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Impact of Catechol-*O*-Methyltransferase on Prefrontal Brain Functioning in Schizophrenia Spectrum Disorders

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The enzyme catechol-*O*-methyltransferase (COMT) has attracted increasing interest regarding a genetic disposition towards schizophrenias and as a modulator of prefrontal brain function. A common SNP in the *COMT* gene causes a Val to Met transition at AA158/AA108 (Val158Met), resulting in reduced COMT activity in Met allele carriers. An impact of *COMT* genotype on cognition has been well established; however, the exact nature of this influence has yet to be elucidated. The aim of this study was to determine whether *COMT* genotype affects an electrophysiological marker of prefrontal activation and neuropsychological frontal lobe measures in schizophrenia. To this end, 56 acutely psychotic in-patients with schizophrenia spectrum disorders were investigated. Patients with the *COMT* 1947AA (Met/Met) genotype ($n = 13$) were compared to a carefully matched sample of patients with a G1947A (Val/Met) genotype ($n = 15$); matching criteria included patients' age, handedness, gender distribution, diagnosis, and medication status. A small group of six homozygous Val allele carriers was additionally included to allow an assessment of possible gene-dosage effects. P300 amplitudes and latencies, as well as an electrophysiological marker of prefrontal brain function (NoGo-Anteriorization/NGA) and neuropsychological measures (Stroop Test, Verbal Fluency, Trail Making Test) were regarded. Homozygous Met allele carriers had significantly increased NGA values and fronto-central Nogo amplitudes compared to patients with at least one Val allele. They also tended to perform better in the Stroop task, as compared to the matched group of Val/Met patients. These results indicate that *COMT* genotype exerts a strong impact on prefrontal functioning and executive control in schizophrenia spectrum disorders.
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INTRODUCTION

Schizophrenia is an etiologically heterogeneous group of disorders that involve serious alteration of the patients' cognitive, emotional, and social functioning. One of their cardinal characteristics is a hypofunctionality of the frontal cortex ('hypofrontality concept'; Ingvar and Franzen, 1974), which has been demonstrated in various studies and with different methodological approaches (eg Andreasen *et al*, 1992; Fallgatter and Mueller, 2001). One of the affected areas is the anterior cingulate cortex (ACC), which has been shown to be deficient at rest (Tamminga *et al*, 1992) as well as during activation with neuropsychological tasks (Andreasen *et al*, 1992; Carter *et al*, 1997). The ACC is critically involved in executive functions such as the monitoring and regulation of ongoing actions, which usually comprise initiation of appropriate and inhibition of inappropriate actions. Tasks that typically involve both these processes are Go–Nogo paradigms that demand the preparation and execution of responses to predefined target stimuli (Go) as well as the inhibition of prepared motor responses (Nogo).

Over the past years, an electrophysiological parameter has been developed and validated that supposedly reflects activation within prefrontal brain areas, particularly involving the ACC (NoGo-anteriorization, NGA; Fallgatter *et al*, 1997). The NGA quantifies the anteriorization of the positive brain electrical field during the inhibition of prepared (motor) responses that is usually observed during Go–Nogo tasks such as the Continuous Performance Test (CPT). Within the event-related potential (ERP), a marked positive component (P300) can be observed about 300 ms after presentation of a stimulus, for both Go and Nogo conditions of such a task. However, the topography of the P300 is usually located significantly more anterior during Nogo (response inhibition) as compared to Go (response execution) trials (eg Bokura *et al*, 2001). This anteriorization of the brain electrical field can now be quantified by the NGA, which represents the geometrical distance (or the arithmetical difference) between the center of gravity

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('centroid') of the Go and Nogo P300 field distribution. In healthy subjects, the NGA was shown to have a very high interindividual stability, excellent short- and long-term test–retest reliability, and it appears to be independent of the subjects' age and gender (reviewed by Fallgatter, 2001). In accordance with the results obtained by brain imaging studies (de Zubicaray *et al*, 2000; Rubia *et al*, 2001; Ford *et al*, 2004; Matthews *et al*, 2004), electrophysiological source localizations (LORETA procedure; Pascual-Marqui *et al*, 1994) have revealed a close relationship between the NGA and a Nogo hyperactivity within prefrontal brain areas, particularly the ACC, in healthy subjects (Strik *et al*, 1998; Fallgatter *et al*, 2002). Based on such findings, the NGA has been suggested to be an electrophysiological correlate of cognitive response control and a neurophysiological marker of ACC function, even though other brain regions, particularly within the prefrontal cortex, are likely to be involved as well (eg Ford *et al*, 2004). In accordance with the hypofrontality concept, schizophrenic patients were found to have a significantly diminished NGA (Fallgatter and Mueller, 2001) and a reduced activation of the ACC during Nogo conditions (Fallgatter *et al*, 2003), findings that are again largely in line with electrophysiological (Kopp and Rist, 1999; Strandburg *et al*, 1999; Kiehl *et al*, 2000; Weisbrod *et al*, 2000; Mathalon *et al*, 2002) and neuroimaging data (Volz *et al*, 1999; Carter *et al*, 2001; Rubia *et al*, 2001; Laurens *et al*, 2003).

