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Dissociable influences of NR2B-receptor related neural transmission on functions of distinct associative basal ganglia circuits

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ABSTRACT

Glutamate is an important excitatory neurotransmitter within functional prefrontal-basal ganglia loops. These distinct loops mediate different cognitive functions. One function of the anterior-cingulate loop is error processing. One function of the orbito-frontal loop is response inhibition. These functions are altered in several neuro-psychiatric disorders like Huntington's disease (HD). Because of the known role of the *GRIN2B* C2664T polymorphism in HD neuropathology, which is partly due to increased glutamatergic neural transmission, we analyze how this polymorphism influences error processing and response inhibition in a sample of healthy probands ($N = 65$).

Combining a genetic approach with event-related potential (ERP) measurements of response inhibition (OFC-loop function) and error processing (ACC-loop function), we provide robust results showing a selective modulation of response inhibition processes by the *GRIN2B* C2664T polymorphism at the behavioural and neurophysiological level. Response inhibition processes were stronger in the CT/TT genotype group, compared to the CC genotype group. Since error processing functions were not affected, the results suggest for differential influences of the *GRIN2B* C2664T polymorphism on response inhibition and error processing functions. The results provide first insight into cognitive-neurophysiological effects of the *GRIN2B* C2664T polymorphism. The dissociation obtained may be due to a differential importance of *N*-methyl-D-aspartate receptors for glutamatergic neural transmission in different striatal compartments (matrix and striosomes). We provide a model on this that may be a target for future research.

Introduction

The basal ganglia are connected to prefrontal areas like the orbitofrontal (OFC) and the anterior cingulate cortex (ACC) (Chudasama and Robbins, 2006) by means of different functional loops. The orbitofrontal cortex and the anterior cingulate cortex are involved in numerous cognitive processes (e.g. Rolls and Grabenhorst, 2008; Wallis, 2007; Bush et al., 2000). However, one function mediated by the anterior cingulate cortex (ACC) is error processing, which is important for behavioural adaptation (Ridderinkhof et al., 2004). Another important function of behavioural control is response inhibition, which has been shown to be related to orbitofrontal cortical areas (OFC) (Schoenbaum et al., 2009; Falkenstein, 2006, or the inferior frontal cortex (Aron et al., 2004). Error processing and response inhibition are altered in several diseases like schizophrenia and Huntington's disease where cortical and basal ganglia structures are compromised in functioning (e.g. Beste et al., 2009a,b). Moreover, glutamatergic neural transmission is affected in these diseases (Coyle, 2006; Beal and Ferrante, 2004), which may also modulate these cognitive processes.

For glutamatergic neural transmission and its influence on cognitive functions *N*-methyl-D-aspartate receptors (NMDA receptors) play an important role (e.g. Beste et al., 2008a; Villmann and Becker, 2007). NMDA receptors can be subdivided into different subunits (e.g. Villmann and Becker, 2007). Glutamatergic *N*-methyl-D-aspartate receptor 2B (NR2B) receptor subunits are expressed within striatal and cortical structures (e.g. Loftis and Janowsky, 2003; Zeron et al., 2002) and important for cognitive functions, like learning and memory (Loftis and Janowsky, 2003; Tang et al., 1999). On a functional level it has been shown that *GRIN2B* over-expression in the forebrain of mice results in increased activation of NMDA receptors facilitating synaptic potentiation by enhanced signal detection of pre-synaptic inputs (Ludwig et al., 2010; Tang et al., 1999). NR2B receptors are highly

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expressed at striatal levels and especially at medium spiny neurons (MSNs) (Küppenbender et al., 2000). These neurons have been suggested to play an important role in response control processes (Wild-Wall et al., 2008; Beste et al., 2008d; Gurney et al., 2004), like error processing and response inhibition (Beste et al., 2006, 2010a). Moreover, increased levels of glutamate have been shown to reduce dopaminergic neural transmission (e.g. Seamans and Yang, 2004; Moghaddam et al., 1997), which is also of relevance for error processing (Holroyd and Coles, 2002) and response inhibition processes (Beste et al., 2010a,b). These reasons identify NMDA receptors as a potential modulator for response inhibition and error processing functions. In order to analyze a potential modulation of these receptor subunits on response inhibition and error processing we combined event-related potential (ERP) analyses with molecular genetic analyses.

Error processing assessed by means of ERPs is reflected by the error negativity (Ne/ERN) (Falkenstein et al., 1991; Gehring et al., 1993), which may trigger behavioral adaptation after an error (e.g. Debener et al., 2005). Response inhibition processes are reflected by the Nogo-N2 and Nogo-P3. Both components reflect various sub-processes of response inhibition; i.e. pre-motor inhibition, or conflict processing (Nogo-N2) (e.g. Beste et al., 2009a,b; Nieuwenhuis et al., 2003; Falkenstein et al., 1999) and the evaluation of inhibition (Nogo-P3) (Schmajuk et al., 2006; Roche et al., 2005).

