DTNBP1 (Dysbindin) Gene Variants Modulate Prefrontal Brain Function in Healthy Individuals

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DTNBP1 (dysbindin) is one of the several putative schizophrenia genes supported by association, neuroanatomical, and cellular studies. These suggest an involvement of *DTNBP1* in the prefrontal cortex and cognitive functions mediated by interaction with neurotransmitter systems, in particular glutamate. The influence of *DTNBP1* gene variation on prefrontal brain function at the systemic neurophysiological level, though, has not been characterized. The NoGo-anteriorization (NGA) as an event-related potential (ERP) measure elicited during the continuous performance test (CPT) has been established as a valid neurophysiological parameter for prefrontal brain function in healthy individuals and patients with schizophrenias. In the present study, we therefore investigated the influence of eight dysbindin gene variants on the NGA as a marker of prefrontal brain function in 48 healthy individuals. Two *DTNBP1* polymorphisms previously linked to schizophrenia (P1765 and P1320) were found associated with changes in the NGA. *Post hoc* analysis showing an influence of genetic variation at these loci on the Go centroid and frontal amplitudes suggest that this might be due to modification of the execution of motor processes by the prefrontal cortex. This is the first report on a role of *DTNBP1* gene variation for prefrontal brain function at a systemic neurophysiological level in healthy humans. Future studies will have to address the relevance of this observation for patients with schizophrenias.
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INTRODUCTION

DTNBP1 (dysbindin) is a recently characterized protein that binds to alpha–beta-dystrobrevins and is part of the dystrophin protein complex in muscle and nonmuscle cells. Variations in the human *DTNBP1* gene located on chromosome 6p22.3 have been reported to be associated with schizophrenia by several groups (eg Straub *et al*, 2002; Schwab *et al*, 2003; van den Oord *et al*, 2003; VanDenBogaert *et al*, 2003; Kirov *et al*, 2004; Williams *et al*, 2004; for a review see Funke *et al*, 2004) and a mutation has been reported to be the cause of Hermansky–Pudlak syndrome type 7 (Li *et al*, 2003).

In the brain, *DTNBP1* may influence glutamatergic neurotransmission by multiple post- and presynaptic

mechanisms, partly independent of the dystrophin protein complex and including modulation of other, for example, GABAergic, nicotinic, and dopaminergic neurotransmitter systems (Harrison and Weinberger, 2005). In primary cortical neuronal culture, *DTNBP1* appears to influence exocytotic glutamate release via upregulation of molecules in the presynaptic machinery and to promote neuronal viability through PI3-kinase-Akt signaling (Numakawa *et al*, 2004). In the hippocampus of schizophrenic patients, reductions of *DTNBP1* expression were observed in terminal fields of intrinsic, glutamatergic afferents of the subiculum, the hippocampus, and the inner molecular layer of the dentate gyrus, which correlated with increases in vesicular glutamate transporter-1 (*VgluT-1*) (Talbot *et al*, 2004). Reductions of *DTNBP1* gene expression were also observed in the dorsolateral prefrontal cortex of schizophrenic patients and expression correlated with *DTNBP1* genotype (Weickert *et al*, 2004). It was suggested that these changes in *DTNBP1* gene expression in the hippocampus and prefrontal cortex contribute to the cognitive deficits observed in patients with schizophrenias.

During the last years, a new field of research in biological psychiatry has emerged, which was coined imaging genomics (eg Hariri and Weinberger, 2003). The basic idea

is that certain genetic variants affecting neurotransmission systems may change brain activity in a way that is measurable by neuroimaging methods, in particular functional magnetic resonance tomography. However, also electrophysiological measures seem suitable for such a purpose, since twin and family studies have shown that characteristics of the spontaneous EEG in general, but also of P300 event-related potentials (ERPs) are highly heritable (van Beijsterveldt and van Baal, 2002). Moreover, P300 abnormalities known from schizophrenic patients are also found in their healthy siblings (Winterer *et al*, 2003).

Accordingly, the first report in that new field of imaging genomics (Fallgatter *et al*, 1999a) described a topographical change in an ERP measure during an inhibitory task associated with the presence of a short allele of the serotonin transporter promoter polymorphism (SERT; Lesch *et al*, 1996). Meanwhile, several other groups reported an influence of this genetic variant on brain activity in the amygdale of healthy subjects (eg Canli *et al*, 2005) as well as an interaction with stressful life events in depressed patients (Caspi *et al*, 2003). A similar research strategy resulted in a series of studies highlighting the influence of a genetic variant affecting the dopaminergic neurotransmission (COMT Val158Met polymorphism) on the function of the dorsolateral prefrontal cortex in healthy subjects (eg Winterer and Goldman, 2003) as well as psychiatric patients (eg Egan *et al*, 2001). Studies investigating the influence of other genetic variants besides SERT and COMT on brain function are rare in general and absent in the case of dysbindin.

