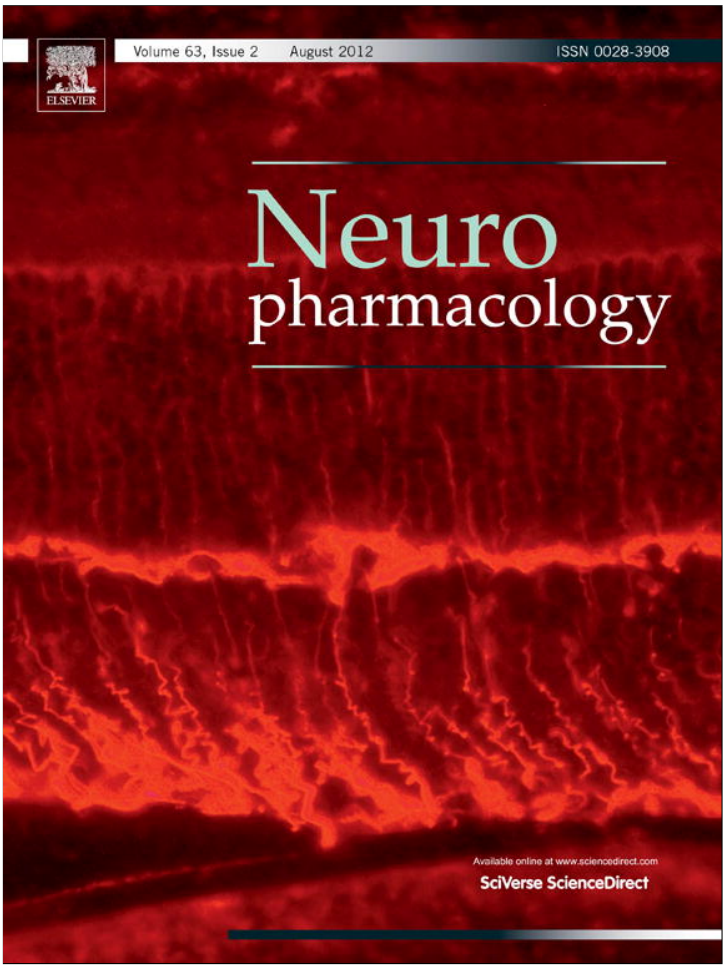

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N-methyl-D-aspartate receptor 2B subunit (*GRIN2B*) gene variation is associated with alerting, but not with orienting and conflicting in the Attention Network Test

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ABSTRACT

Appropriate attention levels are pivotal for cognitive processes, and individual differences in attentional functioning are related to variations in the interplay of neurotransmitters. The attention network theory reflects attention as a non-homogenous set of separate neural networks: alerting, orienting and conflicting. In the present study, the role of variations in *GRIN2B*, which encodes the NR2B subunit of *N*-methyl-D-aspartate (NMDA) receptors, was explored with regard to the regulation of arousal and attention by comparing the efficiency of the three attentional networks as measured with the Attention Network Test (ANT). Two synonymous SNPs in *GRIN2B*, rs1806201 (T888T) and rs1806191 (H1178H) were genotyped in 324 young Caucasian adults. Results revealed a highly specific modulatory influence of SNP rs1806201 on alerting processes with subjects homozygous for the frequent C allele displaying higher alerting network scores as compared to the other two genotype groups (CT and TT). This effect is due to the fact that in the no cue condition faster reaction times were evident in participants carrying at least one of the rare T alleles, possibly as a result of more effective glutamatergic neurotransmission. The results might be further explained by a dissociation between tonic and phasic alertness modulated by the *GRIN2B* genotype and by a ceiling effect, meaning that subjects cannot be phasicly alert in excess to a certain level. Altogether, the results show that variations in *GRIN2B* have to be taken into consideration when examining attentional processes.