We choose a NR2B receptor polymorphism (GRIN2B C2664T (rs1806201)), which is a silent (synonymous) single nucleotide polymorphism (SNP) (Thr888Thr) in the gene region (exon 13) encoding the carboxyl-terminal intracellular domain of the NR2B subunit (Nishiguchi et al., 2000). Despite being synonymous, i.e. not changing the amino acid sequence, in analogy to synonymous mutations in the human DRD2 gene (Duan et al., 2003), this SNP may have drastic functional effects by altering mRNA stability or translation: The CT/TT genotypes may be related to increased glutamatergic neurotransmission, because these genotypes are associated with earlier manifestations of symptomatic Huntington's disease (Arning et al., 2005, 2007) that can be attributed to excitotoxic mechanisms (Beal and Ferrante, 2004) depending on NMDA-receptor mediated mechanisms. The SNP has also been described as a likely candidate involved in the etiology of schizophrenia (for review see: Cherlyn et al., 2010; Quin et al., 2005), and hyperactive symptom dimensions of ADHD (Dorval et al., 2007). The fact that HD is accompanied by changes in error processing and response inhibition (Beste et al., 2006, 2008b, 2008c) further underlines the relevance of this polymorphism to be studied in relation to error processing and response inhibition. Moreover, electrophysiological properties of the NR2B receptor dominate over other subunits within the NR2-receptor family (Loftis and Janowsky, 2003), which underlines the relevance of the NR2B subunit for electrophysiological studies.

Increases in glutamatergic neural transmission reduce dopaminergic functions (e.g. Seamans and Yang, 2004; Moghaddam et al., 1997). If the CT/TT genotypes are related to increased glutamatergic neural transmission, it may be hypothesized that processing of errors should be reduced in these genotype groups. Downregulating dopaminergic neural transmission has on the other hand been shown to increase response inhibition efficacy (Beste et al., 2010a,b). It may therefore be hypothesized that response inhibition processes are enhanced in the CT/TT genotype groups, compared to the CC genotype group.

However, error processing and response inhibition processes may not necessarily be both affected. At a striatal level the ACC seems to project to the striosomal compartment, whereas the OFC seems to project to the striosomal and the matrix compartment, with preponderance for the matrix (Eblen and Graybiel, 1995). These compartments differ in their chemoarchitecture (e.g. Sato et al., 2008; Martin et al., 1993). Postsynaptic NMDA receptors are involved in glutamatergic processing within the matrix, but less so in the striosomes (Bordelon et al., 1999; Blanchet et al., 1998; Dure et al., 1992). If the above-mentioned neuroanatomical dissociations are of functional relevance for error processing and response inhibition, it maybe hypothesized that response inhibition functions may improve with increasing NMDA-receptor related neurotransmission, i.e. in the CT/TT genotype group, while error processing functions may not be altered.

Materials and methods

Subjects

A sample of 65 genetically unrelated healthy participants of Caucasian descent was recruited by newspaper announcements. The mean and standard deviation (SD) are given. The mean age of the subjects was 24.9 years (5.2). The sample consisted of 28 males and 37 females. Hardy–Weinberg equilibrium was examined using the program Finetti provided as an online source (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl; Wienker TF and Strom TM). The distribution of *GRIN2B* C2664T genotypes did not significantly differ from the expected numbers calculated on the basis of observed allele frequencies according to Hardy–Weinberg equilibrium (TT = 9, CT = 26, CC = 30; $p = 0.389$). As the TT genotype had an expectedly low frequency, we combined the TT and CT genotype groups to one group. The sexes were comparably distributed across the different *GRIN2B* C2664T genotype groups (H-Test: $chi^2 = 2.12$; $df = 1$; $p = 0.217$ Monte-Carlo significance). All subjects enrolled into the study had no history of any neurological or psychiatric diseases. The study was approved by decision of the ethics committee of the University of Münster. All subjects gave written informed consent before any of the study procedures were commenced.

Genotyping

Genotyping of *GRIN2B* rs1806201 (synonymous SNP; position: chr12:13,717,508) was carried out following published protocols applying the multiplex genotyping assay iPLEX™ for use with the MassARRAY platform (Oeth et al., 2007), yielding a genotyping completion rate of 97%. Genotypes were determined by investigators blinded for the study.

