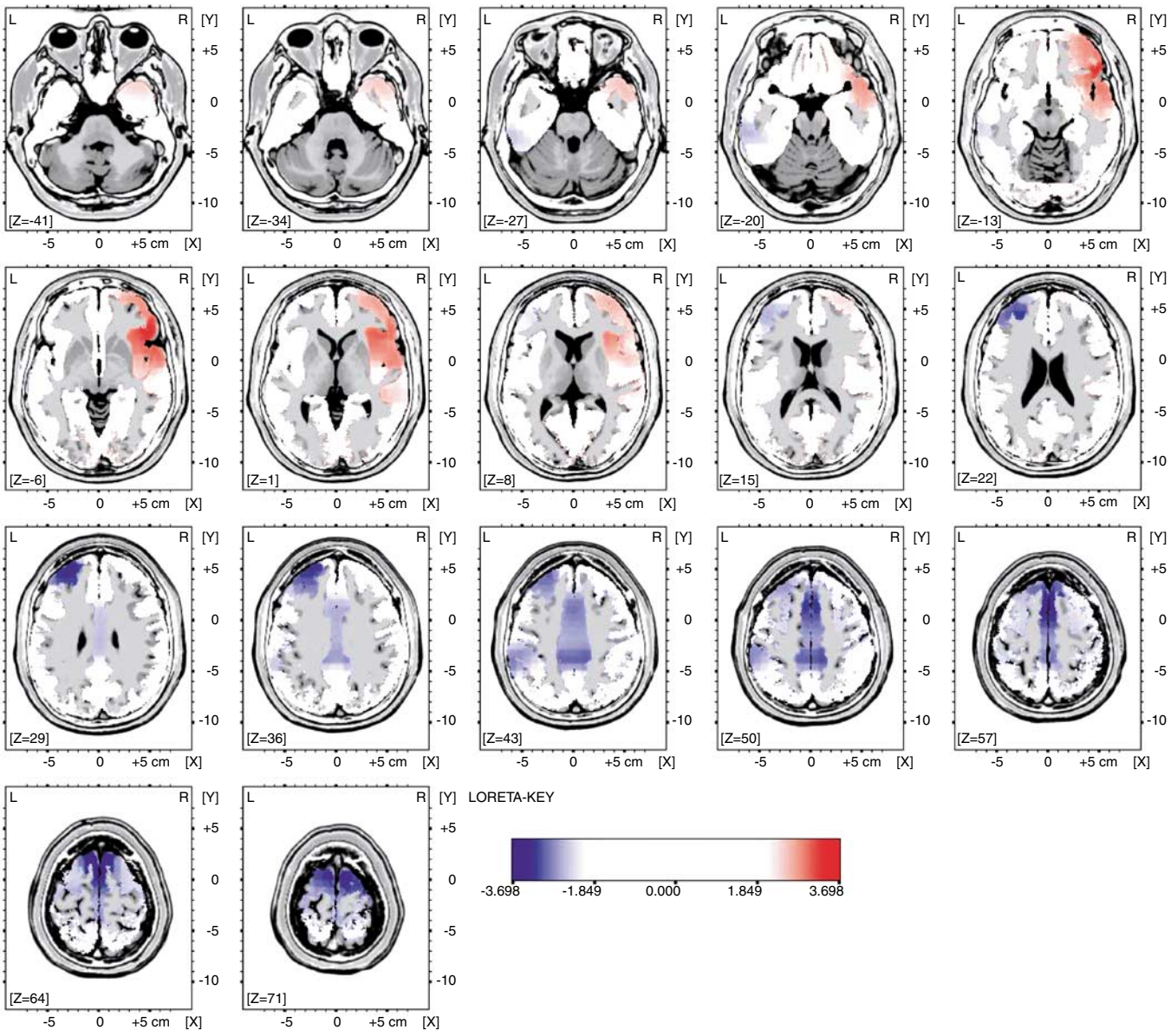

Figure 3 LORETA-nonparametric comparison between the Gly/Gly group and the Ser/Ser group. Red color means increased activity in the Gly/Gly group, blue color increased activity in the Ser/Ser group.

are both not reported in the study by Tsai investigating healthy female Han-Chinese subjects. In this context, it might be of interest that in the recent meta-analysis on the DRD3 Ser9Gly variation in schizophrenia, no association was found in the Asian subsample but only in the European subsample suggesting a possible role of ethnicity in this context. In comparison to other European samples, the observed Ser allele frequency of 66.9 in the present study fits well in the expected range of 65.7–71% (Jonsson *et al*, 2004; Joyce *et al*, 2003).