The enzyme catechol-*O*-methyltransferase (COMT) has gathered increasing interest in recent years with respect to the genetic disposition towards schizophrenia and as a modulator of prefrontal function in schizophrenic patients and healthy controls. COMT degrades catecholamines and is thought to compete for dopamine removal from the synaptic cleft with the dopamine transporter. As the latter is abundantly expressed in the striatum, COMT does not seem to play a major role here; however, in the prefrontal cortex, COMT is thought to be crucially involved in dopamine metabolism (Weinberger *et al*, 2001).

Schizophrenia has a substantial heritability of $>80\%$, as estimated in a recent meta-analysis (Sullivan *et al*, 2003). Thus, huge efforts have been made to identify disease genes. Chromosome 22q has been described as one of the confirmed regions for susceptibility loci (Badner and Gershon, 2002). Interestingly, the gene encoding COMT resides at 22q11, which makes it an attractive candidate gene. A common SNP was found in the *COMT* gene causing a Val to Met transition at amino-acid position 158 (or 108, respectively), which is commonly designated as Val158Met. The Met allele codes for a 'thermo-labile' enzyme displaying lower enzymatic activity, resulting in increased synaptic dopamine and strengthened (prefrontal) dopaminergic tone. Met/Met homozygotes display approximately 25% COMT activity compared to Val/Val homozygotes, with heterozygotes in-between. *COMT* was therefore one of the prime candidate genes to identify disease genes for psychosis, and Val158Met is currently one of the most frequently studied polymorphisms in schizophrenia. The results of association studies, however, are somewhat ambiguous with both positive (eg Wonodi *et al*, 2003) and negative (Inada *et al*, 2003) findings. A recent haplotype analysis (Sanders *et al*, 2005) added further evidence for *COMT* making a contribution to the genetic risk of schizophrenia, as did a study utilizing a large number of Irish schizophrenia high-density families (Chen *et al*, 2004). Importantly, in both cases, the over-transmitted haplotype included the Val allele.

At a functional level, the impact of *COMT* genotype on cognition has gathered increasing interest in recent years. Evidence for the influence of *COMT* on cognitive abilities came from *COMT* knockout mice that were found to display improved performance in a memory task (Kneavel *et al*, 2000). Val158Met thereafter has been repeatedly examined regarding its association with cognitive functioning, and in a seminal study Weinberger and associates showed that the Val allele causes reduced performance in the Wisconsin Card Sorting Test (thought to mirror frontal executive functioning) in schizophrenic patients as well as healthy controls (Egan *et al*, 2001), a finding that could be replicated in numerous subsequent studies. Regarding other neuropsychological functions, Val158Met was also found to influence processing speed and attention in chronic schizophrenic patients (Bilder *et al*, 2002) and working memory in schizophrenics, their siblings, and controls (Goldberg *et al*, 2003). In a recent study (Nolan *et al*, 2004), schizophrenic Met allele carriers displayed better cognitive *stability*, whereas subjects with the Val allele showed better cognitive *flexibility*.

Thus, *COMT* apparently plays a role in some cognitive domains, although its exact contribution to functional skills has yet to be elucidated. One approach to further clarify the meaning of *COMT* for cognitive functioning and underlying cerebral mechanisms is the examination of neurophysiological parameters that reflect basic mechanisms of brain activation during the performance of cognitive operations and that are likely to be more directly linked to underlying genomic variation than a highly variable behavioral phenotype. Compared to association studies with genetically complex behavioral traits, which frequently comprise several hundreds of subjects, robust gene–brain activity correlations allow the investigation of substantially smaller sample sizes (Egan *et al*, 2001; Fallgatter *et al*, 2004). As the NGA has been suggested to be an electrophysiological correlate of prefrontal functioning and is closely related to fundamental cognitive processes of executive control, it might be a promising research parameter in attempts to further examine the impact of *COMT* genotype on such processes. Considering the above-mentioned findings, we reasoned that *COMT* genotype might influence this electrophysiological marker in schizophrenia spectrum disorders. Therefore, the NGA was recorded in 34 carefully selected psychotic patients and correlated to Val158Met.