Over the last years, our research group has developed an electrophysiological method for the quantitative measurement of prefrontal brain function, which is based on the comparison of ERPs elicited during the Go and the NoGo condition of the CPT. The more anterior located P300 topography in NoGo as compared to Go trials has been denoted as NoGo-anteriorization (NGA) and is characterized by a very high interindividual stability (Fallgatter *et al*, 1997). Moreover, the NGA is independent of age and gender (Fallgatter *et al*, 1999b) and has a very high short-term (Fallgatter *et al*, 2001) and long-term (Fallgatter *et al*, 2002a) test–retest reliability. Replicated three-dimensional electrophysiological source location studies with the LORETA method (low-resolution electromagnetic tomography; Pascual-Marqui *et al*, 1994) suggest that the two-dimensional NGA phenomenon is based on a strong electrical activity of the medial prefrontal cortex during the NoGo condition (Fallgatter *et al*, 2002b). Based on these findings, the NGA may be considered as a noninvasive electrophysiological measurement of medial prefrontal brain function involved in response inhibition processes. This association with response inhibition, however, is still under debate and may even be absent in the auditory modality (Salisbury *et al*, 2004).

In fact, patients with schizophrenias (Fallgatter and Müller, 2001; Fallgatter *et al*, 2003) are characterized by a diminished NGA indicating an altered function of the medial prefrontal cortex. Concordant alterations of brain function were obtained when investigating patients with obsessive–compulsive disorders (Herrmann *et al*, 2003) and attention-deficit hyperactivity disorder (ADHD; Fallgatter *et al*, 2004, Fallgatter *et al*, 2005a). In addition, patients with

schizophreniform and schizophrenic psychoses showed parallel improvements of both NGA and cognitive function as measured by performance in a Stroop test during a 6-week trial with atypical but not with typical neuroleptic compounds (Fallgatter *et al*, 2005b).

Based on the above-reported genetic and neurophysiological findings, we hypothesized a modulation of NGA as a marker of prefrontal brain function by variation in the *DTNBP1* gene. To probe this hypothesis, we investigated the association of eight single-nucleotide polymorphisms (SNPs) in the *DTNBP1* gene with NGA in a sample of healthy German individuals.

METHODS

Subjects

After complete description of the study, written informed consent was obtained from 54 healthy volunteers. Six participants had to be excluded due to too many artifacts (less than 20 artifact-free EEG epochs in either the Go or the NoGo condition of the electrophysiological investigation). The remaining 48 participants (26 female, 22 male) had a mean age of 26.5 ± 7.2 years (range 19–63 years). The subjects were healthy, medication -free, and had no lifetime or family history of neurological or psychiatric diseases. In all, 43 of the participants were self-reported right-hander, and five were left-hander. The investigation was approved by the Ethics Committee and by the research conference of the Department of Psychiatry and Psychotherapy of the University of Wüerzburg.

Continuous Performance Test (CPT)

For the electrophysiological investigation, subjects were seated on a comfortable chair in front of a computer screen in an electrically shielded, dimly lit room to perform the CPT. Letters were presented sequentially in a pseudorandomized order and participants were instructed to press a response button whenever the letter 'O' was directly followed by the letter 'X'. Speed and accuracy were emphasized equally during explanation of the test. The whole stimulus set consisted of 400 letters, with 80 primer conditions (O), 40 Go (O–X) and Nogo (O–any other letter) conditions, and 240 distractors (other letters, or letter X without a preceding O). With an interstimulus interval of 1650 ms, each letter was presented for 200 ms. After a short training session, subjects performed this version of the CPT for about 13 min while the EEG was recorded.

EEG Recording and Data Analysis

The EEG was recorded from 21 electrodes according to the International 10–20 system (Fp1, Fp2, F3, F4, F7, F8, T3, T4, C3, C4, T5, T6, P3, P4, O1, O2, Fpz, Fz, Cz, Pz, and Oz). Three additional electrodes were placed at the outer canthi of both eyes and below the right eye to register horizontal and vertical eye movements. Linked mastoids were used as the recording reference and electrode impedances were constantly kept below 5 kΩ. The recording system consisted of an 80-channel DC amplifier (MI System, Prague, Czech

Republic). The hardware filter was set to a band pass of 0.1–70 Hz, and A/D rate was 250 Hz.

A computerized artifact rejection excluded all segments with amplitudes exceeding 50 µV in any of the EEG or EOG channels within the first 500 ms after stimulus onset. The remaining artifact-free EEG segments were then averaged to one Go and one NoGo ERP for each participant, whereby only trials with correct responses were included. In the individual Go and NoGo ERPs, the global field power (GFP; Lehmann and Skrandies, 1980) peaks were determined within a P300 microstate (240–440 ms). Over the electrode position Fz, a positive peak was detected in the time window between 284 ms until 440 ms. The GFP represents the mean of all possible potential differences in a given scalp potential field and is used as an estimator of the electrical field strength in multichannel recordings. 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.

