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DBH −1021C>T and *COMT* Val108/158Met genotype are not associated with the P300 ERP in an auditory oddball task

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HIGHLIGHTS

- The results revealed that neither *COMT* Val108/158Met in isolation nor the interaction with the *DBH* −1021C>T polymorphism accounted for variation in the P300 ERP.
- The present study for the first time demonstrated that the *DBH* −1021C>T polymorphism does not significantly mediate the P300 ERP.
- Earlier contradictory results regarding the potential modulation of the P300 ERP by the *COMT* Val108/158Met gene variant were resolved by showing that no such association exists in a large healthy population sample.

ABSTRACT

Objective: The amplitude and latency of the P300 may be associated by variations in dopaminergic genes. The current study was conducted to determine whether functional variants of the catechol-O-methyltransferase (*COMT*) and dopamine beta-hydroxylase (*DBH*) gene were associated with P300 amplitude and latency in an auditory oddball task.
Methods: The P300 ERP was assessed by a two-tone auditory oddball paradigm in a large sample of 320 healthy volunteers. The Val108/158Met polymorphism (rs4680) of the *COMT* gene and the −1021C>T polymorphism (rs1611115) of the *DBH* gene were genotyped. P300 amplitude and latency were compared across genotype groups using analysis of variance.
Results: There were no differences in demographic characteristics in subjects for genotypic subgroups. No genotype associations were observed for the P300 amplitude and latency on frontal, central and parietal electrode positions.
Conclusions: *COMT* Val108/158Met and *DBH* −1021C>T polymorphisms do not show evidence of association with characteristics of the P300 ERP in an auditory oddball paradigm in healthy volunteers.
Significance: We failed to find evidence for the association between dopaminergic enzymatic polymorphisms and the P300 ERP in healthy volunteers, in the largest study undertaken to date.

1. Introduction

The P300 ERP has been associated with a wide range of prefrontally mediated cognitive processes, psychiatric diseases and pharmacological manipulations (Polich, 2004;Polich and Criado, 2006) and is viewed as an important candidate endophenotype and

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diagnostic marker for schizophrenia and substance use disorders (Hesselbrock et al., 2001;Bramon et al., 2005;de Wilde et al., 2008;Euser et al., 2012). Although the P300 ERP is one of the most intensively investigated ERP components, the underlying mechanism and neurotransmitter systems of the P300 remain elusive (Polich, 2007). Several studies suggest that the P300 originates in part from cortical sources (Johnson, 1993). More specifically, there is evidence that the inferior parietal lobe, the temporoparietal junction, parietal and cingulate cortices are among important brain regions thought to be underlying the generation of the P300 (Linden, 2005; Wronka et al., 2012). Patient and pharmacological studies have suggested that the P300 ERP is mediated – among other systems – by dopaminergic processes (Stanzione et al., 1991;Jeon and Polich, 2003;Kenemans and Kahkonen, 2011;Albrecht et al., 2011). The heritability of the P300 amplitude has been estimated to be about 60% (van Beijsterveldt and van Baal, 2002), but the genes that contribute to the P300 ERP are still unknown.

Two promising candidate genes are the *COMT* and *DBH* genes. Catechol-O-methyltransferase (*COMT*) and dopamine beta-hydroxylase (*DBH*) are two enzymes involved in dopamine (DA) turnover in the prefrontal cortex (Zabetian et al., 2001;Akil et al., 2003). Two functional polymorphisms in the genes, i.e., *COMT* Val108/158Met (rs4680) and *DBH* −1021C>T (rs1611115), lead to variability in DA turnover. *COMT* Val108/158 carriers show increased *COMT* enzyme activity and consequently decreased prefrontal DA levels in comparison to Met homozygotes (Lachman et al., 1996;Gogos et al., 1998). Likewise, the *DBH* −1021T allele is associated with a lower *DBH* enzyme activity relative to the C allele (Zabetian et al., 2001), and it can therefore be assumed that T allele carriers have higher levels of dopaminergic activity (Ji et al., 2011). Notably, the effects of both candidate genes are not limited to the DA system as variable enzymatic activity also affects other systems. The *COMT* enzyme is known to degrade a number of other cathecholamines, e.g. norepinephrine (NE) and epinephrine. *DBH* enzyme converts DA into NE, thereby exerting influence on both systems. Both genes are particularly useful to investigate in relation to cognition because they are mainly expressed in the prefrontal cortex, the main area in the brain involved with cognitive functions (Savitz et al., 2006).