PATIENTS AND METHODS

Patients

A total of 56 acutely psychotic psychiatric in-patients who were suffering from schizophrenia spectrum disorders were investigated. Exclusion criteria were age below 18 and above 60 years, comorbidity with other currently present axis-I disorders, a history of or an actually manifest disease of the CNS, or other severe somatic diseases. Thirteen patients with a *COMT* 1947AA genotype (homozygous Met allele carriers) had a sufficient number of at least 20 artifact-free

ERP epochs, and could therefore be included in the present analysis (eight male, 10 right-handed, mean age 39.8 ± 7.0 years). To ensure proper matching, 15 out of 22 patients with a G1947A genotype (Val/Met allele carriers) were selected to closely resemble the AA sample in age, handedness, and gender distribution (seven male, 13 right-handed, 38.6 ± 11.4 years), as well as diagnoses and medication status (antipsychotics and co-medication, see below). The matching procedure was performed meticulously and before all data analyses. As a result, the selected group of Val/Met carriers did not differ significantly from the Met/Met group in any of the above-mentioned parameters ($t_{age} = 0.34$, $p = 0.74$; t_{cpz} (daily chlorpromazine equivalents in mg (see below)) = 1.05, $p = 0.30$; all $\chi^2 < 0.8$, $p > 0.4$). A small group of six homozygous Val allele carriers (three male, six right-handed, mean age 30.7 ± 9.3 years) was additionally included to allow an assessment of gene-dosage effects, at least on a descriptive level. As this last group was very small and thus could not be strictly matched to resemble the other two genotype groups, it was only included in additional non-parametric statistical analyses (see below), which should be regarded as exploratory.

According to the SKID-I-Interview, patients were diagnosed as disorganized (295.10; $n = 6$), catatonic (295.20; $n = 4$), paranoid (295.30; $n = 11$), and undifferentiated (295.90; $n = 3$) types of schizophrenia, schizophreniform (295.40; $n = 8$), or schizoaffective disorders (295.70; $n = 2$). The mean duration of the disease was 172 ± 104 months (mean ± SD), with an average of 6.9 ± 7.5 admissions to psychiatric hospitals. Five patients had a positive family history of schizophrenia; another 10 patients had first-degree relatives with non-psychotic or unknown psychiatric conditions. No significant current comorbidities were found in either patient sample except for one patient with neuroleptic-induced adiposity and one with hypertonia; in the Val/Met group, two patients had life-time comorbidities (Bulimia nervosa; anxiety disorder), in the Met/Met group one patient had a previous anorexia nervosa.

Neuroleptic treatment consisted of 529 ± 400 mg chlorpromazine equivalents per day; 13 patients were treated with typical antipsychotics, 15 patients received atypical antipsychotics, and six patients no neuroleptic medication. As already mentioned above, Val/Met and Met/Met patients did not differ significantly regarding the diagnoses, nor the amount or type of antipsychotic medication. Co-medications were regarded in the matching procedure as well, so that the mean daily doses did not differ significantly between the two genotypes ($t < 1.2$, $p > 0.25$) for any of the potentially relevant substance groups (carbamazepin, biperiden, lithium, lorazepam, valproic acid, SSRI).

Written informed consent was obtained from all patients after the procedures had been fully explained. The study was approved by the Ethics Committee of the University of Wuerzburg, and the procedures involved were in accordance with the Declaration of Helsinki.

Psychopathological and Neuropsychological Assessment

Each patient underwent an extensive psychometric examination, consisting of the SKID-I-Interview, the Brief Psychiatric Rating Scale (BPRS; Overall and Gorham, 1962), the Positive and Negative Symptoms Scale (PANSS; Kay, 1991), and the Hamilton Depression Rating Scale (HDRS; Hamilton, 1960).

The neuropsychological assessment consisted of the Verbal Fluency Test (VFT), a Stroop Color Word Task, and the Trail Making Test (TMT). For the VFT, patients were instructed to name as many nouns as possible beginning with a certain letter ('letters version') or belonging to a certain category of words ('categories version'). The Stroop task consisted of three parts, with two control conditions ('word reading', 'color naming') and one interference condition (color words were presented in a color that did not correspond to the word meaning of the color word, and patients were instructed to name the ink color of the words). For the TMT, patients had to connect the numbers 1–15, randomly distributed on a sheet of paper, in the correct order (Part A), or connect the numbers 1–8 and the letters A–G in the correct order while consecutively alternating between numbers and letter (Part B).

Electrophysiological Investigation

The electrophysiological investigation took place in an electrically shielded, dimly lit room where the participants performed a CPT. Letters were presented sequentially in a pseudo-randomized order and the patients had to press a response button whenever the letter 'O' (Primer) was directly followed by an 'X' (Go condition). The whole stimulus set consisted of 400 letters, with 114 primer stimuli, 57 Go and Nogo conditions (O followed by any other letter), and 172 distractors. Each letter was presented for 200 ms with an interstimulus interval of 1650 ms. The recording sessions took place between 0800 and 1200.