1. Introduction

Attentional performance in humans appears to be controlled by several interacting brain systems. According to the Attention Network Theory, three distinct networks are distinguished: orienting, conflicting and alerting (Posner and Peterson, 1990). Orienting is defined as the selection of information from sensory input and conflicting can be explained in terms of executive control and the resolving of conflicts among stimuli and responses (Fan et al., 2002, 2009; Posner and Rothbarth, 2007). The alerting network can be further subdivided into phasic and tonic alertness. Whereas tonic alertness describes wakefulness and arousal, phasic alertness depicts the ability to increase alertness and response readiness as the result of a warning stimulus (Fan et al., 2009; Fossella et al., 2002). Based on past fMRI studies, these three networks cannot only be distinguished based on their function, but moreover also on the basis of the brain structures and neurotransmitters belonging to each of the networks (e.g. Fan et al., 2005; Konrad et al., 2005). The relevant neuroanatomical structures belonging to the orienting network are located in the parietal and frontal lobes, the temporal parietal junction, the frontal eye-fields and the superior colliculi. This network has been associated with acetylcholine as the predominant neurotransmitter (see e.g. Davidson and Marocco, 2000; Fan et al., 2002; Posner and Rothbarth, 2007). The conflicting network is controlled by dopamine with the lateral prefrontal cortex and the anterior cingulum as the neuroanatomical regions belonging to this network (Corbetta and Shulman, 2002; Fan et al., 2002; Posner and Rothbarth, 2007). The alerting functions are associated with frontal and parietal areas as well as thalamic regions (Fan et al., 2002, 2005; Posner and Rothbarth, 2007). The most important neurotransmitter for the alerting network is noradrenaline, which is released in the locus coeruleus (LC, Aston-Jones and Cohen, 2005; Marrocco et al., 1994; Marocco and Davidson, 1998). Yet, also glutamate plays an important role in the regulation of arousal and attentional processes (Jones, 2003; Sarter et al., 2006). Glutamate, the most abundant excitatory neurotransmitter in the central nervous system, mediates its excitatory effects by ionotropic and metabotropic receptors.

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The *N*-methyl-D-aspartate (NMDA) receptors are glutamate-gated cation channels that are highly permeable to calcium (Ca^{2+}) and are essential for the regulation of various neuronal processes like synaptogenesis, synaptic remodelling and long-term plastic changes in synaptic efficacy (Lau and Zukin, 2007; Beste et al., 2008).

NMDA receptors are heteromeric complexes, whereby NR1 as obligatory subunit combines with NR2 or NR3 subunits to form a functional receptor. Among the NR2 subunits, NR2A and NR2B subunits predominate. The functional properties of NMDA receptors depend on subunit composition, whereas additional heterogeneity can also arise through alternative splicing (Cherlyn et al., 2010; Hollmann and Heinemann, 1994; Monyer et al., 1992). Over the past years, results from different areas of research emphasize the critical (pathological) role of NMDA receptors in cognition, learning, memory, behaviour and various neurological diseases (Cull-Candy et al., 2001; Kalia et al., 2008; Lau and Zukin, 2007). Additionally, numerous association studies showed that specific single nucleotide polymorphisms (SNPs) of NMDA receptor subunits impact on these phenotypes. In particular, variations in *GRIN2B*, which encodes the NR2B subunit of NMDA receptors, have been associated with a wide range of neurological conditions and neuropsychological phenotypes, confirming the essential role of NMDA receptors in brain function (see e.g. Arning et al., 2005; Beste et al., 2010; Clinton and Meador-Woodruff, 2004a; Kiss et al., 2011; Martucci et al., 2006; Ness et al., 2011; Ocklenburg et al., 2011).

Recently, a modulatory function of glutamate in the noradrenergic system was found in studies using animal models. The firing rate of the LC is modulated by other neurotransmitters with glutamate being one of the most important ones providing a major excitatory input (see e.g. Charléty et al., 1993; Somogyi and Llewellyn-Smith, 2001). Moreover, besides noradrenaline (e.g. Ishikawa and Tanaka, 1977; Kang et al., 2000; Samuels and Szabadi, 2008), glutamate binding sides were found in the diencephalon (Khan et al., 2000; Kiss et al., 2011). By binding at thalamic structures, both, noradrenaline as well as glutamate mediate excitatory thalamocortical in- and output (Ibrahim et al., 2000a; Jones et al., 1998; McBridge and Sutin, 1976; McCormick et al., 1991). Since the thalamus (Fan et al., 2005; Yanaka et al., 2010) as well as the noradrenergic system can be seen as essential parts of the alerting network, it is assumed that glutamate possesses modulatory function in conjunction with tonic and phasic alertness. The importance of glutamate for the modulation of attentional processes was recently suggested by Beste et al. (2011). The authors showed that attentional control is altered by glutamate dependent long-term potentiation (LTP) and long-term depression (LTD)-like perceptual learning.