Two- and Three-Dimensional Analysis of ERP Data

A two-dimensional delineation of the electrode array was used as a coordinate system with the integers 1–5 indicating the electrode positions in the anterior–posterior direction to quantify the centroid locations. Finally, the NGA, defined as the distance between the individual Go and NoGo centroid within this coordinate system, was calculated separately for each subject. For the analyses of the two-dimensional maps, we compared the amplitudes of each of the three midline electrode positions Fz, Cz, and Pz at the individual time points of the GFP peaks between individuals of the different *DTNBP1* genotype groups (Figure 1), and displayed a statistical map using the corresponding *t*-values (*t*-test for independent samples; Figure 2).

For an attempt at three-dimensional delineation of the brain electrical sources of the Go-ERP, we applied the LORETA method (Pascual-Marqui *et al*, 1994; Figure 3). LORETA calculates the current density at each of the 2394 voxels in the gray matter and the hippocampus of a reference brain (MNI 305; Brain Imaging Centre, Montreal
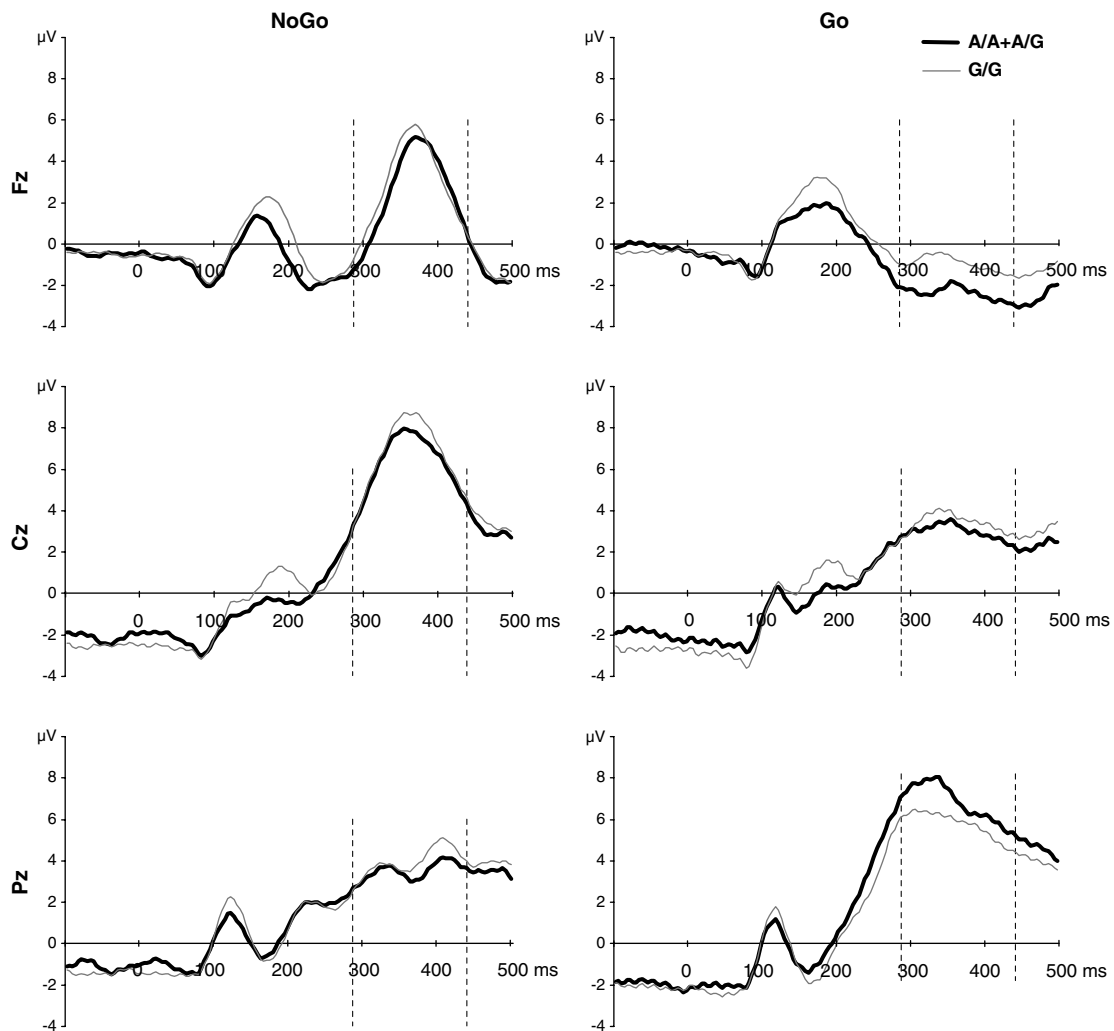

Figure 1 Traditional ERP waveforms from 100 ms before to 500 ms after the NoGo- (left) and the Go-stimulus (right) as derived from midline electrodes (Fz, Cz, and Pz). ERPs from the P1765 G/G group are displayed in thin lines, and from the P1765 A/A + A/G group in heavy lines. Vertical lines mark the beginning and the end for the analyzed time segment (284–440 ms).