The EEG was recorded from 21 scalp electrodes placed according to the International 10–20 system (Jasper, 1958) with three additional electrodes to monitor eye movements. Linked mastoids were used as the recording reference; electrode impedances were below 5 kΩ. The recording system involved a 32-channel DC amplifier (Brain Star System) and the Neuroscan data acquisition software calibrated with an external 100 μV/10 Hz signal. With an A/D rate of 256 Hz, the hardware filter was set to a bandpass of 0.1–70 Hz.

Data Analysis

Data were analyzed offline with the program 'Vision Analyzer' (Brain Products, Munich, Germany). After re-referencing the data to an average reference, they were segmented according to the conditions of the CPT (segments from −100 to 700 ms after stimulus presentation), and the Go and Nogo epochs were further analyzed. A computerized artifact rejection excluded all segments with amplitudes exceeding ± 50 μV; if at least 20 artifact-free EEG epochs were available for the Go and the Nogo condition, the remaining segments were averaged to one Go and one Nogo ERP per patient.

In the individual ERPs, the global field power (GFP; Lehmann and Skrandies, 1980) peaks were determined within a P300 time frame (275–530 ms; based on a visual inspection of the grand average curves). The GFP represents the mean of all possible potential differences in a given

scalp potential field and is used as a measure of the amount of activity in this field. At the individual GFP peaks, the amplitude, latency, and anterior–posterior location of the positive centroid (the amplitude-weighted center of gravity of the positive brain electrical field; Lehmann, 1987) were calculated. The centroid locations were quantified by a coordinate system defined by a two-dimensional delineation of the electrode array (cf Figure 1). For the purpose of the present study, only the centroids in the anterior–posterior direction were of interest, the more anterior locations of the centroids being represented by smaller numbers on this axis (eg '1' represents electrode position Fpz, '5' represents Oz). Finally, the NGA, defined as the distance between the individual Go and Nogo centroid within the coordinate system (unit of the NGA = 'electrode positions'), was calculated.

In addition to this topographical analysis, the P300 peaks were determined for the three midline electrode positions (Fz, Cz, Pz) employing a semi-automatic peak picking procedure and using the same time frame mentioned above (275–530 ms post-stimulus); amplitudes and latencies of these peaks were analyzed.

Genotyping

DNA was extracted from whole blood. Genotyping for *COMT* G1947A SNP was accomplished using standard PCR procedures modified from a previously published protocol (Egan *et al*, 2001); primers were 5′-GGG GCC TAC TGT GGC TAC TC-3′ (forward) and 5′-TTT TTC CAG GTC TGA CAA CG-3′ (reverse). Briefly, PCR reactions were performed in a reaction volume of 25 µl, including approximately 50 ng of template genomic DNA, 10 pmol of each primer, 2.5 mM of each dNTP, 0.75 mM $MgCl_2$, and 1 U of *Taq* DNA polymerase. Annealing temperature was 58°C (35 cycles). PCR products were digested with *Nla*III (3 h at 37°C; fragment sizes: wild-type G1947, 114 bp; 1947A variant, 96 and 13 bp) and subsequently visualized on a 4% agarose gel. G1947 corresponds to the high-activity Val158 allele; 1947A codes for the low-activity Met variant.

Statistical Analysis

Only the data of Met/Met and Val/Met carriers were subjected to parametric statistical analyses, as only these two groups were properly matched and included a sufficient number of patients per group. For the P300 centroids of these two groups, a 2×2 analysis of variance (ANOVA) for repeated measurements was conducted, with the within-subject factor 'condition' (Go, Nogo) and the between-subject factor 'group' (Met/Met *vs* Val/Met). *Post hoc* analyses were conducted by means of two-tailed *t*-tests for matched or independent samples. *T*-tests were also used to compare the single electrode (peak) ERP data, the NGA, and the behavioral performance between the two groups. Equality of variances was tested by means of Levene's test, and corrections for inequality were performed whenever necessary. As the number of commission errors was not normally distributed (Kolmogorov–Smirnov $Z = 1.54$, $p < 0.05$), between-group comparisons for this parameter were conducted by means of Mann–Whitney *U*-tests. All the other electrophysiological and behavioral parameters were normally distributed (Kolmogorov–Smirnov $Z < 1.2$, $p > 0.2$) and were therefore subjected to parametric testing procedures.