Comparing our results of P300-amplitude alterations in the Gly9Gly group with the findings in schizophrenia, a parallel in the finding of reduced parietal amplitudes is obvious: reduced parietal amplitudes are the common finding in a large number of studies investigating patients with schizophrenia (Blackwood, 2000; Bruder *et al*, 2001; Demiralp *et al*, 2002). However, in previous studies it has been suggested that this P300-amplitude attenuation is most pronounced in a subgroup of patients with early onset, increased risk for tardive dyskinesia and insufficient response to neuroleptic medication (Hegerl *et al*, 1995; Juckel *et al*, 1996; Olichney *et al*, 1998). Concerning our finding of increased prefrontal activity in the Gly9Gly-homozygotes and findings in schizophrenia, the situation is more complex: while most authors describe reduced frontal P300 amplitudes (Martin-Loeches *et al*, 2001), some did not find any difference between patients and controls and some even describe increased frontal amplitudes in schizophrenia. Interestingly, in a recent P300 study in 66 schizophrenic patients and 115 healthy siblings, increased frontal amplitudes have been described both in patients and in unaffected siblings (Winterer *et al*, 2003). In addition, in a recent 128-channel P300 study, investigating twenty patients with schizophrenia in comparison to controls using individual MRIs for a statistical LORETA-approach with statistical parametric mapping (SPM), reduced left

Table 3 Statistical Nonparametric Comparisons between the Current Source Density Values of the Gly/Gly and the Ser/Ser Group

Region	X Y Z (Talairach)	Bordmann area	T-value
Increased activity in the Ser/Ser group versus the Gly/Gly group			
Superior frontal gyrus (left)	−3, −17, 64	6	3.70*
Middle frontal gyrus (left)	−31, 45, 29	10	3.29†
Paracentral lobule (right)	11, −39, 50	5	3.08‡
Temporo-parietal junction (left)	−59, −39, 43	40	2.82‡
Middle temporal gyrus (left)	−66, −32, 20	21	2.53‡
Increased activity in the Gly/Gly group versus the Ser/Ser group			
Inferior frontal gyrus (right)	46, 31, −6	47	3.34†
Middle temporal gyrus (right)	46, −39, 1	22	3.05‡
Middle frontal gyrus (right)	39; 59; −13	11	2.82‡

*p-value <0.05, †p-value <0.10; ‡significant cluster.
Analysis was performed in the time-frame 300–600 ms poststimulus.

inferior parietal activity was described in combination with increased right orbito-frontal activity in the schizophrenic group (Pae *et al*, 2003). This pattern seems to be similar to our finding in the Gly9Gly-group in comparison to the Ser9Ser-homozygotes.

Reduced parietal P300 amplitudes are also a common finding in alcoholism (Pfefferbaum *et al*, 1991). In addition frontal amplitudes are also typically reduced (Cohen *et al*, 1995; George *et al*, 2004). The reduced parietal P300 would be in line with our present findings but the right frontal increase in the present study does not fit. However, the number of studies reporting amplitudes of frontal electrodes in alcoholism is relatively small and toxic alcohol effects on frontal lobe function have to be taken into account.

LORETA-Analysis

In the subsequent tomographic current source density approach with LORETA, comparing both homozygote groups (Ser9Ser and Gly9Gly) we did not only investigate a single time-point (eg fixed on the P300 peak at a selected electrode) but used the large timeframe (300–600 ms poststimulus) with a more comprehensive overview about involved brain regions at the expense of lower statistical significance for the any respective finding. Concerning the increased right frontal activity in the Gly9Gly group in comparison to the Ser9Ser group on the scalp electrode level, correspondingly the highest t-value in the LORETA-analysis was found in the right inferior frontal gyrus, Brodmann area 47. Lowering the significance level, an additional cluster in the right middle temporal gyrus turned out to be stronger activated in the Gly9Gly group. On this level, several regions were also less activated in the Gly9Gly group, including the left inferior temporo-parietal junction (Brodmann 40), the paracentral lobule and the left superior frontal gyrus. In addition, significantly increased activity in the superior frontal gyrus was detected in the Ser9Ser group in comparison to the Gly9Gly group.