Neurologic Institute) based on the linear, weighted sum of the scalp electric potentials. LORETA chooses the smoothest of all possible current density configurations throughout the brain volume by minimizing the total squared Laplacian of source strengths. This procedure only implicates that neighboring voxels should have a maximally similar electrical activity, no other assumptions are made. The applied version of LORETA used a three-shell spherical head model registered to the Talairach space. It has been shown that LORETA is able to localize sources for cognitive tasks reliably (eg Herrmann and Fallgatter, 2004). However, interpretations of the neuroanatomical results given by LORETA have to be carried out cautiously since all EEG source localization methods including LORETA can only give approximations but no definitive solutions of the so-called inverse problem (eg Michel et al, 2004). We used

LORETA to calculate the three-dimensional localization of the electrical sources contributing to the electrical scalp field for each subject and condition at the time point of the individual GFP peaks.

Genotyping

DNA was extracted from whole-blood samples using standard protocols. Eight SNPs (Table 1) that cover the *DTNBP1* gene and its 5′- and 3′-flanking region were genotyped by TaqMan 5′ exonuclease allelic discrimination assays (Applied Biosystems, Darmstadt, Germany). Preparation of the PCR reaction mixtures was performed by Genesis Workstation RSP 150 (Tecan, Crailsheim, Germany). For PCR amplification and allelic discrimination, the ABI Prism 7900 HT Sequence Detection System and SDS software version 2.1 (Applied Biosystems, Darmstadt, Germany) were used.

Statistical Analysis

All polymorphisms were in Hardy–Weinberg equilibrium ($p > 0.05$; assessed by online resource www.kursus.kvl.dk/shares/vetgen/_Popgen/genetik/applets/kitest.htm; for genotype frequencies, see Table 1). As for some polymorphisms (P1635, P1325, P1586), only two genotypes were present, and for most with three genotypes, the number of individuals with one genotype was below $N = 5$ (P1730, P1765, P1320, P1140), the genotypes G/C and C/C of P1730, G/C and G/G of P1655, A/G and A/A of P1765, C/T and T/T of P1320 as well as A/C and C/C of P1140 were combined into one group each for the subsequent analysis without *a priori* hypothesis to allow for comparable power in statistical analyses. Owing to the overall low heterozygosity rate with only two individuals with T/C at the P1586 locus, no meaningful statistical analysis of an association with the NGA could be performed for this polymorphism. Linkage disequilibrium (LD) was analyzed by Haploview version 3.2 (http://www.broad.mit.edu/mpg/haploview). The P1765 and P1320 polymorphisms were in complete LD as well as in
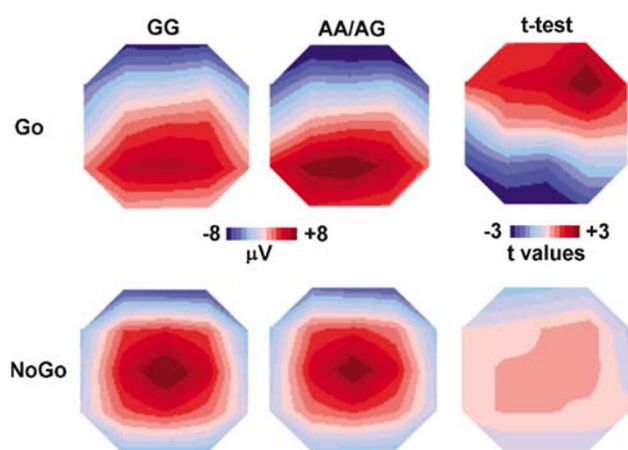