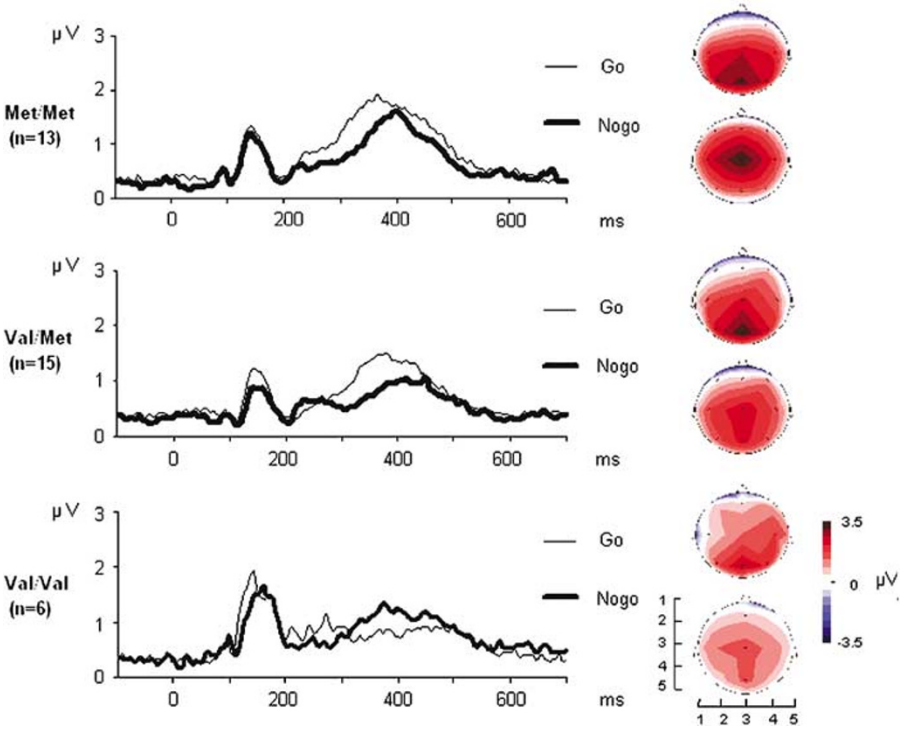

Figure 1 GFP grand average curves. GFP curves for the CPT Go (thin) and Nogo condition (bold line) in patients with Met/Met ($n = 13$), Val/Met ($n = 15$), and Val/Val ($n = 6$). The maps display the brain electrical field at the time point of the GFP peak for the Go (the upper one of each pair of maps) and Nogo (lower maps) condition.

Regarding the third genotype group (Val/Val; $n=6$), supplementary non-parametric test procedures were additionally conducted to investigate an effect of the factor 'genotype' on neurophysiological and behavioral parameters across the three *COMT* polymorphism groups (Kruskal–Wallis tests with Mann–Whitney *U*-tests for *post hoc* comparisons). Because of the small number of patients included in the third genotype group, however, this additional statistical analysis should be regarded as preliminary and the results should be interpreted carefully.

RESULTS

Performance Measures, Psychometry, Neuropsychology

Reaction times, commission errors (button-press after non-target stimulus), and omission errors (no response to Go stimulus) were used as performance measures for the CPT. Statistical testing revealed no significant differences between the two matched groups (Met/Met and Val/Met) for any of the behavioral measures (reaction times: 633 ± 200 *vs* 606 ± 126 ms, $t_{26}=0.44$, NS; omission errors: 8.6 ± 9.5 *vs* 8.5 ± 9.4 errors, $t_{26}=0.02$, NS; commission errors: 2.3 ± 3.3 *vs* 1.1 ± 1.4 errors, $U=84.5$, NS) (Val/Val: RT $=580\pm86$ ms; 3.33 ± 2.50 omission errors; 1.83 ± 2.40 commission errors; Kruskal–Wallis $\chi^2=0.73$, 1.74, and 0.44, respectively; $p>0.4$). Regarding the different psychometric scales, the two groups did not differ significantly either, even though patients with a Met/Met genotype exhibited slightly higher scores on most of them (Table 1).

Regarding the neuropsychological tests, Met/Met and Val/Met patients did not differ significantly in their VFT (letters: 21.7 ± 9.8 *vs* 24.2 ± 12.1 words, $t_{26}=0.60$, NS; categories: 29.1 ± 10.4 *vs* 32.9 ± 10.5 words, $t_{25}=0.97$, NS) (Val/Val: 18.5 ± 7.1 and 29.0 ± 10.6 words, respectively; Kruskal–Wallis $\chi^2<3.8$, $p>0.15$) or TMT performance (A: 28.2 ± 19.4 *vs* 30.7 ± 12.1 s, $t_{26}=0.42$, NS; B: 54.9 ± 34.8 *vs* 70.2 ± 46.0 s, $t_{26}=0.98$, NS) (Val/Val: 21.0 ± 7.6 and 58.0 ± 31.7 s in TMT A and B; Kruskal–Wallis $\chi^2<3.8$, $p>0.15$); however, for the interference condition of the Stroop Test, the group of patients with a heterozygous genotype exhibited a statistical trend for prolonged times compared to the Met/Met group (116.8 ± 35.0 *vs* 146.4 ± 54.7 s, $t_{24}=1.73$, $p<0.1$) (Val/Val: Stroop interference: 138.7 ± 66.7 s; Kruskal–Wallis $\chi^2<3.8$, $p>0.15$). For the two control conditions of the Stroop task, on the other hand, a similar trend could not be detected, the time needed to accomplish the tasks being very similar for all groups (data not shown).