There are indications that both genes may play a role in cognition, and more specifically may potentially modulate the P300 ERP. In general, the variants associated with higher DA functioning, that is carriers of the T allele of the *DBH* gene and Met allele of *COMT* gene respectively, were found to be associated with beneficial effects on a number of cognitive processes. The *COMT* Met allele is generally associated with better performance on tasks of executive functioning (Egan et al., 2001;Joober et al., 2002;Barnett et al., 2007; Tunbridge et al., 2006) and working memory (Goldberg et al., 2003;Bruder et al., 2005; Tunbridge et al., 2006). Furthermore, Bilder et al. (2002) indicated within a population of schizophrenic patients, that the Met allele was associated with faster processing speed. Previous work on the P300 ERP has indicated that carriers of the Val allele of the *COMT* genotype showed longer P300 latencies (Kang et al., 2010; Tsai et al., 2003). Similarly, carriers of the −1021T allele of the *DBH* gene have shown better performance on executive functioning, working memory and sustained attention (Kieling et al., 2008), although the number of studies addressing the association between this functional variant and cognitive processes is still low.

Establishing the association between the two dopaminergic candidate genes of the current investigation, the *COMT* and *DBH* gene, and the P300 ERP, has turned out to be complicated. Although there are indications that DA affects the P300 ERP (Stanzione et al., 1991;Jeon and Polich, 2003;Kenemans and Kahkonen, 2011;Albrecht et al., 2011), it is unlikely that this neurotransmitter affects the P300 in isolation. Moreover, both *COMT* and *DBH* enzymes affect the signaling of NE for which there is recent evidence of involvement in the generation of the P300 ERP (e.g. Murphy et al., 2011; Nieuwenhuis et al., 2005). This implies that the potential association between the candidate genes and the P300 cannot solely be attributed to variations in the dopamine system.

Several earlier studies that have examined *COMT* gene-P300 associations have highly variable study designs and results. Given the potential of the P300 ERP as a promising endophenotype, the majority of the studies have been performed in clinical populations such as schizophrenia (Gallinat et al., 2003; Golimbet et al., 2006; Bramon et al., 2006; Kang et al., 2010). Both the ERP itself and the variability in the enzymatic activity as coded by the candidate genes, may be very different in disease and therefore these results cannot be extrapolated to healthy subjects. This is corraborated by a meta-analysis by Barnett et al. (2008), who reported larger effect sizes between *COMT* gene and cognitive processes in patient populations compared to healthy controls. The studies that were performed in healthy volunteers (most often in addition to a patient population) have raised some other concerns. It was observed that many studies included a relatively small number of subjects (<120) (Bramon et al., 2006;Yue et al., 2009; Tsai et al., 2003; Gallinat et al., 2003) and may therefore be underpowered. Further limiting the comparability between studies, is the variability in paradigms. Among the paradigms that have been used are the Visual n-back task (Yue et al., 2009) and the auditory oddball task (Gallinat et al., 2003; Kang et al., 2010; Tsai et al., 2003). Finally, the inclusion of ethnic backgrounds between studies differs; some have included subjects with an Asian background (Yue et al., 2009; Tsai et al., 2003; Kang et al., 2010), others included subjects from European background (Gallinat et al., 2003). In sum, each of the studies discussed suffer from a different design and set-up, making valid comparisons between studies impossible.