This complex tomographic activation pattern is for the most part in line with the scalp EEG data and is offering a more precise localization of the right frontal and left parietal P300-amplitude findings. In addition it also expands the limited view of a peak picking approach in taking into account an increased time-frame and an increased number of electrodes.

Limitations

Since the Gly allele is relatively rare, the total number of 11 subjects in the critical Gly/Gly group with only three males seems to be critically low. In addition, there are probably multiple causes for reduced P300 amplitudes in both schizophrenia and alcoholism. The definite role of the DRD3 Ser9Gly polymorphism for P300 amplitude reductions in schizophrenia and alcoholism cannot be finally determined by this investigation of healthy volunteers. Concerning the LORETA-analyses, besides the more detailed description of reduced parietal and increased right frontal activity in the Gly/Gly group additional results (eg in temporal regions) have been found. This might well be due to the fact, that in the tomographic all channel information is included. However, since these findings are significant only at liberal p-values, further confirmation and replication is necessary.

Summarizing our results, we could find a relationship between the DRD3 9Gly and the P300 amplitude. The alterations in the Gly9Gly homozygotes (reduced parietal and increased frontal activity) have some similarity with typical findings in schizophrenia and alcoholism. This suggests that the P300 findings might be in fact regarded as an intermediate or endophenotype. Very large-scale family studies are necessary to investigate the possibility that reduced parietal and increased frontal amplitudes are present in DRD3 9Gly homozygotes.

REFERENCES

Almasy L, Porjesz B, Blangero J, Chorlian DB, O'Connor SJ, Kuperman S *et al* (1999). Heritability of event-related brain potentials in families with a history of alcoholism. *Am J Med Genet* **88**: 383–390.

Anderer P, Saletu B, Semlitsch HV, Pascual-Marqui RD (2003). Non-invasive localization of P300 sources in normal aging and age-associated memory impairment. *Neurobiol Aging* **24**: 463–479.

Basile VS, Masellis M, Badri F, Paterson AD, Meltzer HY, Lieberman JA *et al* (1999). Association of the *Msc*I polymorphism of the dopamine D3 receptor gene with tardive dyskinesia in schizophrenia. *Neuropsychopharmacology* **21**: 17–27.

Black KJ, Hershey T, Koller JM, Videen TO, Mintun MA, Price JL *et al* (2002). A possible substrate for dopamine-related changes in mood and behavior: prefrontal and limbic effects of a D3-preferring dopamine agonist. *Proc Natl Acad Sci USA* **99**: 17113–17118.

Blackwood D (2000). P300, a state and a trait marker in schizophrenia (In Process Citation). *Lancet* **355**: 771–772.

Boyce JM, Risinger FO (2002). Dopamine D3 receptor antagonist effects on the motivational effects of ethanol. *Alcohol* **28**: 47–55.

Bramon E, McDonald C, Croft RJ, Landau S, Filbey F, Gruzelier JH *et al* (2005). Is the P300 wave an endophenotype for

schizophrenia? A meta-analysis and a family study. *Neuroimage* **27**: 960–968.

Bruder GE, Kayser J, Tenke CE, Friedman M, Malaspina D, Gorman JM *et al* (2001). Event-related potentials in schizophrenia during tonal and phonetic oddball tasks: relations to diagnostic subtype, symptom features and verbal memory. *Biol Psychiatry* **50**: 447–452.

Cohen HL, Wang W, Porjesz B, Begleiter H (1995). Auditory P300 in young alcoholics: regional response characteristics. *Alcohol Clin Exp Res* **19**: 469–475.

Crocq MA, Mant R, Asherson P, Williams J, Hode Y, Mayerova A *et al* (1992). Association between schizophrenia and homozygosity at the dopamine D3 receptor gene. *J Med Genet* **29**: 858–860.

Demiralp T, Ucok A, Devrim M, Isoglu-Alkac U, Tecer A, Polich J (2002). N2 and P3 components of event-related potential in first-episode schizophrenic patients: scalp topography, medication, and latency effects. *Psychiatry Res* **111**: 167–179.

Eischen SE, Polich J (1994). P300 from families. *Electroencephalogr Clin Neurophysiol* **92**: 369–372.

Fallgatter AJ, Bartsch AJ, Zielasek J, Herrmann MJ (2003). Brain electrical dysfunction of the anterior cingulate in schizophrenic patients. *Psychiatry Res* **124**: 37–48.

First MB, Spitzer RL, Gibbon M, Williams JB (1997). *Structured Clinical Interview for DSM-IV Axis I and II Disorders*. American Psychiatric Press: Washington, DC.