Moreover, investigations in psychiatric populations provide useful indicators regarding the importance of the glutamatergic system for particular cognitive functions. In this context, changes in NMDA receptor mediated glutamatergic neurotransmission were observed in patients with schizophrenia (e.g. Cherlyn et al., 2010; Coyle, 2006; Goff and Coyle, 2001; Marsman et al., in press; Quin et al., 2005), where disturbed functioning of the glutamatergic system has been reported with the thalamus and the prefrontal cortex showing abnormalities in NMDA receptor functioning (Clinton and Meador-Woodruff, 2004b; Dracheva et al., 2001; Hong et al., 2001; Ibrahim et al., 2000b; Kim et al., 1980; Kornhuber, 1990; Matute et al., 2005; Smith et al., 2001). It is widely known, that schizophrenic patients suffer from attentional deficits (Goldberg and Gold, 1995). In this context, a deficit in the alerting network was postulated by Nestor et al. (2007). In line with this finding, reaction times (RTs) were slowed down in schizophrenic patients in another study using a task measuring alertness by distinguishing between tonic and phasic alertness (Amado et al., 2011). In addition to patient studies, pharmacological studies shed light on the role of glutamate for attentional processes. Pharmacological studies using hallucinogens have been frequently used as a model for psychosis in the course of schizophrenia. Drug derivates of the NMDA antagonist phencyclidine (PCP) type (e.g. Ketamine) are frequently employed to induce a state of undifferentiated or disorganized psychosis and attention impairments (Daumann et al., 2010; Morgan et al., 2004; Musso et al., 2011; Newcomer et al., 1999; Umbricht et al., 2000). In this context, Daumann et al. (2008) found evidence for attention regulation in an inhibition of return paradigm with enhanced brain activity after s-Ketamine administration.

The neurobiological basis of cognitive processes explored by using the Attention Network Test (ANT) has been subject to other behavioural genetic studies (e.g. Fossella et al., 2002; Reuter et al., 2007) and also to studies using functional imaging (e.g. Fan et al., 2003). In this context only recently, Thimm et al. (2010) showed the importance of the glutamatergic system for processes controlled by the conflicting network. The authors found that individual variation in the dystrobrevin-binding protein 1 (*DTNBP1*) gene impact executive functioning via modulation of a left prefrontal network regulated among others by glutamate (see also Fallgatter et al., 2010).

In the present study, the role of glutamate in the regulation of arousal and attention was further explored by comparing the efficiency of the three attentional networks measured by the ANT, alerting, orienting and conflicting in 324 healthy subjects carrying different allelic variations of the NR2B subunit gene *GRIN2B* (rs1806201 and rs1806191). The rationale for selecting these particular SNPs was based on previously reported significant associations with diverse cognitive processes and psychiatric disorders related to cognitive dysfunctions (Beste et al., 2010; Cherlyn et al., 2010; Ness et al., 2011; Ocklenburg et al., 2011; Quin et al., 2005). Based on the described interactions between the noradrenergic and glutamatergic systems and the important role of glutamate regarding cognitive impairments of psychiatric patients together with the findings by Thimm et al. (2010), we might expect to find a modulatory effect of GRIN2B genotypes on the alerting and conflicting networks, but not on the orienting network, since the latter is not supposed to depend on glutamatergic processing.

2. Materials and methods

2.1. Participants

324 young adults (144 males/180 females) of Caucasian descent, with a mean age of 23.91 years (23.91 ± 2.73, range: 17–31years) participated in this study and received course credits or financial compensation. 292 subjects where right handed and 32 left handed as measured with the Edinburgh Handedness Inventory (Oldfield, 1971). None of the subjects reported a history of any neurological or psychiatric disorder, and all participants had normal or corrected to normal vision. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