In this study, we examined the single and combined effects of the *COMT* Val108/158Met and *DBH* −1021C>T polymorphisms on the amplitude and latency of the P300 ERP in a two-tone auditory oddball task. Higher dopaminergic activity has generally been related to improved performance on information processing tasks. We therefore hypothesized that subjects carrying genetic variants associated with more dopamine signaling, i.e. Met allele carriers of the *COMT* and T allele carriers of the *DBH* gene, would show larger P300 amplitudes. We also investigated the effect of the *COMT* and *DBH* polymorphism on the latency of the P300. Likewise, as increased dopaminergic signaling was associated with faster information processing speed, we hypothesized that carriers of a Met allele of the *COMT* or T allele of the *DBH* gene would show shorter P300 latencies.

2. Methods

2.1. Subjects

A total of 320 healthy subjects aged 18–70 years with complete P300 data consisting of amplitude and latency for three selected electrode sites of interest (Fz, Cz and Pz) were selected from the Brain Resource International Database administered by BRAINnet for scientific access [BRID; http://www.brainnet.net] (see Table 1 for demographics, and (Gordon et al., 2005) for more detailed information about the BRID). Only subjects genotyped for at least one of the genes were included. All subjects were originally recruited from metropolitan regions in the USA, Europe and Australia. To increase sample homogeneity, only subjects with self-reported European ethnicity were included. Prior to invitation to the research centre, subjects received a structured interview by phone in which inclusion and exclusion criteria were assessed by means of a standard questionnaire (also see Sumich et al., 2006

Table 1
Means and standard deviations of demographics and behavioral results for each genotype.

Measure	*COMT* genotype			*DBH* genotype	
	ValVal	ValMet	MetMet	CC	T carriers (CT/TT)
N	74	142	85	201	115 (101/14)
Gender (m/f)	39/35	67/75	42/43	100/101	58/57
Age (years)	41.6 ± 15.8	38.0 ± 16.1	40.3 ± 14.9	39.8 ± 15.8	40.1 ± 15.7
Years of education	14.5 ± 2.7	14.4 ± 2.6	14.4 ± 2.8	14.6 ± 2.7	14.0 ± 2.7
RT (ms)	340.7 ± 60.9	339.6 ± 47.8	339.7 ± 46.3	340.5 ± 47.0	337.0 ± 54.6
Total errors	0.81 ± 1.7	0.84 ± 1.6	0.75 ± 1.6	0.80 ± 1.6	0.82 ± 1.5

RT = reaction time, ms = milliseconds, Total errors = false positives + false negatives.

for questionnaire). After inclusion, all subjects completed several web-based questionnaires which contained, among others, questionnaires concerning mental health i.e. the SPHERE-12 (Hickie et al., 2001) and medical history. All inclusions were retrospectively verified and excluded if deviation from the normalcy score on the SPHERE and/or medical history questionnaires gave indication one or more of the exclusion criteria were present. Exclusion criteria were: personal or family history of psychiatric disorders like schizophrenia, attention deficit hyperactivity disorder (ADHD), bipolar disorder or other psychiatric condition, personal history of neurological disorder including stroke, Parkinson's Disease, epilepsy, Alzheimer's disease or Multiple Sclerosis, history of past or current substance dependency or extensive abuse, major health problems related to the heart, thyroid gland or cancer, a blood borne illness, severe impediment to vision, hearing or hand movement and loss of consciousness for more than 10 min. All participants were asked to refrain from drinking caffeinated beverages and smoking cigarettes for 2 h, smoking marijuana for 6 h and drinking alcohol for up to 12 h before data collection. All subjects gave written informed consent for inclusion into the study and usage of their data as part of the international database and for all sites Independent Review Board (IRB) approval was obtained.