Figure 2 The distribution of the averaged brain electrical activity at the time point of GFP peaks for subjects with the *DTNBP1* gene polymorphism P1765 genotypes G/G (left) and A/A + A/G (middle) for the Go (above) and the NoGo condition (below). Additionally, on the right side the statistical *t*-maps comparing the amplitudes of the G/G group with the A/A + A/G group are displayed for both conditions.
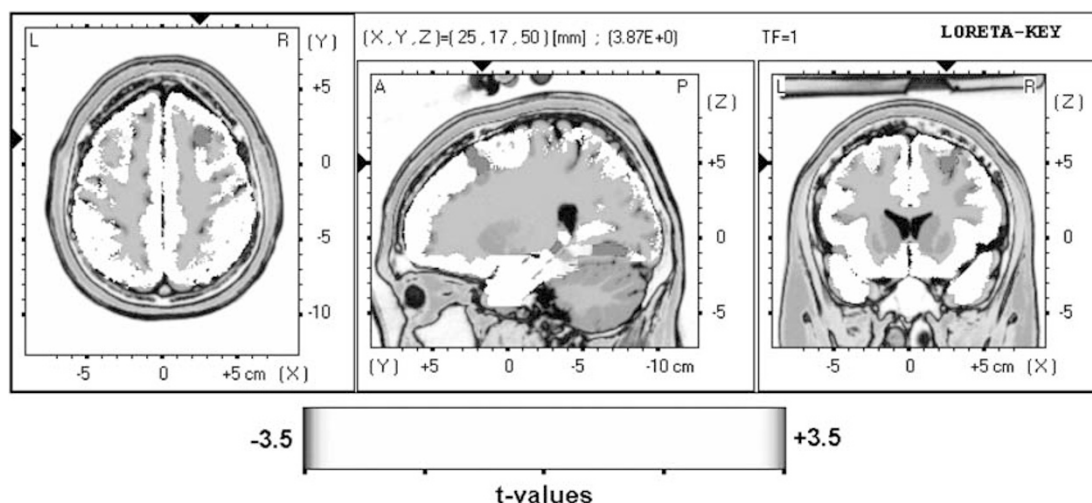

Figure 3 Comparison of LORETA source localization between subjects with the *DTNBP1* gene polymorphism P1765 genotypes G/G and A/A + A/G for the Go condition. Red (black) color indicates significantly higher brain activity for G/G subjects.

Table I Polymorphisms of the *DTNBP1* (Dysbindin) Gene

SNP[a], dbSNP ID[b]	Allele	SNP location	SNP position[c]	Genotype frequencies
P1730, rs1474588	G/C	3′-flanking region	15 569 688	G/G = 23, G/C = 22, C/C = 3
P1655, rs2619539	C/G	Intron 5	15 728 834	C/C = 18, G/C = 21, G/G = 9
P1635, rs3213207	A/G	Intron 4	15 736 081	A/A = 37, A/G = 11, G/G = 0
P1325, rs1011313	G/A	Intron 4	15 741 411	G/G = 40, A/G = 8, A/A = 0
P1765, rs2619528	G/A	Intron 3	15 757 808	G/G = 30, A/G = 17, A/A = 1
P1320, rs760761	C/T	Intron 3	15 759 111	C/C = 30, C/T = 17, T/T = 1
P1586, rs885773	C/T	5′-flanking region	15 777 465	C/C = 46, T/C = 2, T/T = 0
P1140, rs1000117	A/C	5′-flanking region	15 981 968	A/A = 26, A/C = 18, C/C = 4

[a]Straub *et al* (2002).
[b]NCBI number (http://www.ncbi.nlm.nih.gov/SNP).
[c]UCSC Genome Browser, Human May 2004 Assembly (http://genome.ucsc.edu/cgi-bin/hgGateway).

Table 2 Pairwise LD in *DTNBP1* Polymorphisms as Measured by D' (Top Diagonal) and r^2 (Bottom Diagonal)

	P1730	P1655	P1635	P1325	P1765	P1320	P1586	P1140
P1730	—	0.15	*1.0*	*1.0*	0.02	0.02	0.52	0.28
P1655	0.01	—	*1.0*	*1.0*	0.05	0.05	*1.0*	0.04
P1635	0.05	0.19	—	0.03	*1.0*	*1.0*	0.36	0.47
P1325	0.04	0.13	0.0	—	*1.0*	*1.0*	*1.0*	0.2
P1765	0.0	0.0	*0.52*	0.02	—	*1.0*	0.18	0.09
P1320	0.0	0.0	*0.52*	0.02	*1.0*	—	0.18	0.09
P1586	0.0	0.03	0.02	0.23	0.0	0.0	—	*1.0*
P1140	0.07	0.0	0.01	0.01	0.0	0.0	0.06	—

D' values > 0.8 and r^2 values > 0.5 are in boldface italic.

nearly complete LD with the P1635 polymorphism (for all $D' = 1.0$ and $r^2 > 0.5$; see Table 2). Haplotype blocks were assigned using the solid spine of LD method of Haploview, which resulted in three different blocks containing P1730–P1655–P1635 (block 1), P1325–P1765–P1320 (block 2), and P1586–P1140 (block 3). Haplotypes were reconstructed on an individual level by PHASE, version 2.1 (Stephens *et al*, 2001; Stephens and Donnelly, 2003), based on pairwise standardized LD coefficient $D' > 0.95$ and a probability of reconstructed pairs of haplotypes for each individual $\geqslant 0.91$. One individual with a probability of > 0.60 for the haplotype P1586–P1140 was excluded from the respective analysis. By this, three haplotypes of P1325–P1765–P1320 (A-G-C, G-A-T, and G-G-C) and two haplotypes of P1586–P1140 (C-C and C-A) could be assessed. Individual haplotypes were coded as two copies (2), one copy (1), or not (0) present in an individual. Because of haplotype frequencies less than $N = 5$, for haplotype G-A-T groups (1) and (2) were combined, for haplotype G-G-C groups (0) and (1), for haplotype C-C groups (1) and (2) as well as for haplotype C-A groups (0) and (1).