Frodl-Bauch T, Bottlender R, Hegerl U (1999). Neurochemical substrates and neuroanatomical generators of the event-related P300. *Neuropsychobiology* **40**: 86–94.

Gallinat J, Bajbouj M, Bajobouji M, Rentzsch J, Senkowski D, Schunter J *et al* (2003). Association of the G1947A COMT (Val(108/158)Met) gene polymorphism with prefrontal P300 during information processing. *Biol Psychiatry* **54**: 40–48.

Gallinat J, Mulert C, Sander T, Schlattmann P, Xu K, Ferro EF *et al* (2002). Frontal and temporal dysfunction of auditory stimulus processing in schizophrenia. *Neuroimage* **17**: 110–127.

George MR, Potts G, Kothman D, Martin L, Mukundan CR (2004). Frontal deficits in alcoholism: an ERP study. *Brain Cogn* **54**: 245–247.

Glatt SJ, Faraone SV, Tsuang MT (2003). Association between a functional catechol *O*-methyltransferase gene polymorphism and schizophrenia: meta-analysis of case–control and family-based studies. *Am J Psychiatry* **160**: 469–476.

Gottesman II, Gould TD (2003). The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* **160**: 636–645.

Gray CM, Konig P, Engel AK, Singer W (1989). Oscillatory responses in cat visual cortex exhibit inter-columnar synchronization which reflects global stimulus properties. *Nature* **338**: 334–337.

Gurevich EV, Bordelon Y, Shapiro M, Arnold SE, Gur RE, Joyce JN (1997). Mesolimbic dopamine D3 receptors and use of antipsychotics in patients with schizophrenia. A postmortem study. *Arch Gen Psychiatry* **54**: 225–232.

Gurevich EV, Joyce JN (1999). Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. *Neuropsychopharmacology* **20**: 60–80.

Hegerl U, Juckel G, Muller-Schubert A, Pietzcker A, Gaebel W (1995). Schizophrenics with small P300: a subgroup with a neurodevelopmental disturbance and a high risk for tardive dyskinesia? *Acta Psychiatr Scand* **91**: 120–125.

Heidbreder CA, Andreoli M, Marcon C, Thanos PK, Ashby Jr CR, Gardner EL (2004). Role of dopamine D3 receptors in the addictive properties of ethanol. *Drugs Today (Barc)* **40**: 355–365.

Heinz A (2002). Dopaminergic dysfunction in alcoholism and schizophrenia—psychopathological and behavioral correlates. *Eur Psychiatry* **17**: 9–16.

Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001). Replication validity of genetic association studies. *Nat Genet* **29**: 306–309.

Jonsson EG, Flyckt L, Burgert E, Crocq MA, Forslund K, Mattila-Evenden M *et al* (2003). Dopamine D3 receptor gene Ser9Gly variant and schizophrenia: association study and meta-analysis. *Psychiatr Genet* **13**: 1–12.

Jonsson EG, Kaiser R, Brockmoller J, Nimgaonkar VL, Crocq MA (2004). Meta-analysis of the dopamine D3 receptor gene (DRD3) Ser9Gly variant and schizophrenia. *Psychiatr Genet* **14**: 9–12.

Joyce JN (2001). D2 but not D3 receptors are elevated after 9 or 11 months chronic haloperidol treatment: influence of withdrawal period. *Synapse* **40**: 137–144.

Joyce PR, Rogers GR, Miller AL, Mulder RT, Luty SE, Kennedy MA (2003). Polymorphisms of DRD4 and DRD3 and risk of avoidant and obsessive personality traits and disorders. *Psychiatry Res* **119**: 1–10.

Juckel G, Muller-Schubert A, Gaebel W, Hegerl U (1996). Residual symptoms and P300 in schizophrenic outpatients. *Psychiatry Res* **65**: 23–32.

Katsanis J, Iacono WG, McGue MK, Carlson SR (1997). P300 event-related potential heritability in monozygotic and dizygotic twins. *Psychophysiology* **34**: 47–58.

Krebs MO, Sautel F, Bourdel MC, Sokoloff P, Schwartz JC, Olie JP *et al* (1998). Dopamine D3 receptor gene variants and substance abuse in schizophrenia. *Mol Psychiatry* **3**: 337–341.

Landwehrmeyer B, Mengod G, Palacios JM (1993). Dopamine D3 receptor mRNA and binding sites in human brain. *Brain Res Mol Brain Res* **18**: 187–192.