2.2. P300 recording

EEG recordings were performed using a 32-channel EEG cap (QuickCap, Neuroscan), with electrode positions according to the international 10–20 system and a sampling rate of 500 Hz. Scalp EEG electrodes were off-line referenced to the digital average of A1 and A2 (mastoid) electrode sites. Horizontal and vertical eye movements (EOG) were recorded and all signals were digitized and amplified by NuAmps (Compumedics, Inc.). For all channels, sintered Ag/AgCl electrodes were used and impedances were brought below 5 k Ω at each electrode prior to recording. The P300 ERPs were elicited with an auditory oddball task. In this task, subjects were presented with two tones, either high (1000 Hz) or low (500 Hz) pitched tones. The 'high' tones were the targets and were presented less frequently (60 target tone vs. 280 standard tones). Subjects were instructed to press a button box with the index fingers of both hands whenever a target was presented, and speed and accuracy were equally stressed. For the 'low' tone stimuli no response was required. Subjects were furthermore instructed to sit still and relaxed and keep their eyes open and focus on a red dot in the middle of the screen. All tones were presented at 75 dB for 50 ms with an inter-stimulus interval of 1 s. The total duration of the task was 6 min. ERPs were automatically computed from stimulus-locked EEG data for each participant. Single-trial epochs to target stimuli from the auditory oddball task were filtered with a low-pass Tukey (cosine taper) filter function. A cosine ramp from 1 down to 0.5 between 25 Hz and 35 Hz was used as an envelope on the FFT data in the Tukey filter. The single-trials were averaged to form conventional ERPs. P300 ERP was detected by an automated algorithm that searched for the most positive voltage over a 270–550 ms time window following presentation of the target. The 300 ms preceding the stimulus was considered baseline. The EEG was corrected for horizontal and vertical eye movement by means of the method developed by Gratton et al., 1983. An epoch rejection algorithm was employed, which rejected epochs in which three or more channels exceed a maximum threshold of 100 uV for all sites except Oz, O1 and O2, for which the threshold was 250 uV. Only ERP epochs in which response to target stimuli were correct, were used. If more than 50% of the epochs had to be rejected the data were not included. The P300 recorded from the main midline electrode sites (Fz, Cz and Pz) were of interest for this study. Data was collected from different labs; each of the labs used the exact same methodology and equipment to ensure standardization. Test–retest reliability of the P300 ERP data across sites has previously been shown to be high (.78–.81) and to be site-consistent (Williams et al., 2005;Paul et al., 2007). Site differences on P300 amplitude and latency in the current sample were unlikely as univariate ANOVAs on amplitude and latency at the electrode site of largest amplitude (Pz), and site as between-subject factor (5 levels), showed no significant differences ($F < 1$, p = ns).

2.3. Genotyping

Genomic DNA was extracted from cheek swab samples by standard proteinase K digestion and chloroform extraction. The rs4680 genotype (*COMT* Val108/158Met) was determined using polymerase chain reaction (PCR) amplification of participant DNA using primers 5′-TGTCACCAGGGGCGAGGCTCAT-3′ and 5′-CGGCCCTT TTTCCAGGTCTGAC-3′ under standard conditions. Amplified fragments were digested with the restriction enzyme *Nla*III and digests were separated on 4% agarose gels. The rs1611115 genotype (*DBH* −1021C>T) was determined using primer extension followed by mass spectrometry analysis on the Sequenom MassARRAY system (Sequenom, San Diego, CA) by the Australian Genome Research Facility (http://www.agrf.org.au/). More detailed genotyping protocols are available from the authors upon request. Genotype distributions were checked for accordance with Hardy–Weinberg equilibrium. There were three *COMT* genotype groups (ValVal, ValMet and MetMet) and three *DBH* genotype groups (CC, CT and TT). Due to the small prevalence of the *DBH* TT genotype in the population, we grouped CT and TT into one group of 'T carriers'. We also formed groups based on a composite score of 'high' dopamine (*COMT* MetMet and *DBH* T-carriers, 31 subjects) and 'low' dopamine (*COMT* ValVal and *DBH* CC, 52 subjects) and compared those groups on the P300 amplitude and latency.