2.2. Stimuli and experimental procedure

The ANT was used for the assessment of the three attention networks alerting, orienting and conflicting. The test was used in its original form as it has been developed by Fan et al. (2002). E-Prime 2.0 was utilized for presentation on a personal computer with a 17 inch monitor and participants viewed the screen from a distance of approximately 65 cm. The first block was a practice block in which the subjects got feedback about their reaction accuracy and reaction time (24 trials). The remaining three blocks were test blocks with 96 trials each (288 trials in total). Between all runs, the subjects were allowed to take breaks as long as wanted. A computer mouse was used as response panel. The subjects had to press either the left or the right mouse button with the index and middle finger of their right hand. A black fixation cross on a gray background in the middle of the computer screen was present during all blocks and had to be fixated the whole time. The subject's task was to respond as quickly and correctly as possible to the direction of a left- or

rightward pointing black arrow which served as the target. This target was either flanked by two dashed black lines on the left and right (1/3 trials, neutral condition) or by black arrows pointing to the same (1/3, trials congruent condition) or opposite direction (1/3 trials, incongruent condition, see Fig. 1a). One single line or arrow consisted of 0.55° of visual angle, and the contours of adjacent arrows or lines were separated by 0.06° of visual angle. One central arrow with four flankers consisted of 3.08° of visual angle.

In addition to the two flanker conditions, four feasible cueing conditions were employed (Fig. 1b), with a little asterisk serving as the cue. In the no cue condition, only the target stimuli were presented without a cue beforehand. The center and double cue only contained information about the immediate occurrence of the target. However, it is important to note that in the center cue condition alerting was involved, because participants were shown an asterisk at the location of the fixation cross for 100 ms (Fan et al., 2002). In the double cue condition, the time course was the same, but the attentional field was larger, because two cues were presented at the two possible target positions above and below the fixation cross instead of one cue in the middle of the screen as in the center cue condition. Furthermore, in contrast to the center cue, the double cue tends to keep the attention diffused between the two possible target locations. Fan et al. (2002) assumed that even if an alerting effect was involved in the center cue condition, this effect is supposed to be larger in the double cue condition, because of the larger attentional field evoked by the two simultaneously occurring cues. In the fourth possible cue condition, the spatial cue condition, the cue also contained information about the location at which the target will occur. All four cuing conditions occurred equally often, 25% (=72 trials) each. Based on these different conditions three calculations could be made to determine the efficiency of each of the three attention networks (Fig. 1c).

Each ANT trial consisted of five components. At first, a fixation period was presented lasting variably between 400 ms and 1600 ms (D1). Afterwards a cue was presented for 100 ms in most of the trials. Following a 400 ms lasting fixation period, target and flanker occurred simultaneously until the subject responded, but for 1700 ms at maximum. Upon the subject's response, the flanker and the target disappeared instantly, followed by a post target fixation period (Fig. 1d). All trials were presented in a pseudorandom order. For further details about the ANT see Fan et al. (2002).

2.3. Genotyping

For non-invasive sampling, exfoliated cells were brushed from the oral mucosa of the participants. DNA isolation was performed with QIAamp DNA mini Kit (50) (Qiagen GmbH, Hilden, Germany). Two synonymous SNPs in *GRIN2B*, rs1806201 (T888T) and rs1806191 (H1178H) were genotyped by polymerase chain reaction (PCR) and differential enzymatic analysis with the PCR restriction fragment length polymorphism method. Linkage disequilibrium (LD) between SNPs was assessed using Haploview (http://www.broad.mit.edu/mpg/haploview/). Further details of methodology and primer sequences are available upon request.

2.4. Statistical analysis

Our analyses were performed assuming a co-dominant or a recessive effect for the polymorphisms. In the co-dominant model, all three genotype groups were analysed. In the recessive model, both the heterozygous (rs1806201:CT/rs1806191:GA) and the rarely observed homozygous (rs1806201:TT/rs1806191:AA) variations were combined. To explore network score differences depending on the genotype or rather possible interactions between the cueing conditions and the genotype, repeated measures ANOVAs were conducted, including the experimental factors as factors into the model (e.g. no cue and double cue as within subject factors and genotype as between-subject factor). Significant interactions were further explored by using post-hoc *t*-tests to determine the variable accounting for the effect. Significances were Greenhouse Geisser corrected if necessary. One-way between-groups analyses of variance (ANOVAs) with *GRIN2B* genotypes as between-subject factor were calculated for error rates and reaction times (RT). Responses which occurred 1700 ms after the target onset were categorized as misses. Numbers of correct responses are given in percentage values. The significance level was $<.05$ for all statistical tests. Mean (M) and standard error (SEM) are indicated ($M \pm SEM$). All analyses were computed with Predictive Analytics Software (PASW) 18.0.