Modified Flanker Task

Response inhibition and error processing were examined in one experimental paradigm. In order to provoke response errors a flanker paradigm was applied, in which the flankers (triangles pointing to the left or right) preceded a centrally presented target stimulus by 100 ms to maximize premature responding to the flankers. Target stimuli were also triangles pointing to the left or right. This configuration would provoke error especially in the incompatible condition, where arrowheads of flankers and the target point in opposite directions. The target stimulus was displayed for 300 ms. The response–stimulus interval was 1600 ms. Flankers and target were switched off simultaneously. Time pressure was administered by asking the subjects to respond within 600 ms. In trials with reaction times exceeding this deadline a feedback stimulus (1000 Hz, 60 dB SPL) was given 1200 ms after the response; this stimulus had to be avoided by the subjects.

To measure response inhibition processes the target stimulus was randomly changed to a circle. This circle served as Nogo-stimulus signalling that the response had to be suppressed. This central circle was presented with the identical SOA as the central target arrowheads in the response trials. The paradigm is comparable to Kopp et al. (1996). Four blocks of 105 stimuli each were presented in this task. Compatible (60%) and incompatible stimuli (20%) and Nogo stimuli (circle) (20%) were presented randomly.

EEG recording and analysis

During the task the EEG was recorded from 24 Ag-AgCl electrodes (Fpz, Fp1, Fp2, Fz, F3, F4, F7, F8, FCz, FC3, FC4, FC5, FC6, C3, C4, C7, C8, Pz, P3, P4, P7, P8, Oz, O1, O2, left mastoid—M1, right mastoid—M2) against a reference electrode located at Cz at a sampling rate of 500 Hz applying a filter bandwidth 0–80 Hz to the EEG. Electrode impedances were kept below 5 kΩ. EEG was filtered off-line from 0.5 to 16 Hz. The EEG data were pre-processed by means of a standard protocol. Eye movements were monitored and recorded by means of two lateral and four vertical EOG electrodes. These EOG electrodes were used to correct trials for ocular artifact by means of the Gratton–Coles-Algorithm (Gratton et al., 1983). Results of the ocular correction procedure were visually inspected to be sure that the regression method did not distort frontal channels. Artifact rejection procedures were applied twice: automatically, with an amplitude threshold of ±70 μV, and visually by rejecting all trials contaminated by technical artifacts.

The response-locked Ne/ERN was quantified in its amplitude and latency at electrodes Fz and FCz, based upon the scalp topography. Both electrodes were quantified separately. The Ne/ERN was defined as the most negative peak occurring in a time range between 50 and 120 ms after an erroneous response, against a pre-response baseline from −200 ms till 0 (i.e. response). Potentials on correct trials (Nc/CRN) were quantified similarly, i.e. the CRN was defined as the most negative peak between 50 and 120 ms with the amplitude being measured against a pre-response baseline from −200 ms till 0. The Ne was only quantified in trials where arrowheads were presented as targets. Errors in Nogo-trials were left out of analysis, to avoid any confoundation of the analysis.

The stimulus-locked Nogo-N2 was quantified at electrode Fz and FCz. The Nogo-P3 (stimulus-locked) was quantified at electrode FCz and Pz. Each electrode was quantified separately. These electrode locations were also chosen based upon the scalp topography. Amplitudes were measured against a pre-stimulus baseline −200 until 0 (i.e. time point of stimulus presentation). Potentials on Go-trials were quantified in similar manner. The N2 was defined as the most negative peak occurring 200 till 300 ms after stimulus onset. The P3 was defined as the most positive peak occurring 350–500 ms after stimulus onset.

Statistical analysis

Amplitudes and latencies were analysed using repeated measures ANOVAs. The factors “electrode” and “condition” (correct/error or Go/Nogo) were used as within-subject factors, “genotype” (CT/TT vs. CC) was used as between subject factor. All variables subjected to analyses of variance were normal distributed as indicated by Kolmogorov–Smirnow Tests (all z's<0.8; $p>0.3$). In all analyses, Greenhouse–Geisser corrections were applied when appropriate. Post-hoc tests were Bonferroni-corrected.

In a second step, all analyses were cross-validated. Doing this, participants in each genotype group were randomly divided randomly into two subgroups. Then, all ANOVAs were repeated using these randomly created subgroups as an additional between-subject factor “split-half subgroup”.

Results

Behavioural data: response inhibition

The mean rate of false alarms was 3.92 (0.26). While there were no genotype differences in the reaction times (RTs) on false Nogo trials ($F(1,63)=0.43$; $p>0.5$) (CC: 343 ms ± 16; CT/TT: 329 ms ± 15), the mean rate of false alarms was higher for the CC (5.65 ± 0.25) genotype group, compared to the CT/TT genotype group (2.44 ± 0.23) ($F(1,63)=85.99$; $p<0.001$; $\eta=0.577$). The effect in false alarm rates is illustrated in Fig. 1A. A Kruskal–Wallis test was calculated to account for possible gene–dose effects. This non-parametric test was chosen, because of the small sample size of the TT genotype group. It is shown that gene–dose effects were evident (H-test: $chi^2=39.91$, $df=2$; $p<0.001$) in which the CC genotype group showed the highest rate of false alarms (5.65 ± 0.25). The rate of false alarms was lower in the CT genotype group (2.65 ± 0.27) and lowest in the TT (1.86 ± 0.23) genotype group. The CT and TT genotype groups also differed from each other, as indicated by a Mann–Whitney U test ($Z=-1.79$; $p=0.036$). This indicates a deficient response inhibition in the CC genotype group, relative to the CT and TT genotype group. Adding the factor “split-half subgroup” did not change the pattern of results (all F's related to “split-half subgroup”<1.1; $p>0.2$), underlining the robustness of effects. Similar, also the factor “sex” did not affect the pattern of results (all F's related to “sex”<0.6; $p>0.5$).