To reduce the number of statistical tests, we compared in a first step only the NGA between genotypes of seven SNPs and between groups of five haplotypes using *t*-tests for independent samples with a corresponding corrected

$p < 0.05/7 = 0.0071$ for single-marker analyses and $p < 0.05/5 = 0.01$ for haplotype analyses, respectively. For significant differences between genotypes and haplotypes, *post hoc t*-tests and Wilcoxon's rank-sum tests for independent samples were used to elaborate the electrophysiological parameters that might contribute to these differences.

RESULTS

The mean reaction time for Go trials in the CPT was 428.6 ± 87.7 ms. The error rate was very low. On average, participants made 0.6 ± 0.8 commission errors (ie button presses after a NoGo trial) and 0.2 ± 0.4 omission errors (ie missed button presses after a Go trial).

The NGA as our primary measure of prefrontal brain function had a mean value of 0.75 ± 0.53 electrode distances for the whole group of 48 healthy subjects. This result was nearly identical with the mean NGA values reported in three previous normative samples (0.78 ± 0.47, Fallgatter *et al*, 1997; 0.76 ± 0.23, Fallgatter and Strik, 1999; 0.76 ± 0.33, Fallgatter *et al*, 2000). However, in the present study, subjects with genotypes containing the A-allele (A/A and A/G) of the P1765 polymorphism are characterized by a significantly higher NGA as compared to subjects homozygous for the G-allele (1.00 ± 0.44 *vs* 0.58 ± 0.52; $t = 2.84$, $p < 0.0068$). The neighboring and completely linked SNP P1320 also shows the same association, with the NGA being significantly higher in subjects with the T-allele (C/T and T/T) as compared to subjects homozygous for the C-allele. Consistent with this, subjects having one or two copies of the haplotype G-A-T of block 2 are characterized by significantly higher NGA as compared to subjects in whom no copy is present (1.00 ± 0.44 *vs* 0.58 ± 0.52; $t = -2.84$, $p < 0.0068$). In contrast, the neighboring SNPs in the 3′ and in the 5′ directions as well as the other haplotypes do not show any association with the NGA (Tables 3 and 4).

As the subjects in the P1765 subgroup A/A + A/G and the P1320 subgroup C/T + T/T are identical due to the complete LD between both SNPs, only the *post hoc* tests for the P1765 polymorphism are reported.

The differential NGA between subjects with P1765 A/A + A/G and subjects with P1765 G/G variants seems to be

Table 3 Comparing *DTNBP1* Gene Allele Combinations with respect to the Electrophysiological Parameter of Prefrontal Brain Activation NGA

SNP	Allele	N	NGA mean	SD	t	p
P1730	C/G+C/C	25	0.80	0.53		
	G/G	23	0.68	0.53	0.76	0.45
P1655	C/C	18	0.77	0.57		
	C/G+G/G	30	0.72	0.51	0.28	0.77
P1635	A/A	37	0.69	0.55		
	A/G	11	0.90	0.41	−1.13	0.27
P1325	A/G	8	0.65	0.41		
	G/G	40	0.76	0.55	−0.50	0.62
P1765	A/A+A/G	18	1.00	0.44		
	G/G	30	0.58	0.52	2.84	0.0068
P1320	C/C	30	0.58	0.52		
	C/T+T/T	18	1.00	0.44	−2.84	0.0068
P1140	C/C+C/A	22	0.71	0.56		
	A/A	26	0.77	0.51	−0.39	0.70

SD = standard deviation.

caused predominantly by the localization of the Go centroid (Table 5, Figure 2) with a more frontal localization of the Go centroid resulting in a reduced NGA in the subgroup with G/G ($t = 2.53$, $p < 0.05$). This result is underscored by significantly higher amplitudes in the G/G than in the A/A + A/G subgroup as measured at electrode position Fz in the investigated P300 time window (1.34 ± 2.39 *vs* $-0.26 \pm 1.97 \mu V$; $t = 2.38$, $p < 0.05$; Table 5, Figure 1). In contrast, no clear differences between groups occur in the respective P300 time window at the electrode positions Cz and Pz (Figure 1). Furthermore, no significant differences between genotypes regarding GFP amplitudes and latencies are observed. Haplotype analysis again confirmed genotype results by demonstrating distinctly more frontal localization of the Go centroid and distinctly higher amplitudes at electrode position Fz for subjects having no copy of the G-A-T haplotype of block 2 ($Z = 2.64$, $p < 0.05$ and $Z = -2.65$, $p < 0.05$; Table 6), which corresponds to significantly reduced NGA (shown above).