3. Results

3.1. Genotyping

Genotyping *GRIN2B* SNP rs1806201 revealed a prevalence of homozygous subjects for CC of 52% ($n = 169$), heterozygous for CT of 39% ($n = 127$) and homozygous for TT of 9% ($n = 28$). 30% ($n = 97$) were homozygous for the rs1806191 GG genotype, 46% ($n = 146$) heterozygous GA and 24% ($n = 77$) homozygous for AA. Genoytping SNP rs1806191 was not possible for 4 subjects. The two SNPs in linkage disequilibrium (LD, $D' = 0.89$, $r^2 = 0.27$) are separated by 870bp.The distributions for both SNPs were in Hardy–Weinberg equilibrium ($p > .13$). We identified three haplotypes (rs1806201–rs1806191) with a frequency of 46% (CA), 26% (CG) and

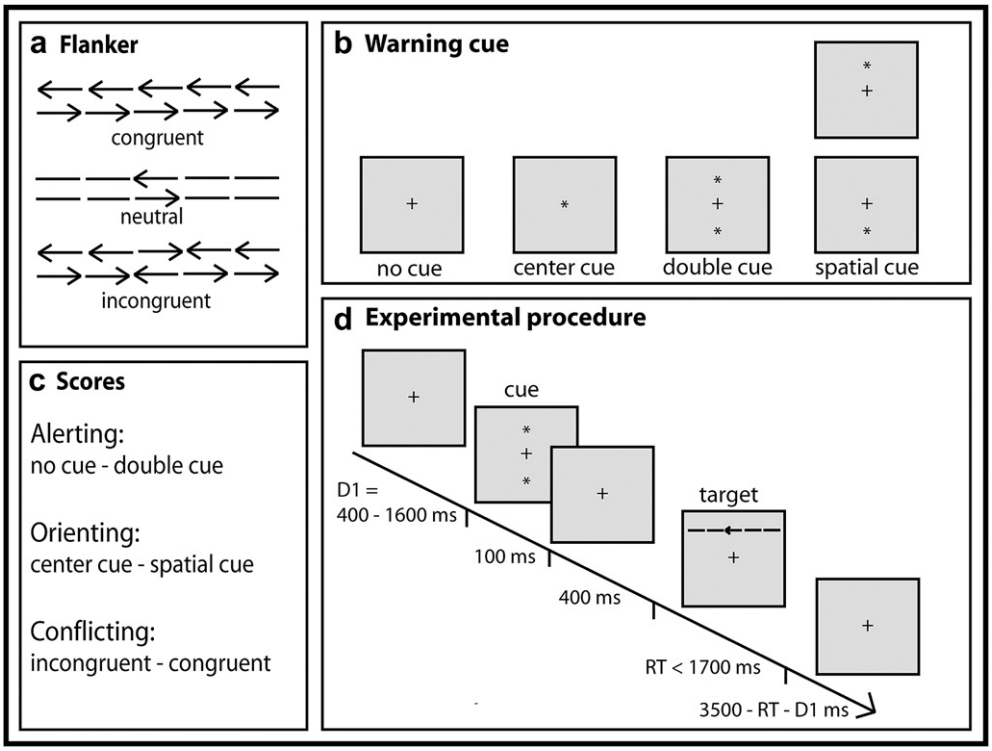

Fig. 1. Schematic overview of the stimulus set-up and experimental procedure adapted and modified according to Fan et al. (2002). Subjects had to indicate the direction of the arrow head of the target stimulus with a button press. Difficulty of the task was varied by different cuing and flanker type conditions.

28% (TG). Yet, none of these haplotypes (all p's $> .24$) or any diplotype (all p's $> .09$) explained the association substantially more than rs1806201 alone.