Lerer B, Segman RH, Fangerau H, Daly AK, Basile VS, Cavallaro R *et al* (2002). Pharmacogenetics of tardive dyskinesia: combined analysis of 780 patients supports association with dopamine D3 receptor gene Ser9Gly polymorphism. *Neuropsychopharmacology* **27**: 105–119.

Llinas RR (1988). The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. *Science* **242**: 1654–1664.

Martin-Loeches M, Molina V, Munoz F, Hinojosa JA, Reig S, Desco M *et al* (2001). P300 amplitude as a possible correlate of frontal degeneration in schizophrenia. *Schizophr Res* **49**: 121–128.

Mash DC, Staley JK (1999). D3 dopamine and kappa opioid receptor alterations in human brain of cocaine-overdose victims. *Ann NY Acad Sci* **877**: 507–522.

Meador-Woodruff JH, Little KY, Damask SP, Watson SJ (1995). Effects of cocaine on D3 and D4 receptor expression in the human striatum. *Biol Psychiatry* **38**: 263–266.

Mulert C, Gallinat J, Pascual-Marqui H, Dorn H, Frick K, Schlattmann P *et al* (2001). Reduced event-related current density in the anterior cingulate cortex in schizophrenia. *Neuroimage* **13**: 589–600.

Mulert C, Jager L, Schmitt R, Bussfeld P, Pogarell O, Moller HJ *et al* (2004a). Integration of fMRI and simultaneous EEG: towards a comprehensive understanding of localization and time-course of brain activity in target detection. *Neuroimage* **22**: 83–94.

Mulert C, Jager L, Juckel G, Rujescu D, Giegling I, Rupp D *et al* (2005). Sound level dependence of the primary auditory cortex: Simultaneous measurement with 61-channel EEG and fMRI. *Neuroimage* **28**: 49–58.

Mulert C, Pogarell O, Propp S, Karch S, Stormann S, Pogarell O *et al* (2004b). The neural basis of the P300 potential. Focus on the time-course of the underlying cortical generators. *Eur Arch Psychiatry Clin Neurosci* **254**: 190–198.

Olichney JM, Iragui VJ, Kutas M, Nowacki R, Morris S, Jeste DV *et al* (1998). Relationship between auditory P300 amplitude and age of onset of schizophrenia in older patients. *Psychiatry Res* **79**: 241–254.

Pae JS, Kwon JS, Youn T, Park HJ, Kim MS, Lee B *et al* (2003). LORETA imaging of P300 in schizophrenia with individual MRI and 128-channel EEG. *Neuroimage* **20**: 1552–1560.

Pascual-Marqui RD, Lehmann D, Lehmann D (1999). Low resolution brain electromagnetic tomography (LORETA) functional imaging in acute, neuroleptic-naive, first-episode, productive schizophrenia. *Psychiatry Res* **90**: 169–179.

Pascual-Marqui RD, Michel CM, Koenig T, Kochi K, Merlo MC, Hell D *et al* (1994). Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *Int J Psychophysiol* **18**: 49–65.

Pfefferbaum A, Ford JM, White PM, Mathalon D (1991). Event-related potentials in alcoholic men: P3 amplitude reflects family history but not alcohol consumption. *Alcohol Clin Exp Res* **15**: 839–850.

Polich J, Pollock VE, Bloom FE (1994). Meta-analysis of P300 amplitude from males at risk for alcoholism. *Psychol Bull* **115**: 55–73.

Reynolds GP, Yao Z, Zhang X, Sun J, Zhang Z (2005). Pharmacogenetics of treatment in first-episode schizophrenia: D3 and 5-HT2C receptor polymorphisms separately associate with positive and negative symptom response. *Eur Neuropsychopharmacol* **15**: 143–151.

Rice JP, Reich T, Bucholz KK, Neuman RJ, Fishman R, Rochberg N (1995). Comparison of direct interview and family history diagnoses of alcohol dependence. *Alcohol Clin Exp Res* **19**: 1018–1023.

Rietschel M, Krauss H, Muller DJ, Schulze TG, Knapp M, Marwinski K *et al* (2000). Dopamine D3 receptor variant and tardive dyskinesia. *Eur Arch Psychiatry Clin Neurosci* **250**: 31–35.

Roth WT, Pfefferbaum A, Kelly AF, Berger PA, Kopell BS (1981). Auditory event-related potentials in schizophrenia and depression. *Psychiatry Res* **4**: 199–212.