Behavioural data: error processing

Reaction times (RTs) were faster on error (328 ms ± 10), compared to correct trials (403 ms ± 9) ($F(1,63)=183.05$; $p<0.001$; $\eta=0.744$). This effect was not different for the examined genotype groups, as indicated by the non-significant interaction ($F(1,63)=0.02$; $p>0.8$; $\eta=0.001$). Similarly, RTs across all response conditions (compatible and incompatible) were not different for genotype groups ($F(1,63)=0.41$; $p>0.5$; $\eta=0.006$). As expected, error rates were higher for the incompatible (8.14 ± 0.36), compared to the compatible condition (2.62 ± 0.12) ($F(1,63)=221.7$; $p<0.001$; $\eta=0.779$). However, this effect was not different for genotype groups, as revealed by the non-significant interaction ($F(1,63)=1.10$; $p>0.2$; $\eta=0.017$). Interestingly, across all response trial types (compatible and incompatible), there was a difference between genotype groups ($F(1,63)=19.15$; $p<0.001$; $\eta=0.233$), with the combined CT/TT genotype group showing fewer errors (4.5 ± 0.2), than the CC genotype group (6.2 ± 0.3).

The prolongation of RTs after an error as occurred (post-error slowing) reflects the behavioral adaptation after an error (Rabbitt, 1966). To calculate this post-error slowing, the mean reaction time of

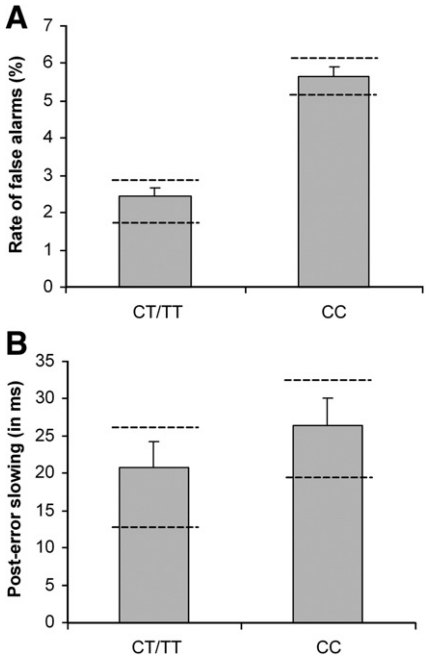

Fig. 1. (A) Mean rates of false alarms (i.e. responses on Nogo trials) for the CT/TT and CC genotype groups. (B) The mean time of post-error slowing (in ms) for the CT/TT and CC genotype groups. Error bars denote the standard error of the mean (SEM). Dashed black lines denote the upper and lower bound of the 99%-confidence interval.