2.4. Statistical analyses

Statistical computations were performed with SPSS (version 16.0, Chicago, IL). Potential effects of genotype differences on gender, age, education and behavior were analyzed through Pearson's chi-square test or univariate ANOVA, as appropriate. A chi-square test was also performed to examine the interaction between

polymorphisms. The P300 amplitude and latencies were subjected to four separate mixed model analyses of ANCOVA in which either the *COMT* (ValVal, ValMet and MetMet) or *DBH* genotype (CC and 'T-carriers') was entered as a between-subject factor. Electrode position (Fz, Cz and Pz) was entered as the within-subject factor in each of the analyses. Furthermore, two additional exploratory analyses on the amplitude and latency were performed in which the composite 'high' and 'low' dopamine group was entered as between subject factor. In each model we used age and gender as covariates. Additional analyses with 'smoking status' as covariate were performed. For all analyses, results were considered significant in case of P (2-sided) < 0.05. Post hoc power calculations were made using the G*Power program (Faul et al., 2007). Cohen's d was used to calculate the effect size.

3. Results

3.1. Demographics

In Table 1 the demographics and behavioral outcomes are listed. The observed distribution of both genotypes was in agreement with expected values according to the Hardy–Weinberg equilibrium (p_{COMT} = 0.39; p_{DBH} = 0.77). *COMT* and *DBH* genotypes were available for 94.1% and 98.1% of the subjects, respectively. There were no genotype differences for age and sex (F < 1, p = ns). There was no *COMT* genotype difference for years of education (F < 1, p = ns) and a trend *DBH* genotype difference for education although this did not reach significance [$F(1,314)$ = 2.8, p = 0.096]. The chi-square test reported that the interaction between the two genotypes was not significant ($\chi^2(2)$ = .726, p = .695). In the post hoc power analyses we found that our sample sizes, given the genotype distribution and a power of 80%, were large enough to detect a small effect size (Cohen's d) of 0.16 for the *COMT* and 0.14 for the *DBH* analyses.

3.2. P300 ERP

Table 1 shows the average reaction times and error rates, which were as expected in a two-tone auditory oddball task (Polich and Heine, 1996). The behavioral outcomes were not significantly related to either *COMT* or *DBH* genotype (F < 1, p = ns). The P300 parameters are presented in Table 2. There was no main effect for electrode (F < 1, p = ns), nor was there an interaction effect between electrode and each of the genotypes (F < 1, p = ns), nor was there an interaction between the two genotypes (F < 1, p = ns).

Separate ANCOVAs on each genotype showed that there was no significant association of P300 latency with either *COMT* or *DBH* genotype (all F < 1, p = ns).

Fig. 1 depicts the grand average ERPs elicited by the target stimuli for each of the different variants. The analysis of the P300 amplitude showed a main effect of electrode [$F(2,288)$ = 72.2, p < 0.001]. The post hoc tests showed that the P300 amplitudes over the posterior electrode site (13.9 μV) were significantly higher than over the central and frontal electrode sites (9.4 and 7.9 μV, p < 0.001) and that the amplitude over the central site was significantly higher than over the frontal site (p < 0.001). No significant interactions between electrodes and genotypes, nor significant interactions between *COMT* and *DBH* genotype were found (F < 1, p = ns). Separate analyses on *COMT* and *DBH* genotype indicated no effect of *COMT* or *DBH* genotype on the P300 amplitude (all F < 1, p = ns). Also the composite genotype grouping of 'high' vs. 'low' dopamine showed no effect on either amplitude or latency (F < 1, p = ns).

Replication of all analyses without inclusion of age and gender as covariates yielded essentially the same results (all F < 1, p = ns). Also, replication of the analyses in a more restricted age range (18–40, N = 167) did not show any different effects. Additional analyses that included smoking status as covariate still did not demonstrate a significant effect of genotype in any of the analyses (F < 1, p = ns). However, we did find a significant electrode by smoking status interaction on the P300 amplitude [$F(2,289)$ = 4.2, p = 0.016] indicating lower P300 amplitudes in smokers compared to non-smokers, especially at the more frontal electrode sites.