NGA and ERP Data

Regarding the topographical ERP analysis, the ANOVA for the positive centroids revealed a significant main effect of the factor 'condition' ($F_{1,26}=9.18$, $p<0.01$) and a significant interaction 'condition × group' ($F_{1,26}=5.99$, $p<0.05$).

Regarding the main effect, the centroid was located more anteriorly in CPT Nogo trials (3.2 ± 0.1) as compared to Go conditions (3.5 ± 0.1), which is a common finding ('NGA') even more markedly visible in healthy subjects. Regarding the interaction, the Nogo-related anteriorization of the brain electrical field was much more pronounced in patients carrying Met/Met (Go *vs* Nogo centroid: 3.54 ± 0.66 *vs* 3.08 ± 0.43, $t_{12}=3.75$, $p<0.01$) than in the Val/Met group (3.41 ± 0.62 *vs* 3.36 ± 0.69, $t_{14}=0.43$, NS; Figure 1).

This finding is confirmed by a significantly reduced NGA in the group of Val/Met patients compared to the Met/Met group (Table 2). Homozygous Val allele carriers ($n=6$) showed an even further reduced mean NGA (-0.37 ± 1.00), a Kruskal–Wallis test confirming an effect of the factor 'genotype' on the NGA across the three groups ($\chi^2=7.25$, $p<0.05$) with a significantly larger NGA in Met/Met patients as compared to Val allele carriers (Val/Met: $U=48.0$, $p<0.05$; Val/Val: $U=15.0$, $p<0.05$). Thus, the Val allele caused a reduced anteriorization of the brain electrical field during Nogo conditions in a linear way (gene-dose effect).

These topographical findings were largely reflected by the single electrode ERP data (Table 2). Regarding the two matched groups of patients, Val/Met and Met/Met subjects did not differ significantly regarding any of the ERP measures elicited by Go trials. For the Nogo condition, however, Val/Met patients exhibited significantly decreased P300 amplitudes at fronto-central electrode sites, and it is this failure to generate a robust fronto-central P300 during the CPT Nogo condition that underlies the reduced NGA we observed in this group of patients. When taking into

Table 1 Psychometric Data

	Val/Val ($n=6$)	Val/Met ($n=15$)	Met/Met ($n=13$)	t (Val/Met *vs* Met/Met)	Kruskal-Wallis (χ^2)
HDRS	9.50 ± 3.99	8.00 ± 4.83	11.77 ± 6.85	1.70, NS[a]	2.21, NS
BPRS	37.00 ± 6.51	36.80 ± 8.00	41.54 ± 13.37	1.12, NS	0.53, NS
PANSS	56.00 ± 7.18	57.33 ± 10.53	67.85 ± 21.38	1.61, NS[a]	1.74, NS
Pos	15.50 ± 5.17	12.87 ± 4.41	14.92 ± 4.89	1.17, NS	2.48, NS
Neg	13.33 ± 2.42	14.80 ± 4.66	18.00 ± 8.15	1.30, NS	2.79, NS
Global	27.17 ± 3.54	29.67 ± 5.29	34.92 ± 11.19	1.55, NS[a]	2.16, NS
GAF	41.50 ± 9.05	39.93 ± 6.86	38.15 ± 7.16	0.66, NS	0.48, NS

[a] $0.1<p<0.2$.

Table 2 ERP Data

	Val/Val ($n=6$)	Val/Met ($n=15$)	Met/Met ($n=13$)	t (Val/Met *vs* Met/Met)	Kruskal-Wallis (χ^2)
Go					
Fz	1.20±0.75	1.05±0.84	1.18±1.28	0.30, NS	0.13, NS
Cz	1.73±1.11	2.68±1.20	3.22±1.53	1.05, NS	3.96, NS
Pz	2.80±1.12	3.73±1.20	3.67±1.85	0.11, NS	2.37, NS
GFP	2.15±0.79	2.29±0.74	2.58±0.91	0.91, NS	1.41, NS
Centroid	2.86±0.92	3.41±0.62	3.54±0.66	0.52, NS	3.48, NS
Nogo					
Fz	1.49±0.95	1.28±0.72	1.96±0.80	2.37, $p<0.05$	3.95, NS
Cz	2.64±0.91	2.54±1.06	3.81±1.45	2.66, $p<0.05$	5.01, $p<0.1$
Pz	2.22±0.88	2.23±0.91	2.26±1.25	0.06, NS	0.09, NS
GFP	1.81±0.62	1.68±0.48	2.13±0.86	1.71, $p<0.1$	2.51, NS
Centroid	3.22±0.30	3.36±0.69	3.08±0.43	1.27, NS	2.46, NS
NGA	−0.37±1.00	0.05±0.44	0.45±0.44	2.45, $p<0.05$	7.25, $p<0.05$