Subjects of the P1765 A/A + A/G subgroup do not differ from the subjects of the P1765 G/G group with respect to omission errors (A/A + A/G: $m = 0.33 \pm 0.7$; G/G: $m = 0.70 \pm 0.9$; $t[46] = -1.5$, $p = 0.15$), commission errors (A/A + A/G: $m = 0.22 \pm 0.5$; G/G: $m = 0.13 \pm 0.3$; $t[46] = -0.7$, $p = 0.49$), or reaction times (A/A + A/G: $m = 435.1 \pm 106.0$ ms; G/G: $m = 424.7 \pm 79.0$ ms; $t[46] = 0.7$, $p = 0.70$).

To illustrate the more frontal activation during the Go condition for the P1765 G/G subjects compared to the P1765 A/A + A/G subjects, we displayed the distribution of the brain electrical activity by means of a statistical map for differences. The higher right frontal activity for the G/G subjects during the Go condition can be clearly discerned (Figure 2). At a three-dimensional level, the source localization (LORETA) revealed significant ($p < 0.05$) differences between subjects with P1765 G/G compared to subjects with P1765 A/A + A/G in five different regions including the right hemispheric middle frontal gyrus and

Table 4 Haplotype Frequencies and Comparison of Haplotype Groups with respect to the Electrophysiological Parameter of Prefrontal Brain Activation NGA

Haplotype	Copy number	N	NGA mean	SD	t	p
Block 2						
A-G-C	0	40	0.76	0.55		
	1	8	0.65	0.41	0.50	0.62
G-A-T	0	30	0.58	0.52		
	1+2	17+1	1.00	0.44	−2.84	0.0068
G-G-C	0+1	3+21	0.90	0.47		
	2	24	0.58	0.54	2.24	0.0302
Block 3						
C-C	0	26	0.77	0.51		
	1+2	18+3	0.71	0.58	0.38	0.70
C-A	0+1	3+18	0.71	0.58		
	2	26	0.77	0.51	−0.38	0.70

SD = standard deviation.

Table 5 *Post Hoc* Test for the Electrophysiological Parameters between Allelic Variations of the *DTNBP1* Gene Polymorphism P1765

Parameter	Condition	Allele	N	Mean	SD	t/Z[a]	p
NGA		A/A+A/G	18	1.00	0.44	2.84	0.0068
		G/G	30	0.58	0.52		
Centroid	Go	A/A+A/G	18	4.00	0.28	2.64[a]	0.008[a]
		G/G	30	3.69	0.47		
	NoGo	A/A+A/G	18	3.00	0.40	−0.50[a]	0.62[a]
		G/G	30	3.11	0.51		
GFP (μV)	Go	A/A+A/G	18	5.80	1.71	1.38	0.18
		G/G	30	5.11	1.67		
	NoGo	A/A+A/G	18	4.72	1.00	−1.52	0.14
		G/G	30	5.33	1.49		
Latency (ms)	Go	A/A+A/G	18	343.6	40.3	1.41	0.17
		G/G	30	327.1	38.7		
	NoGo	A/A+A/G	18	368.2	32.6	0.59	0.56
		G/G	30	362.3	35.0		
Fz amplitudes (μV)	Go	A/A+A/G	18	−0.26	1.97	−2.65[a]	0.008[a]
		G/G	30	1.34	2.39		
	NoGo	A/A+A/G	18	5.93	1.56	0.33[a]	0.74[a]
		G/G	30	6.50	3.37		

[a]Wilcoxon's rank-sum test.
SD = standard deviation.

the hippocampus (hippocampus, Talairach space in mm: $X = 32$, $Y = -39$, $Z = -6$, t-value $= 3.96$; lingual gyrus, Brodman area (BA) $= 19$; $X = 18$, $Y = -67$, $Z = -6$, $t = 3.93$; middle frontal gyrus, BA $= 8$, $X = 25$, $Y = 17$, $Z = 50$, $t = 3.87$; fusiform gyrus, BA $= 37$, $X = 39$, $Y = -60$, $Z = -20$, $t = 3.62$; postcentral gyrus, BA $= 3$, $X = 66$, $Y = -11$, $Z = 29$, $t = -3.52$; Figure 3).

Table 6 *Post Hoc* Test for the Electrophysiological Parameters Centroid and Fz Amplitudes (μV) with the Condition Go between the G-A-T Haplotype Groups of Block 2 (P1325–P1765–P1320)

Parameter	Haplotype	Copy number	N	Mean	SD	Z	p
Centroid	G-A-T	0	30	3.69	0.47		
		1+2	17+1	4.00	0.28	2.64	0.008
Fz amplitudes	G-A-T	0	30	1.34	2.39		
		1+2	17+1	−0.26	1.97	−2.65	0.008

SD = standard deviation.