4. Discussion

Our results demonstrate that neither the *COMT* Val108/158Met nor the *DBH* −1021C>T polymorphism, nor the combined presence of both, were associated with the parameters of the P300 ERP in an auditory oddball task. To our knowledge, this is the largest study on the *COMT* gene polymorphism and the auditory oddball P300 in healthy volunteers. The discrepancies found in the literature may be due to different choices in experimental design, such as electrode positions, (clinical) populations, genetic background or small sample sizes (Bramon et al., 2006; Gallinat et al., 2003; Golimbet et al., 2006; Kang et al., 2010; Tsai et al., 2003). In this study we had 80% power to detect an effect size of 0.14–0.16 for the *COMT* Val108/158Met and *DBH* −1021T > C polymorphisms. The current cohort size is therefore more than sufficient to detect effects typically seen in gene-cognition studies (Barnett et al., 2007; Munafo et al., 2008; Beste et al., 2010; Mier et al., 2010).

The obtained findings also have implications for other dopaminergic gene-auditory oddball P300 studies. The observed findings in the healthy population may point towards potentially distinct auditory oddball P300 – genotype relationships for healthy volunteers and subjects with psychiatric conditions. Despite our null finding, *COMT*-auditory oddball P300 and *DBH*-auditory oddball P300 effects may exist in disease populations. It has been argued that the potential association between dopaminergic candidate genes and the P300 ERP would be disease-dependent (Lin et al., 2001), because more consistent *COMT*-auditory oddball P300 associations have been found in schizophrenic patients (Gallinat et al.,

Table 2
Means and standard deviations of P300 amplitude and latency stratified by genotype.

Measure	*COMT* genotype			*DBH* genotype	
	ValVal	ValMet	MetMet	CC	CT/TT
P300 latency (ms)					
Fz	346.9 ± 37.9	344.2 ± 34.2	344.1 ± 32.3	345.1 ± 35.4	344.6 ± 33.1
Cz	349.1 ± 39.4	345.6 ± 39.1	347.3 ± 36.2	347.6 ± 38.5	345.4 ± 38.7
Pz	356.4 ± 30.6	354.4 ± 31.6	357.3 ± 32.0	356.7 ± 30.3	354.1 ± 32.0
P300 amplitude (μV)					
Fz	7.9 ± 6.2	7.7 ± 6.5	8.2 ± 6.3	7.9 ± 6.2	8.0 ± 6.6
Cz	9.0 ± 7.3	9.1 ± 7.8	9.2 ± 7.0	9.0 ± 7.1	9.6 ± 8.1
Pz	13.6 ± 6.7	13.8 ± 6.7	13.8 ± 6.5	13.7 ± 6.6	14.1 ± 6.8