account the group of Val/Val patients as well, a Kruskal–Wallis test confirmed a statistical trend for a genotype effect on the Cz amplitude only ($\chi^2=5.01$, $p<0.1$), Met/Met patients showing increased amplitudes as compared to Val allele carriers (Val/Met: $U=54.5$, $p<0.05$; Val/Val: $U=19.0$, $p<0.1$) All three groups exhibited very similar P300 latencies, with no significant differences between the different genotypes (data not shown).

To ensure that between-group differences were not caused by differences in the number of ERP epochs included in the analyses, the number of artifact-free ERP epochs was determined for each group. With a mean of 43.2±11.2, 43.2±9.2, and 49.0±5.7 Go epochs, as well as 48.6±8.1, 47.6±7.6, and 51.0±3.0 Nogo segments included in the analysis of patients with a Met/Met, Val/Met, and Val/Val genotype, respectively, the three groups did not differ significantly in either condition (Go: $F_{2,31}=0.91$; $p=0.41$; Nogo: $F_{2,31}=0.47$; $p=0.63$).

DISCUSSION

We conducted an electrophysiological and neuropsychological assessment of patients with schizophrenia spectrum disorders stratified for COMT Val158Met polymorphism. In contrast to rather small effects on neuropsychological measures, the Val allele impacted heavily on the NGA, an electrophysiological marker of prefrontal brain function. Val allele carriers had a virtually absent anteriorization of the brain electrical field during Nogo trials, indicating reduced activation within prefrontal brain regions during conditions that impose increased demands on cognitive response control. This effect occurred in a gene-dosage-dependent manner, with Met/Met patients showing the largest, Val/Met patients an intermediate, and Val/Val patients a very small, even negative mean NGA. This indicates that patients with a particularly strong dopaminergic tone showed most consistently the expected functional activation pattern in a task involving prefrontal engagement. This is in accordance with previous studies pointing towards a role of *COMT* genotype in cognition, particularly with respect to prefrontal brain functions (see Introduction). This topographical effect was also partially reflected in the single electrode ERP data, which showed a modulation by *COMT* genotype in a very similar way, albeit not as strongly and consistently. In line with the present findings, we showed in a preliminary report that two subjects suffering from 22q11 deletion syndrome and thus hemizygous for *COMT* featured an absent NGA as well (Reif *et al*, 2004), further underscoring the results of the present study.

In the present study, only patients with schizophrenia spectrum disorders were investigated. Thus, no statement on the electrophysiological impact of the COMT Val158Met polymorphism in healthy control subjects as compared to schizophrenic patients can be made on the basis of the present data. There are, however, numerous studies reporting a detrimental effect of the Val allele on prefrontal functioning (neuropsychological and neuroimaging data) in both schizophrenic patients and healthy controls (for review see Tunbridge *et al*, 2006; Craddock *et al*, 2006). As an underlying explanatory model, Weinberger and colleagues suggested an inverted U-shaped relation between cortical dopamine and prefrontal cortex function, the precise effect of COMT activity depending on the basic dopaminergic tone of a given individual on this U-shaped curve. They furthermore suggest that in healthy controls without pharmacological intervention, individuals with a Met/Met phenotype are located around the peak of the inverted U-shaped curve, with Val/Met and Val/Val carriers located slightly further down along the curve's rising 'left' arm (Tunbridge *et al*, 2006). This would account for the replicated finding of a dose-dependent positive influence of the Met allele on prefrontal (cognitive) functioning. Assuming that schizophrenic patients show hyperactive mesolimbic dopamine projections, but *hypoactive* mesocortical dopamine projections to the prefrontal cortex (eg Abi-Dargham and Moore, 2003), they should generally be

located further down towards the beginning of the rising part of the inverted U-shaped curve. The principal effect of *COMT* genotype should therefore be similar in healthy controls as compared to the group of patients investigated here, although it is probably enhanced in schizophrenic patients because of their abnormal dopaminergic state (steeper gradient of the curve towards its beginning). In summary, based on these considerations, healthy controls should be 'superior' to schizophrenic patients regarding their prefrontal functioning irrespective of *COMT* genotype; but *within* the group of healthy controls, *COMT* should exert a similar—albeit weaker—effect as compared to patients with schizophrenia spectrum disorders.