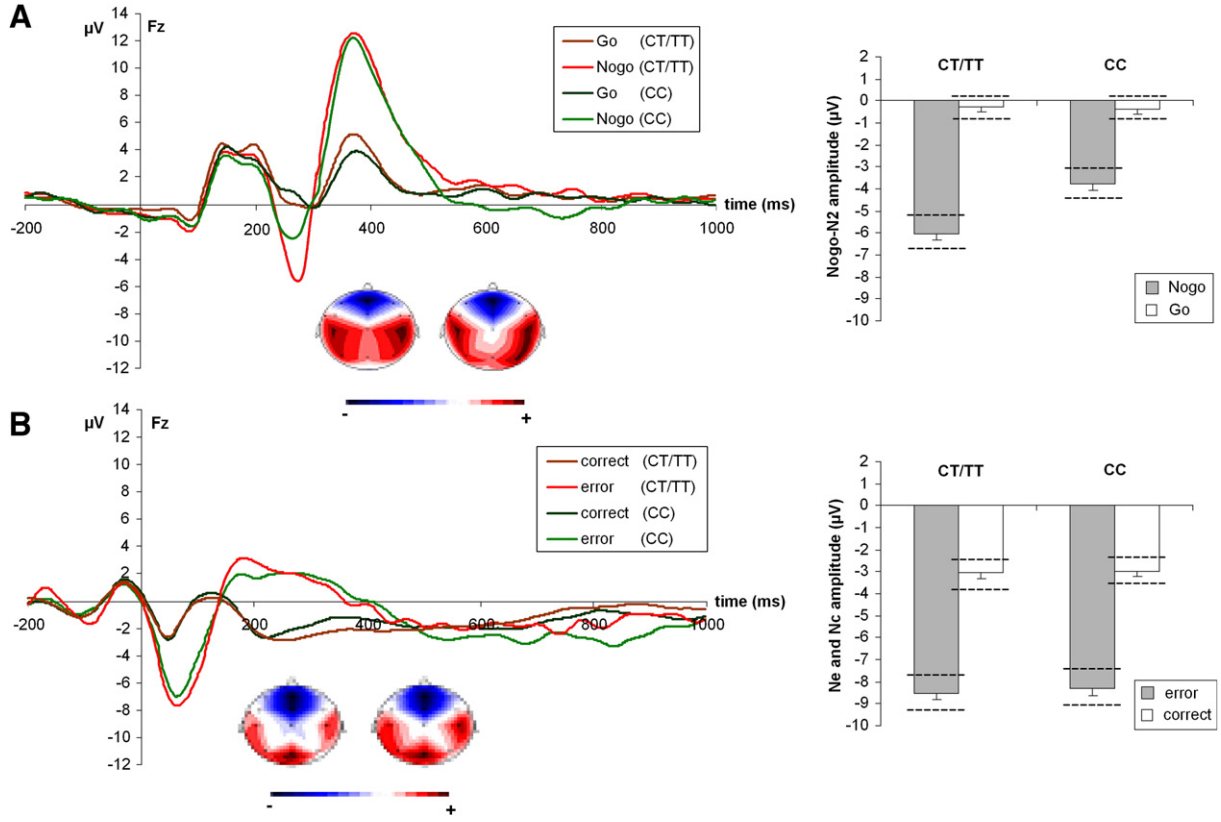

Fig. 2. Potentials at electrode Fz are shown. (A) On the left, the stimulus-locked event-related potential (ERP) on Go and Nogo trials is given together with the topographies of the Nogo-N2. The time point 0 denotes the time point of stimulus presentation. On the left the grand average waveforms of the N2-potential on Go and Nogo trials is given, separated for the different genotype groups (CT/TT and CC). (B) On the left, the grand averaged response-locked ERP on correct and error trials is given. The time point 0 denotes the time point of the response. Error bars denote the standard error of the mean (SEM). Dashed black lines denote the upper and lower bound of the 99%-confidence interval.

correct responses in succession and those after an error ("sequence") were subjected to a repeated measures ANOVA. Despite a slowing effect after error trials ($F(1,63)=89.2$; $p<0.001$; $\eta=0.233$), it was not different for genotype groups, as indicated by the non-significant interaction ($F(1,63)=1.23$; $p>0.2$; $\eta=0.021$). The post-error slowing effects are illustrated in Fig. 1B. This suggests that the behavioural consequences after an error were not different for genotype groups. The whole pattern of behavioural results remained stable even after cross-validation procedure (all F's related to "split-half subgroup"<0.8; $p>0.3$). As with the behavioural data on Nogo-trials (see above) no influence of "sex" was evident for the data concerning error processing (all F's related to "sex"<0.9; $p>0.3$).

Neurophysiological data: response inhibition

Stimulus-locked potentials on Go and Nogo-trials are given in Fig. 2A. *N2-effects*: the repeated measures ANOVA showed that N2-potentials were larger at electrode Fz (−1.25 μV ± 0.12), compared to FCz (−2.62 μV ± 0.12) ($F(1,63)=105.8$; $p<0.001$; $\eta=0.625$). Moreover, the N2 was larger on Nogo, compared to Go trials ($F(1,63)=190.94$; $p<0.001$; $\eta=0.801$). It is shown that the Go/Nogo effect was different for genotype groups, as indicated by the interaction "Go/Nogo × group" ($F(1,63)=28.62$; $p<0.001$; $\eta=0.341$)[1]. Subsequent Bonferroni-corrected post-hoc tests showed that only the Nogo-N2 differed between the groups ($F(1,63)=55.38$; $p<0.001$; $\eta=0.468$), but not the Go-N2 ($F(1,63)=0.13$; $p>0.7$; $\eta=0.002$). The combined CT/TT genotype group revealed a stronger Nogo-N2 (−5.65 μV ± 0.20) than the CC genotype group (−3.37 μV ± 0.22). The main effect "group" was also significant ($F(1,63)=24.53$; $p<0.001$; $\eta=0.280$), underlining generally stronger N2 potentials in the combined CT/TT genotype group (−2.48 μV ± 0.14), compared to the CC genotype group (−1.38 μV ± 0.16). At least there was an interaction "electrode × Go/Nogo" ($F(1,63)=24.36$; $p<0.001$; $\eta=0.279$) which was due to stronger Go/Nogo effects at electrode Fz ($\eta=0.880$), compared to FCz ($\eta=0.700$).