3.2. Attention networks

The data was first analysed assuming a co-dominant effect for the *GRIN2B* polymorphisms. These analyses did not reveal any significant genotype effects for SNP rs1806191 (all p's $> .16$). For SNP rs1806201, a significant interaction cueing × genotype was found for the alerting network ($F_{(2,321)} = 6.39$, $p < .003$, $\eta^2 < .038$). This interaction was also reflected in the networks scores. The alerting network score was higher in subjects homozygous for the C allele (40.15 ± 1.55 ms) as compared to the heterozygous CT group (31.67 ± 1.81 ms) and subjects carrying two T alleles (33.51 ± 4.59 ms). No cueing × genotype effects were observed for the orienting ($F_{(2,321)} < 1$, $p = .96$, $\eta^2 < .001$) or conflicting networks ($F_{(2,321)} < 1$, $p = .91$, $\eta^2 < .002$). Yet, the CT and TT carriers did not differ from each other significantly in the alerting network score ($F_{(1,153)} < 1$, $p = .68$, $\eta^2 < .002$) and were therefore pooled in one group for further analyses. Taking the recessive model as basis for the statistical analyses, no significant cueing × genotype interactions were obtained for SNP rs1806191 with regard to the three attention networks (all p's $> .10$). For SNP rs1806201, the following group differences between subjects homozygous for the C allele and the combined CT/TT genotype group were obtained:

No statistically significant cueing × genotype interaction was evident for the orienting (CC: 37.20 ± 1.93 ms; CT/TT: 37.17 ± 1.98 ms) ($F_{(1,322)} < 1$, $p = .99$, $\eta^2 < .001$) and conflicting network scores (CC: 109.13 ± 3.18 ms; CT/TT: 111.04 ± 3.15 ms) ($F_{(1,322)} < 1$, $p < .68$, $\eta^2 = .001$). Yet, for the alerting network, a significant cueing × genotype interaction was obtained ($F_{(1,322)} = 12.63$, $p < .001$, $\eta^2 = .038$), which was also displayed by a higher alerting network score in the CC (40.15 ± 1.55 ms) group than in the CT/TT group (32.00 ± 1.69 ms; see Fig. 2a). A one-sided post-hoc two sample t-test showed that this interaction was due to a significant genotype group difference in the no cue condition ($t_{(321.96)} = 1.85$, $p < .04$). Carriers of the CT/TT genotypes reacted faster (539.72 ± 5.34 ms) as compared to subjects homozygous for the C allele (553.94 ± 5.15 ms). No such group difference was found in the double cue condition (CC: 513.80 ± 5.08 ms; CT/TT: 507.72 ± 5.07 ms) ($t_{(321.45)} = .85$, $p < .40$) (Fig. 2b).

The overall accuracy in the ANT was 96.7% (range: 71%–100%), and error rates between the two genotype groups differed neither overall ($F_{(1,322)} < 1$, $p < .58$, $\eta^2 = .001$) nor in the no cue ($F_{(1,322)} = 2.46$, $p < .12$, $\eta^2 = .008$) and double cue ($F_{(1,322)} < 1$, $p < .74$, $\eta^2 = .001$) conditions as specific conditions measuring alertness. The overall RTs did not vary statistically significantly between both genotype groups (CC: 519.97 ± 5.11 ms; CT/TT: 510.89 ± 5.34 ms) ($F_{(1,322)} <= 1.51$, $p < .23$, $\eta^2 = .005$).

4. Discussion

This study examined the modulation of attentional processes as measured with the ANT by variations of the NR2B subunit gene *GRIN2B*. The main findings can be summarized as follows: For rs1806191 no genotype effects on attentional networks were evident. Regarding rs1806201, however, a significant group effect was found for the alerting network with subjects homozygous for the frequent C allele displaying higher alerting network scores as compared to subjects carrying at least one rare T allele. This effect could be explained by a significant genotype group difference in the no cue condition, in which faster reaction times were evident in participants heterozygous or homozygous for the T allele. Yet, no such association was evident with respect to the orienting or conflicting networks.

The pattern of results underlines recent findings suggesting that especially the rs1806201 SNP is of relevance for diverse cognitive processes (Ness et al., 2011; Ocklenburg et al., 2011; Beste et al., 2010), even though also rs1806191 has been shown to be associated with cognitive functioning (Ness et al., 2011).