Two previous reports focused on electrophysiological measures as a function of *COMT* genotype. Tsai *et al* (2003) examined healthy female subjects and found that Met allele carriers had significantly reduced P300 latencies in a gene-dose-dependent manner. Gallinat *et al* (2003), on the other hand, investigated schizophrenic patients and healthy controls by means of an auditory oddball paradigm without finding an effect of COMT genotype on P300 latencies. However, fronto-central P300 amplitudes were lower in 158Met homozygous subjects, particularly in schizophrenia. This led the authors to the conclusion that in schizophrenics homozygous for the Met allele, less prefrontal cortical 'noise' occurred, which might be involved in the superior performance of Met allele carriers with regard to working memory and information processing. As the authors employed a paradigm in which the frontal component of the P300 can be considered as a correlate of cortical noise, whereas other tasks (such as the CPT) involve frontal P300 components with strong 'signal' properties, the results reported by Gallinat *et al* do not contradict the present data. In fact, both their results and our own findings indicate better prefrontal functioning in Met allele carriers, whereat two different correlates of prefrontal brain function were used (electrophysiologically assessed prefrontal noise *vs* ERP components related to prefrontal inhibitory control).

Regarding the psychopathological data, patients homozygous for the Met allele tended to be more severely ill, which is in accordance with previous studies (Bilder *et al*, 2002). For two of the three neuropsychological tests of frontal lobe function, no influence of *COMT* genotype was found, whereas for the Stroop Test, a statistical trend indicated prolonged interference times in patients carrying Val/Met as compared to the matched group of Met/Met patients. As both the Stroop interference condition (Gruber *et al*, 2002) and the NGA (Fallgatter *et al*, 2002) have been associated with ACC functioning and our two groups of patients differed regarding their mean NGA, an accompanying difference in Stroop interference performance is highly plausible. As the Stroop task was the only task we used that is thought to specifically involve the ACC, a missing effect in the other two tests—which have been shown to preferentially activate other frontal areas such as the dorsolateral prefrontal cortex or Broca's area (Gaillard *et al*, 2000; Moll *et al*, 2002)—appears to be plausible as well.

Based on the electrophysiological findings, one might expect Val allele carriers to display an increased CPT error rate, particularly during Nogo trials (ie commission errors). The reason for the absence of such a finding might well be the paradigm used: The version of the CPT employed in the present study was adapted for application in psychiatric samples, that is, with low difficulty to avoid high error rates, making it less sensitive for respective performance differences.

When interpreting the present findings, it has to be considered that the patient sample was diagnostically heterogeneous, as not only patients with narrow-definition schizophrenia but also some patients with schizophreniform or schizoaffective disorders were included. Although these diagnoses were equally distributed across the different genotype groups (see Patients and methods) so that the observed group differences cannot be attributed to diagnostic between-group differences, it cannot be ruled out that the impact of *COMT* genotype on prefrontal brain function would be different in a homogenous sample. Future studies with more homogenous patient samples are therefore required; considering, however, that schizophrenia in itself is heterogeneous, and that many genetic studies investigate broad-spectrum schizophrenia with greater success than narrow-spectrum schizophrenia, it is unlikely that those studies would yield differing results.

In conclusion, the present data show a strong impact of *COMT* genotype on an electrophysiological correlate of cognitive response control and prefrontal functioning (NGA) in a group of patients with schizophrenia spectrum disorders. Carriers of the *COMT* allele related to a particularly high activity of the enzyme and thus shorter availability of dopamine in the synaptic cleft (Val) showed a significantly diminished NGA, indications of reduced fronto-central Nogo amplitudes, and an impaired Stroop performance, suggesting impaired functional activation of prefrontal structures probably including the ACC. These findings thus verify the impact of COMT Val158Met on prefrontal functioning and confirm the relevance of prefrontal dopaminergic tone for cognition. As the NGA was virtually absent in Val allele carriers, this might correspond to a genetically driven endophenotype in schizophrenic illnesses, possibly contributing to the cognitive deficits in schizophrenia. Strong genetic influences on prefrontal functioning might also account for the partly inconsistent results regarding a specifically frontal pathology ('hypofrontality') in schizophrenias, that is, for the highly variable symptomatology of disorders from the schizophrenic spectrum. Even though the present results need to be replicated in a larger sample and should also be confirmed by neuroimaging methods, neurophysiological approaches such as the present one are valuable tools in attempts to further elucidate the impact of genetic variations on cognitive functioning and functional brain activation in healthy subjects and neuropsychiatric disorders.

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