There were no latency effects (all F's<0.3; $p>0.7$) and sex did not alter the pattern of results (all F's related to "sex"<0.6; $p>0.3$). Applying the cross-validation procedure, the between-subject factor "split-half subgroup" did not modify the above pattern of results (all F's related to "split-half subgroup" < 1; $p>0.3$) which underlines the robustness of the effects.

Similar to the behavioural data, a Kruskal–Wallis test was calculated to account for possible gene–dose effects. Also for the Nogo-N2 gene–dose effects were evident (H-test: $chi^2=45.49$, $df=2$; $p<0.001$) in which the CC genotype group revealed the weakest Nogo-N2 (−3.37 μV ± 0.22). The Nogo-N2 was stronger in the CT genotype group (−4.95 μV ± 0.15) and strongest in the TT (−7.70 μV ± 0.48) genotype group. The CT and TT genotype groups differed from each other, as indicated by a Mann–Whitney U test ($Z=-3.92$; $p<0.001$).

A correlational analysis across the whole sample revealed that the amplitude of the Nogo-N2 was related to the rate of false alarms. It shown that increases in the strength of the Nogo-N2 were related to decreases in the rate of false alarms ($r=0.777$; $R^2=0.49$; $p<0.001$). Also within the CC ($r=0.525$; $R^2=0.25$; $p=0.001$) and the combined CT/TT genotype group ($r=0.623$; $R^2=0.32$; $p<0.001$) a similar correlation was evident. Fig. 3 denotes the correlation between amplitude of the Nogo-N2 and rate of false alarms for the CC and CT/TT genotype groups.

P3-effects: the repeated measures ANOVA revealed stronger P3 potentials at electrode Pz, compared to FCz ($F(1,63)=10.18$;

[1] The Nogo-N2 was calculated over 84 trials (±8). This number of trials was not different for the genotype groups ($p>0.3$).

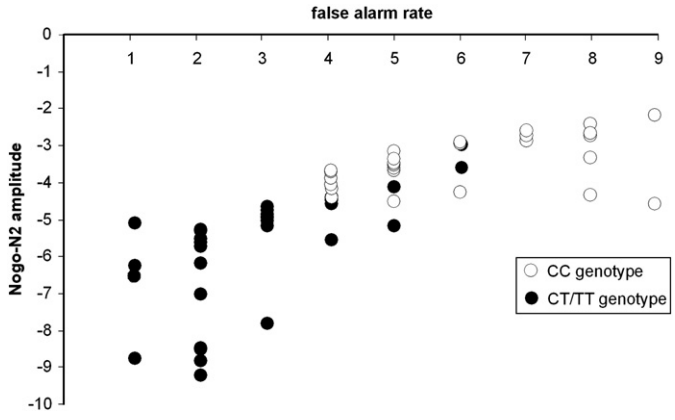

Fig. 3. Correlation of the amplitude of the Nogo-N2 and the rate of false alarms for the CC (white dots) and CT/TT (black dots) genotype group.

$p=0.002$; $\eta=0.139$). Moreover, there was the well-known interaction "electrode×Go/Nogo" ($F(1,63)=88.57$; $p<0.001$; $\eta=0.584$). Regarding this interaction repeated-measures ANOVAs showed that the Nogo-P3 was larger at FCz (14.74 μV±0.41), compared to Pz (10.94 μV±0.44) ($F(1,64)=35.09$; $p<0.001$; $\eta=0.354$). The reverse pattern is shown for the Go-P3, which was larger at Pz (14.79 μV±0.41), compared to FCz (8.87 μV ± 0.41) ($F(1,64)=94.19$; $p<0.001$; $\eta=0.595$). The main effect Go/Nogo itself was also significant, showing that the P3 was larger Nogo (12.85 μV±0.28), compared to Go-trials (11.86 μV±0.28) ($F(1,63)=9.36$; $p=0.003$; $\eta=0.129$).

As opposed to the N2 results, no main or interaction effect with "group" (all F's<0.9; $p>0.3$) was observed. Similar to the N2 results there were also no latency effects (all F's<0.8; $p>0.3$). Again, sex did not alter the pattern of results (all F's related to "sex"<0.5; $p>0.5$) and remained unchanged after cross-validation procedure (all F's related to "split-half subgroup"<1.3; $p>0.2$).