Results of previous studies suggest that the rare T allele of the *GRIN2B* rs1806201 polymorphism could be associated with enhanced glutamatergic transmission (Arning et al., 2005; Beste et al., 2010; Ness et al., 2011; Ocklenburg et al., 2011). Regarding the present results, it can be assumed that the possibly increased glutamatergic neurotransmission in the combined CT/TT genotype group results in larger excitatory input into the LC. As a consequence, more noradrenaline may be released (e.g. Charléty et al., 1993; Singewald and Philippu, 1998; Somogyi and Llewellyn-Smith, 2001) followed by higher levels of arousal and thus modulation of alerting network functions. On the other hand, it can be argued that the faster reaction times of the CT/TT genotype subjects in the no cue condition are evoked by elevated glutamatergic transmission that entails increases in noradrenergic turnover. Yet, until now the precise function of this synonymous (T888T) SNP is still unknown. Synonymous SNPs might be causal through influencing messenger RNA splicing, stability, and structure as well as changing the rate of protein folding (Hunt et al., 2009), but this has yet to be proven for rs1806201.

Interestingly, the *GRIN2B* genotype effect in this study is restricted to the condition measuring tonic alertness in the ANT.

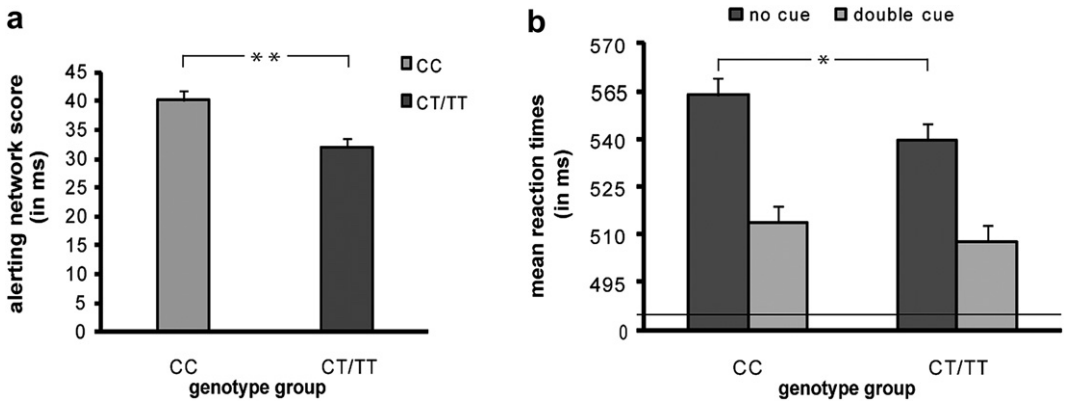

Fig. 2. (a) Alerting network scores depending on the *GRIN2B* polymorphsim rs1806201; error bars depict the standard error. (b) Mean reaction times in the no and double cue conditions of the ANT with respect to the *GRIN2B* genotypes; error bars depict the standard error.

In the phasic alertness condition, no modulatory influence of the *GRIN2B* genotype was obtained. Due to this pattern of RT disparities between the CC and CT/TT genotype groups in the alerting conditions, the alerting network score (RT no cue − RT double cue) was lower in the combined CT/TT genotype group. The alerting network score reflects the relation between tonic and phasic alertness and hence the RT difference between trials with (phasic alertness) and trials without (tonic alertness) a warning cue. One possible explanation for our results deals with the fact that the lower alerting network score in the CT/TT group may be due to impairment in using warning cues to effectively speed up RTs. However, CC and CT/TT carriers did not differ in their absolute amount of phasic alertness, but in the relation between tonic and phasic alertness, whereas subjects homozygous for the C allele appear to be less intrinsically alert in the no cue condition. Based on past research, it can be argued that tonic and phasic alertness are presumably not two poles of the same scale, since differential impairments of both alertness modes were found (see e.g. Johnson et al., 2008). Additionally, partly different brain structures are involved in these two kinds of alertness functions (Sturm and Willmes, 2001). Along this line, our results might also suggest a dissociation between tonic and phasic alertness modulated by the *GRIN2B* genotype: CT/TT carriers can increase their alertness and decrease their reaction times in response to the warning cue as effectively as subjects homozygous for the C allele. In contrast, without a warning cue subjects homozygous for the C allele react slower and are less tonicly alert than the CT/TT carriers.

Moreover, the observed lower alerting network score in the CT/TT group might be influenced by a ceiling effect. However, it has to be mentioned, that reaction times around 500 ms are certainly not the physical ceiling effect, but in contrast may describe the fastest possible reaction in the double cue condition of the ANT. In this line, reaction times around 500 ms in the double cue condition of the ANT were also reported in the very first publication explaining the ANT as an attention test (Fan et al., 2002) and were commonly reported in other studies (see e.g. Oberlin et al., 2005) as well.