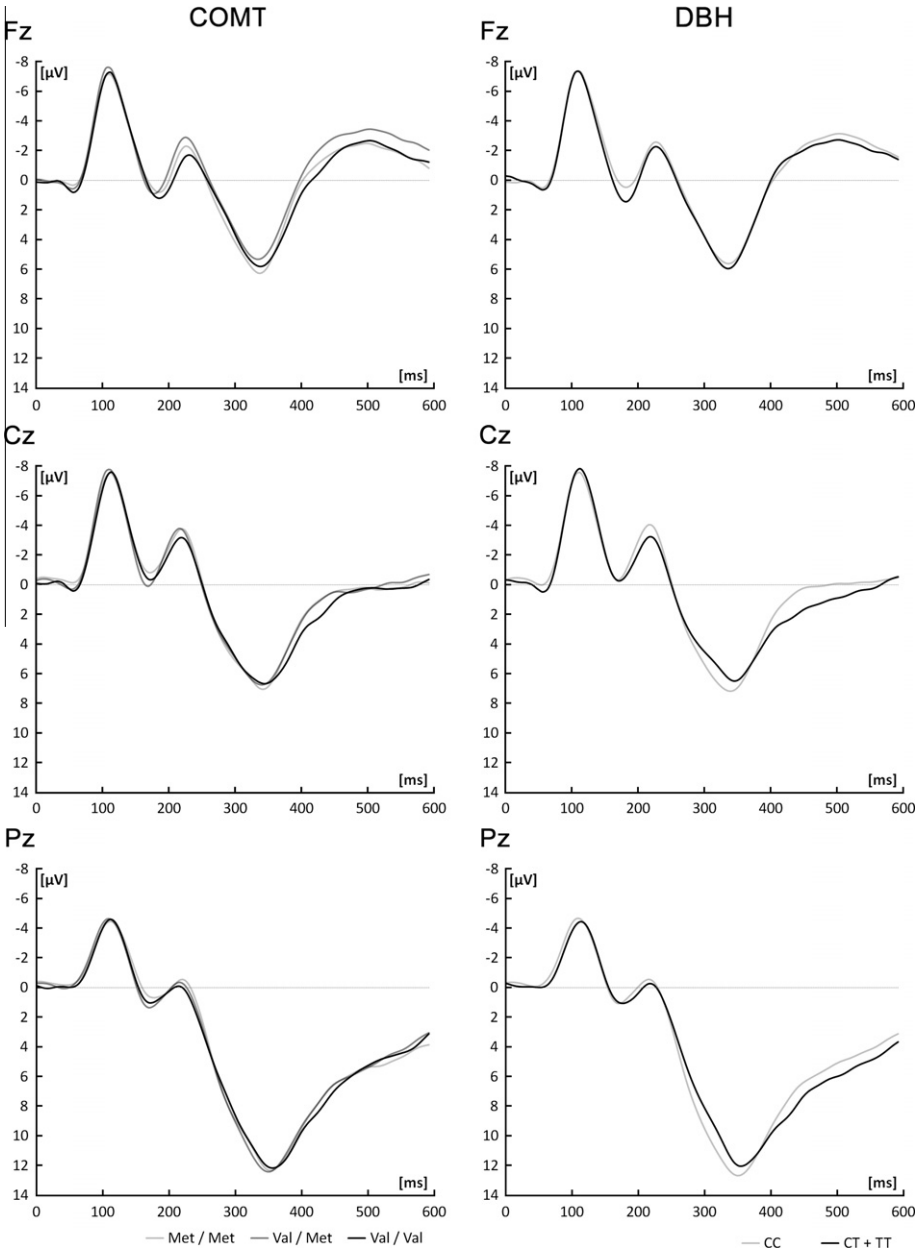

Fig. 1. Grand averages of the P300 potentials at Fz, Cz and Pz related to the target tone for *COMT* (left) and *DBH* (right) genotype.

2003; Golimbet et al., 2006). However, one study did not support this finding (Bramon et al., 2006). Investigating genotype-ERP associations in disease as well in the healthy population is of relevance for the field of endophenotype research. The auditory oddball P300 ERP is considered to be an endophenotype candidate for several clinical conditions like schizophrenia (Bramon et al., 2005; de Wilde et al., 2008) and substance use disorders (Hesselbrock et al., 2001; Bramon et al., 2005; Euser et al., 2012). We argue that genotype-endophenotype associations should also be investigated in healthy volunteers, as their brains are undisturbed by chronic disease and long-term treatment.

Additionally, although we investigated two dopaminergic candidate genes, there are multiple other ways to investigate if and how dopaminergic modulations affect the P300 ERP in an auditory oddball paradigm. We selected polymorphisms of the *COMT* and *DBH* genes because of their relation to variable cortical prefrontal DA levels. However, there are multiple other dopaminergic candidate genes. Polymorphisms of the genes encoding the dopamine D_2, D_3, D_4 receptors have already been associated with variations in P300 amplitude and latency (Noble et al., 1994; Hill et al., 1998; Mulert et al., 2006; Birkas et al., 2006; Berman et al., 2006). Similarly, a recent study demonstrated that striatal dopamine D_2/D_3-receptor availability as measured with SPECT was correlated with the auditory oddball P300 amplitude and latency (Pogarell et al., 2011). Furthermore, although we selected the most well investigated functional polymorphism in each gene for this study, other functional polymorphisms of the *COMT* and *DBH* genes are also known (Stein et al., 2005; Chen et al., 2012). All these factors could modulate characteristics of the auditory oddball P300, but have not been extensively investigated yet. Notably, as already stated in the introduction, both genes affect other neurotransmitters like norepinephrine, a neurotransmitter which is also known to affect the P300 ERP in an auditory oddball paradigm (e.g. Murphy et al., 2011; Nieuwenhuis et al., 2005). The

interpretation of the results can therefore never be based on altered dopaminergic signaling exclusively. A comprehensive understanding of the dopaminergic modulation of the auditory oddball P300 ERP needs to consider the contributions of multiple dopaminergic genes, dopaminergic imaging methods as well as dopaminergic drug manipulations.