DISCUSSION

To our best knowledge, this study is the first to show an association of a variant of the *DTNBP1* gene with a neurophysiological parameter of prefrontal brain function in healthy subjects.

DTNBP1 (dysbindin) is considered as one of the best-validated candidate genes for schizophrenic diseases (Harrison and Weinberger, 2005). The molecular mechanisms by which *DTNBP1* acts in the brain and their functional consequences in subjects with variants in the *DTNBP1* gene, however, are far from being understood. One way to further investigate these functional consequences is to correlate *DTNBP1* gene variants with measurements of functional intermediate phenotypes (endophenotypes), which are well characterized in healthy subjects and disturbed in schizophrenic patients. These two prerequisites are particularly true for the NGA, a robust and reliable ERP marker of prefrontal brain function that has been described disturbed with markedly reduced NGA in patients with schizophrenias (Fallgatter and Müller, 2001; Fallgatter et al, 2003). In this context, the significantly reduced NGA in healthy subjects with the G/G variant of SNP P1765 as well as the C/C variant of SNP P1320 may be cautiously interpreted as an expression of mildly disturbed prefrontal brain function. The P1765 and P1320 polymorphisms as well as the haplotype block P1325–P1765–P1320 associated with disturbed prefrontal brain function in these healthy German individuals are identical to those associated with schizophrenia in a German schizophrenia sample (Schwab et al, 2003). Our observations of a *DTNBP1* genotype effect on prefrontal brain function are not only consistent with a series of linkage studies in schizophrenia (for a review see Funke et al, 2004) but also with findings describing an association of *DTNBP1* gene polymorphisms with other cognitive intermediate phenotypes such as IQ, working, and episodic memory (Straub et al, 2003).

A closer look at the electrophysiological results reveals that the differential NGA in this study in contrast to previous findings in patients with schizophrenia is mainly caused by a change of the centroid location in the Go condition and not in the NoGo condition. This suggests that in the healthy P1765 G/G or P1320 C/C subjects, on a neurophysiological level, the execution of a motor response is the affected process and not the inhibition (NoGo condition). The significantly higher amplitudes as measured at electrode position Fz (Table 5; Figure 1) as well as the two-dimensional (brain maps; Figure 2) and three-dimensional (LORETA; Figure 3) indications for a stronger

activation of right hemispheric prefrontal brain areas in the P1765 G/G group further support the notion that the described alterations in execution-related processes are at least in part due to differences in frontal brain function. However, it has to be mentioned that the source analyses with LORETA found not only the right hemispheric middle frontal gyrus but also four more regions to be significantly different between *DTNBP1* P1765 genotypes. Prominent among them is the hippocampus, where *DTNBP1* expression levels have been shown to be reduced in patients with schizophrenia similarly to prefrontal cortex (Talbot et al, 2004; Weickert et al, 2004). As *DTNBP1* expression levels are inversely correlated with *VgluT-1* expression (Talbot et al, 2004), it is conceivable that cognitive processes involving the hippocampus and prefrontal cortex are affected by *DTNBP1* gene variation.

It might be argued that differences in brain function should also be reflected in behavioral measures. This is not the case in our study where both genotype samples did not differ in terms of error rates or reaction times. This missing behavioral effect might be explained by the easiness of the applied task with very long interstimulus intervals (1650 ms) and presentation times (200 ms). This particularly undemanding task has been designed to produce clearcut electrophysiological results, but is rather unsuited for the detection of performance differences between different patient samples and control subjects (eg schizophrenias: Fallgatter and Müller, 2001; Fallgatter et al, 2003; writer's cramp: Berg et al, 2001; ADHD: Fallgatter et al, 2005a; OCD: Herrmann et al, 2003). It is therefore plausible to expect differences in the brain function without accompanying behavioral effects when comparing two healthy samples with this particular task.

Another point of criticism may be that the sample size with 48 individuals is small compared to sample sizes employed in classical association studies based on categorical phenotypes such as schizophrenia. However, it has to be noted that the sample size is quite comparable to those replicating linkage findings in schizophrenia by utilizing dimensional intermediate phenotypes of the hippocampal and prefrontal cortex function (Egan et al, 2004). Nevertheless, replication in independent and preferably larger samples, possibly also with other functional paradigms of prefrontal brain function, is warranted.

In summary, this is the first report of a modulation of prefrontal brain function at the neurophysiological level by *DTNBP1* gene variants. Obviously, the reported differences in healthy subjects with different variants of the *DTNBP1* gene as measured by ERPs during the execution of a motor response needs replication in a sample of patients with schizophrenia. Such ERP measures elicited during Go–NoGo paradigms may reflect one mechanism by which *DTNBP1* gene variants contribute to the pathophysiology underlying schizophrenic diseases.

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