In this study, a specific modulation of the alerting network was found without modulatory influences of the *GRIN2B* rs1806201 polymorphism on neither the orienting nor the conflicting network. This result is in line with previous studies, in which dissociations between attention network functions measured with the ANT and different variations of genes important for cognitive processes were also shown by other researchers in healthy individuals (see e.g. Fossella et al., 2002; Reuter et al., 2007; Thimm et al., 2011). Additionally, dissociative effects for the three attention networks were also frequently obtained in patient studies, as e.g. in studies with patients showing symptoms of attention deficit hyperactivity disorder, schizophrenia or multiple sclerosis (e.g. Gooding et al., 2006; Johnson et al., 2008; Urbanek et al., 2010; Wang et al., 2005;). Along this line, it was shown in the past that the three attention networks work independently (Fan et al., 2002, 2009; Posner, 2008), which may partly explain why not all three attention networks are influenced by the *GRIN2B* polymorphism in our study.

However, in contrast to Thimm et al. (2010), no modulatory impact of the glutamatergic system on conflicting network functioning was found in the present study. In this context, it has to be mentioned that Thimm et al. (2010) studied the impact of *DTNBP1* gene variation on executive functions. As stated by several authors in the past (e.g., Fallgatter et al., 2010; Harrsion and Weinberger, 2005; Talbot et al., 2006), *DTNBP1* may modulate glutamatergic transmission not directly, but e.g. via gabaergic and dopaminergic transmitter systems. Thus, it is possible that the effects found by Thimm et al. (2010), are at least partly due to the modulatory influences of neurotransmitters other than glutamate. But this point is only speculative and has to be addressed in further studies. Beyond this, the missing modulatory effect for the conflicting network can be explained by considering the brain structures involved in resolving conflicts and incongruency. One of the most important brain structures within the conflicting network is the anterior cingulum (ACC) with dopamine as the most important neurotransmitter (Posner and Rothbarth, 2007). With respect to this region, Beste et al. (2011) showed that functions mediated by the ACC are unaffected by *GRIN2B* rs1806201 genotypes, underlining that cognitive functions mediated via the ACC are not affected by this polymorphism.

Regarding the missing genotype group differences for the orienting network score, Loftis and Janowsky (2003) postulated that compared to other brain regions only moderate levels of NR2B receptors are expressed in the superior colliculi, which play a crucial role in processes related to the orienting of attention (Posner, 2008; Posner et al., 1982; Posner and Rothbarth, 2007). Based upon the dissociative pattern of results across functionally independent attentional networks it may be speculated that the interaction between glutamate and noradrenaline (e.g. Charléty et al., 1993; Somogyi and Llewellyn-Smith, 2001) is more powerful as compared to the interaction with dopamine (see e.g. Castner and Williams, 2007; Javitt, 2007; Moghaddam et al., 1997; Seamans and Yang, 2004; Verma and Moghaddam, 1996) or acetylcholine (Fadel et al., 2001; Rasmusson et al., 1994, 1996; Turchi and Sarter, 2001), which are the important neurotransmitters within the conflicting and orienting attentional networks (Posner and Rothbarth, 2007). However, we tested only 324 subjects and it cannot be ruled out, that effects for the orienting and conflicting network scores would be found if more subjects were tested. Yet, this study is limited by the relatively small sample size for genetic analyses even it is relatively large for a neuropsychological study. Thus, the results need to be replicated in further studies in order to exclude the possibility that our findings are due to chance.

In summary, our data suggests that variations in *GRIN2B* specifically modulate alerting functions in the ANT, and that this modulation is possibly due to a strong linkage between the noradrenergic and glutamatergic systems. The effects of the *GRIN2B* rs1806201 genotypes seem to be highly specific: Subjects homozygous for the frequent C allele showed a higher alerting network score and differed from subjects carrying at least one rare T allele only with respect to RTs when tonic alertness was measured. The CT/TT subjects were higher tonicly alert and this effect was possibly evoked by a greatly effective glutamatergic neurotransmission. The results might be explained by a dissociation between tonic and phasic alertness modulated by *GRIN2B* variation. A second possible explanation deals with a ceiling effect, which means that subjects cannot be phasicly alert in excess of a certain level. The results show that variations in *GRIN2B* have to be taken into consideration when examining attentional processes.

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