A few limitations that could have affected the conclusions should be noted. First, we only used a two-tone auditory oddball design to examine the gene-P300 association. The P300 ERP elicited in a typical auditory oddball paradigm as employed is thought to involve overlapping activities from P3a and P3b components (Polich and Criado, 2006). Therefore, the results specifically concern the modulation of the auditory oddball P300 potential as obtained in this specific paradigm, but do not exclude potential associations with other P300 ERPs which are functionally and physiological different e.g. the 'Novelty P300' (Polich and Criado, 2006). It is possible that *COMT*/*DBH*-auditory oddball P300 associations will be observed after administrating different paradigms. This may also apply to tasks in other sensory modalities. For example, Yue et al. (2009) showed strong *COMT*-auditory oddball P300 associations in a continuous performance task with visual stimuli, even in a small subject sample. Task simplicity may also play a role. In a previous P300 investigation, an increased neuronal activity was observed in a P300 task requiring larger cognitive load compared to a simpler task (Wild-Wall et al., 2011). Higher difficulty may imply more allocation of underlying physiology, thereby magnifying the effect of the polymorphism on the ERP.

Second, there was a great diversity in demographics in our sample. The age range is 18–70. It is known that the activation of the dopamine system reduces across age (e.g. Kumakura et al., 2010), thereby introducing fluctuations in dopaminergic signaling in addition to the dopamine changes associated with the investigated candidate genes. Similarly, a subgroup of the population reported using substances that affect the brain and potentially also the auditory oddball P300, i.e. alcohol, nicotine and marijuana. It should be noted that none of the alcohol and marijuana users reported a history of dependence, and the number of subjects that reported to use marijuana consisted of a very small number (<2.5% of the total population). Prior to testing, abstinence criteria were applied in order to limit the acute effects. The 6 h abstinence for marijuana was limited, however, all users in our normative control group had reported not having used marijuana within 24 h prior to testing. Despite, the potential modulation of marijuana use on the auditory oddball P300 cannot be completed ignored as long-term use is associated with a cannabinoid build-up in the brain, which in turn is thought to influence the P300 (Solowij et al., 1995).

The concerns of potential distortions by age and smoking could be largely accounted for. No difference in results were observed after performance of additional analyses in a more restricted age range, and inclusion of age and smoking status as covariates. The inclusion of smoking status as covariate, did demonstrate lower auditory oddball P300 amplitudes in the smokers group, which was in accordance with expectation (Guney et al., 2009; Mobascher et al., 2010). Despite, the sample should ideally have been divided in more additional subgroups. Thus, regardless of the large sample size, our study suffers from insufficient power to address the *COMT*/*DBH*-P300 association in more homogenous subgroups. In contrast, we ensured that all participants were of European ancestry, as it is known that ethnicity largely affects genetic variations. It does confine, however, the generalizability of our findings to other populations. It is therefore important for future research to replicate this in other ethnic groups.

In conclusion, the current work provides relatively strong evidence that the *COMT* Val108/158Met and *DBH* −1021C>T polymorphisms are not associated with P300 amplitude or latency in an auditory oddball paradigm in the healthy population. The results of this study extend the knowledge of the underlying mechanisms of the auditory oddball P300 ERP as well as the effect of dopaminergic candidate genes on cognitive functioning. Establishing whether the auditory oddball P300 ERP is modulated by certain genotypes is only the first step towards a comprehensive view on the auditory oddball P300 in which clear relationships between genetic, neurophysiological and behavioral constructs are incorporated.

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