Neurophysiological data: error processing

Response-locked ERPs on correct and error trials are given in Fig. 2B. The repeated-measures ANOVA showed that potentials were larger at electrode Fz (−5.71 μV±0.14), compared to FCz (−4.93 μV±0.20) ($F(1,63)=12.40$; $p<0.001$; $\eta=0.165$). Potentials on error trials (Ne/ERN) were larger (−7.77 μV±0.22) than on correct trials (Nc) (−2.87 μV±0.17) ($F(1,63)=289.16$; $p<0.001$; $\eta=0.870$). There was no main or interaction effect with "group" (all F's<0.6; $p>0.4$). The pattern remained unchanged, when the sex was taken into account (all F's<0.3; $p>0.6$).

The analysis of the latencies only revealed known effects of "electrode" ($F(1,63)=6.74$; $p=0.012$; $\eta=0.097$) and "correctness" ($F(1,63)=136.63$; $p<0.001$; $\eta=0.684$) (e.g. Beste et al., 2009a,b; Falkenstein et al., 2000). Latencies were prolonged at Fz (66 μV±2), compared to FCz (62 μV±2) and shorter for correct (51 μV±2), compared to error trials (77 μV±4). There were no main or interaction effects with the factor "group" (all F's<1.3; $p>0.2$), or any modulation with the factor "sex" (all F's<0.9; $p>0.3$).

Similar to the response inhibition data, also the data pattern concerning error processing remained stable during the cross-validation procedure (all F's<0.8; $p>0.3$).

Discussion

In the current study we examined associations of the *GRIN2B* C2664T polymorphism with error processing and response inhibition processes. This analysis was inspired by the fact that the glutamatergic system has known influences on cognitive processes (Villmann and Becker, 2007) and that two distinct basal-ganglia-prefrontal loops (the orbito-frontal and anterior-cingulate loop) can be dissociated with respect to the glutamatergic chemoarchitecture of their striatal target areas.

We show a selective association of the *GRIN2B* C2664T polymorphism with the Nogo-N2. The Nogo-N2 was larger for the combined CT/TT genotype group, which was accompanied by a lower rate of false alarms, compared to the CC genotype group. Response inhibition subprocesses reflected by the Nogo-P3 were not modulated by this polymorphism. There were no differences in potentials on Go-trials, suggesting that the results are specific for response inhibition processes. The specificity of results is underlined by the analysis of error processing (Ne/ERN) and general response monitoring functions (Nc/CRN) that were not associated with the *GRIN2B* C2664T polymorphism. The electrophysiological results were completely paralleled by the behavioural data. The results seem to be robust as indicated by large effect sizes, confidence bounds and the cross-validation procedure.

The observed increase of the Nogo-N2 with a concomitant decrease in the rate of false alarms fits well to the pre-motor inhibition hypothesis of the Nogo-N2 (Beste et al., in press; Beste et al., 2009a,b; Falkenstein et al., 1999). This theory states that the Nogo-N2 reflects inhibition of a mistakenly selected motor program. It reflects inhibition that is exerted before the actual motor process. Increases in this inhibition may reduce the tendency to respond on Nogo-trials (Falkenstein et al., 1999). This is underlined by our behavioural data, showing a reduction of the false alarm rates in the CT/TT genotype group. Until now, not only the behavioural, but also the electrophysiological effects of the *GRIN2B* C2664T polymorphism have been elusive. The stronger Nogo-N2 in the CT/TT genotype group provides first evidence that the CT/TT genotype is most probably related to enhanced electrophysiological activity. Enhanced NMDA-receptor activity is well known to be related to increases in excitatory synaptic transmission, resulting in stronger neuronal activity (e.g. Villmann and Becker, 2007). The CT/TT genotype may be related to increased glutamatergic neurotransmission as also suggested by other findings (Arning et al., 2005). Increased levels of glutamate and NMDA-receptor mediated neural transmission have been shown to reduce dopaminergic neural transmission (e.g. Seamans and Yang, 2004; Moghaddam et al., 1997). Recent research suggests the Nogo-N2 is increased and response inhibition performance in enhanced in conditions with decreased dopaminergic functioning, likely because of a shift of striatal circuits towards inhibitory states (Beste et al., 2010a,b): decreases in dopaminergic activity may render the direct pathway less active while the indirect pathway becomes more active (Gale et al., 2008). This may lead to a predominating inhibitory effect (e.g. Gale et al., 2008). By means of this glutamatergic–dopaminergic interaction the CT/TT genotypes may ultimately be associated with increases of pre-motor inhibition process efficacy.

Interestingly, neuropsychiatric disorders with impaired impulse control have previously been related to the *GRIN2B* gene and therefore suggest a clinical relevance of the reported results. For example, deficits in impulse control in Parkinson's disease (PD) have been shown to be associated with *GRIN2B* and further with dopamine 3 receptors polymorphisms (DRD3) (Lee et al., 2009). While *GRIN2B* is a likely candidate gene involved in the etiology of schizophrenia (for review see Cherlyn et al., 2010; Quin et al., 2005), a recent study by Dorval et al. (2007) supports the clinical relevance of the *GRIN2B* gene specifically with the inattentive and hyperactive symptom dimensions of ADHD. Taken together, these studies support the view of a clinical relevance of *GRIN2B* for neuropsychiatric disorders and for impaired impulse control in particular.