Sabate O, Campion D, d'Amato T, Martres MP, Sokoloff P, Giros B *et al* (1994). Failure to find evidence for linkage or association between the dopamine D3 receptor gene and schizophrenia. *Am J Psychiatry* **151**: 107–111.

Schuckit MA, Kelsoe JR, Braff DL, Wilhelmsen KC (2003). Some possible genetic parallels across alcoholism, bipolar disorder and schizophrenia. *J Stud Alcohol* **64**: 157–159.

Segman R, Neeman T, Heresco-Levy U, Finkel B, Karagichev L, Schlafman M *et al* (1999). Genotypic association between the dopamine D3 receptor and tardive dyskinesia in chronic schizophrenia. *Mol Psychiatry* **4**: 247–253.

Silva LR, Amitai Y, Connors BW (1991). Intrinsic oscillations of neocortex generated by layer 5 pyramidal neurons. *Science* **251**: 432–435.

Staley JK, Mash DC (1996). Adaptive increase in D3 dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci* **16**: 6100–6106.

Stanzione P, Fattapposta F, Tagliati M, D'Alessio C, Marciani MG, Foti A *et al* (1990). Dopaminergic pharmacological manipulations in normal humans confirm the specificity of the visual (PERG-VEP) and cognitive (P300) electrophysiological alterations in Parkinson's disease. *Electroencephalogr Clin Neurophysiol Suppl* **41**: 216–220.

Stanzione P, Fattapposta F, Giunti P, D'Alessio C, Tagliati M, Affricano C *et al* (1991). P300 variations in parkinsonian patients before and during dopaminergic monotherapy: a suggested dopamine component in P300. *Electroencephalogr Clin Neurophysiol* **80**: 446–453.

Steen VM, Lovlie R, MacEwan T, McCreadie RG (1997). Dopamine D3-receptor gene variant and susceptibility to tardive dyskinesia in schizophrenic patients. *Mol Psychiatry* **2**: 139–145.

Strik WK, Dierks T, Franzek E, Stober G, Maurer K (1994). P300 in schizophrenia: interactions between amplitudes and topography. *Biol Psychiatry* **35**: 850–856.

Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Sedvall G (1998). D3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res* **779**: 58–74.

Takeshita S, Ogura C (1994). Effect of the dopamine D2 antagonist sulpiride on event-related potentials and its relation to the law of initial value. *Int J Psychophysiol* **16**: 99–106.

Talairach J, Tournoux P (1988). *Co-Planar Stereotaxic Atlas of the Human Brain*. Stuttgart: Thieme.

Towle VL, Bolanos J, Suarez D, Tan K, Grzeszczuk R, Levin DN *et al* (1993). The spatial location of EEG electrodes: locating the best-fitting sphere relative to cortical anatomy. *Electroencephalogr Clin Neurophysiol* **86**: 1–6.

Tsai SJ, Yu YW, Chen TJ, Chen CM, Hong CJ (2003). Association analysis for dopamine D3 receptor, dopamine D4 receptor and dopamine transporter genetic polymorphisms and P300 event-related potentials for normal young females. *Psychiatr Genet* **13**: 51–53.

van Beijsterveldt CE, van Baal GC (2002). Twin and family studies of the human electroencephalogram: a review and a meta-analysis. *Biol Psychol* **61**: 111–138.

Winterer G, Egan MF, Raedler T, Sanchez C, Jones DW, Coppola R *et al* (2003). P300 and genetic risk for schizophrenia. *Arch Gen Psychiatry* **60**: 1158–1167.

Wittchen HU, Zaudig M, Fydrich T (1997). *Strukturiertes Klinisches Interview für DSM-IV, Achse I und II: Persönlichkeitsstörungen (Structured clinical interviews for DSM-IV Axis I and II disorders)*. Hogrefe: Goettingen, Germany.

Woo SI, Kim JW, Rha E, Han SH, Hahn KH, Park CS *et al* (2002). Association of the Ser9Gly polymorphism in the dopamine D3 receptor gene with tardive dyskinesia in Korean schizophrenics. *Psychiatr Clin Neurosci* **56**: 469–474.

Wright MJ, Hansell NK, Geffen GM, Geffen LB, Smith GA, Martin NG (2001). Genetic influence on the variance in P3 amplitude and latency. *Behav Genet* **31**: 555–565.