However, Nogo-trials were infrequent in this task (20%) and may therefore evoke a target effect that also activates attentional networks. It has been shown that the orbitofrontal cortex is part of the ventral attention system (e.g. Corbetta and Shulman, 2002) and the inferior frontal cortex (IFC) is activated when targets are detected controlling for inhibitory processes (Hampshire et al., 2009).

Processes reflected by the Nogo-N2 have frequently been related to the orbitofrontal cortex (OFC). Sources of the Nogo-N2 have been shown in the OFC using source analysis (Bokura et al., 2001; Lavric et al., 2004; but see: Bekker et al., 2005; Nieuwenhuis et al., 2003). Also other studies using fMRI (Garavan et al., 2002; Liddle et al., 2001), or TMS (Chambers et al., 2006) support the role of the OFC in response inhibition functions (overview: Schoenbaum et al., 2009; Falkenstein, 2006), even though some studies also suggest the inferior frontal cortex to be of relevance (Aron et al., 2004).

It can therefore not completely be ruled out that attentional processes may play a role, too. However, errors occur infrequently and may hence also activate attentional networks. Despite of this, the Ne, which has consistently been shown to be related to the ACC (e.g. Ridderinkhof et al., 2004), was not modulated by the *GRIN2B* C2664T polymorphism. Thus, even though attentional processes cannot fully be ruled out, they do not seem to drive the effects obtained, since otherwise similar effects for the Nogo-N2 and Ne should have been obtained. Therefore, the results strongly suggest a dissociation of the NR2B receptor subunit for error processing and pre-motor inhibition functions. In the following paragraph, we propose a theoretical model that may explain the pattern of results:

Cortico-striatal projections preserve a strong neuroanatomical segregation at the striatal level (Haber, 2003). Here, projections from the ACC seem to target the striosomes, whereas fibres originating from the OFC seem to target both compartments, with preponderance for the matrix (Eblen and Graybiel, 1995). This segregation continues at a neurochemical level, since glutamatergic neural transmission in the matrix (targeted by OFC projections), but not the striosomes (targeted by ACC projections) is mediated via NMDA receptors (Bordelon et al., 1999; Blanchet et al., 1998; Martin et al., 1993; Dure et al., 1992). Due to these neuroanatomical and neurochemical dissociations of orbitofrontal and anterior cingulate loop projections at a striatal level, the dissociation observed at a neurophysiological and behavioural level may emerge. NR2B receptors are expressed in the frontal cortex (e.g. Ludwig et al., 2010), but no study reports differences between the OFC and ACC. With respect to the observed dissociation, it is hence probable that the most important point of dissociation of *GRIN2B* C2664T polymorphism effects lies within striatal structures. Clearly, the above model is theoretical and will need further validation in future studies.

It has to be acknowledged that at a striatal and mesencephalic level the parallelism of cortical projections is weakened by intrastriatal collaterals and interneurons (Pennartz et al., 2009; van Dongen et al., 2005; Haber, 2003), allowing communication across functionally distinct circuits in order to coordinate behaviour. Yet, even though parallelism of cortical projections is weakened at a striatal level, their effect seems to be less important, as otherwise the dissociated pattern would have been unlikely.

Based on our initial hypothesis we investigated particularly the NR2B NMDA receptor. Future studies may examine other receptors belonging to the NMDA-receptor family (e.g. NR1, NR2A,C,D and NR3A-B; for rev: Villmann and Becker, 2007). It is known that the NR2B receptor determines electrophysiological properties of NMDA receptors more strongly than other NR2 subunits (Loftis and Janowsky, 2003). With respect to the current findings, this makes it unlikely that other NR2-receptors may be of similar importance for the examined cognitive functions. This fact may also explain the robustness of effects obtained.

In summary, the results provide insights into the functional relevance of the *GRIN2B* C2664T polymorphism and thereby NR2B receptor activity for complex cognitive processes: pre-motor processes of response inhibition seem to be modulated by the *GRIN2B* C2664T polymorphism, whereas error processing functions are not modulated. Glutamatergic effects observed here may be explained by a model of NR2B receptor mediated glutamatergic neurotransmission selectively modulating orbitofrontal loop functions, but not anterior cingulate loops functions. The model proposed implies that striatal compartments cannot only be characterized with respect to neuroanatomy and neurochemistry, but also with respect to their differential role in the mediation of diverse cognitive processes. The results call for additional studies examining possible differential involvements of striatal compartments directly e.g. using single cell recording techniques.

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