A functional promoter polymorphism of neuronal nitric oxide synthase moderates prefrontal functioning in schizophrenia

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Abstract

Cognitive deficits in tasks involving the prefrontal cortex such as working memory or verbal fluency are a key component of schizophrenia. This led to the hypofrontality hypothesis of schizophrenia, which is widely accepted even though molecular underpinnings are elusive. While disturbances of glutamatergic neurotransmission might play a role, other components have rarely been investigated. Recently, the promoter region of nitric oxide (NO) synthase-I (NOS-I, encoded by the gene NOS1), impacting on prefrontal glutamate transmission, has repeatedly been associated with schizophrenia. We thus tested whether an associated schizophrenia risk variant (rs41279104), leading to reduced expression of the transcript, influences prefrontal brain functioning. Forty-three patients suffering from chronic schizophrenia and 44 controls were genotyped for NOS1 rs41279104 and investigated by means of functional near-infrared spectroscopy (fNIRS), while completing a working-memory task (2-back test) and a verbal fluency test (VFT). After matching for genotype, behavioural and brain activation data of 26 patients and 28 comparable controls were correlated to rs41279104. Healthy controls showed significant activation of large parts of the lateral prefrontal cortex during both tasks, whereas task-related changes in oxygenation were significantly reduced in patients. Schizophrenia patients also performed worse in both tasks. The NOS1 schizophrenia risk genotype rs41279104 AA/AG was associated with slower reaction time in the 2-back task, as well as with reduced right-hemispheric activation of the frontal cortex for VFT in patients only.

Our fNIRS data extend previous studies suggesting disturbed prefrontal functioning in schizophrenia and suggest that genetic variation of NOS1 has a role in cognitive dysfunction, probably by mediating glutamatergic tone.

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Introduction

Cognitive deficits are considered a key component of schizophrenia. Specifically, impaired performance in tasks indicative of prefrontal functioning is found frequently. Along with biochemical and molecular data demonstrating a wide range of abnormalities in the prefrontal cortex and a multitude of neuroimaging studies arguing for decreased activity of (pre-)frontal brain regions, these neuropsychological findings make a case for the so-called ‘hypo-frontality concept’ (Ingvar & Franzen, 1974; Rodriguez-Sanchez et al. 2005; Snitz et al. 2005). However, despite significant effort only a few studies convincingly link genetic data to cognition and prefrontal functioning, as most of the scrutinized polymorphisms are not functional, so that their biological significance remains uncertain. The cellular mechanisms underlying hypo-frontality in schizophrenia are therefore unclear. The prevailing theory suggests that a hyper-glutamatergic state of the prefrontal cortex corresponds to hypo-activation of
this structure, and that this is caused, *inter alia*, by a disruption of glutamatergic signalling of the NMDA receptor complex. This notion put the NMDA synapse in the centre of current concepts on the aetiology of schizophrenia, supported also by genetic studies.

The gaseous molecule nitric oxide (NO) is a second messenger of the NMDA receptor with unusual physical properties (Snyder & Ferris, 2000). It interacts with the dopaminergic as well as the serotonergic systems, thereby linking glutamatergic to monoaminergic signalling, and affects a wide range of effector molecules. In animal studies, NO has been implicated in the mechanism of phencyclidine (PCP) psychosis (Fejgin et al. 2008, 2009; Palsson et al. 2009) as well as cognitive dysfunction (Bennett et al. 2007; Harooni et al. 2009; Wultsch et al. 2007). In neurons, NO is produced by an isoform of NO synthase termed NOS-4, encoded by the gene NOS1. The NOS1 locus on 12q24.3 harbours one of the most complex genes in the human genome: a 125-kb region containing 28 coding exons, whereas another 125-kb region, termed ‘variable region’, includes at least 11 distinct first exons transcribed into mRNA but thereafter removed by splicing. This locus has repeatedly been found to be linked to schizophrenia (Reif et al. 2006); thus, NOS1 is a relevant functional and positional candidate gene for schizophrenia. We have previously shown that a functional promoter polymorphism in the alternative exon 1c of NOS1 (rs41279104), which decreases expression of this exon by 30% (Saur et al. 2004) in *vivo* and by 50% in *vivo* [human post-mortem brain, dorsolateral prefrontal cortex (Cui et al. 2010)], is associated with schizophrenia (Reif et al. 2006). In the meantime our sample was extended to *n*=251 [68 (27%) carriers of the schizophrenia risk allele] patients and *n*=1032 controls [199 (19%) schizophrenia risk allele carriers] from the same catchment area, and the association still remains significant (*p*=0.006; OR 1.56, 95% CI 1.13–2.14; A. Reif et al. unpublished data). Furthermore, this finding was recently replicated in a Japanese sample consisting of 343 patients (*p*=0.001) (Cui et al. 2010). NOS1 exon 1c is expressed in high levels in the frontal cortex (Reif et al. 2006) in GABAergic interneurons, and the hippocampus, where NO functions as the second messenger of the NMDA receptor. As the schizophrenia risk allele of rs41279104 results in a decrease of reporter gene expression, presence of this allele might also go along with decreased expression of the alternative first NOS1 exon 1c. Such reduction of NOS1 expression might contribute to glutamatergic dysfunctions found in schizophrenia: in the hippocampus, where NO is the second messenger of glutamate, it might directly result in a compromised function of these neural circuits (*hypo*-active state of glutamatergic hippocampal neurons). On the other hand, in the prefrontal cortex, NO has a different role as it inhibits GABAergic interneurons. By reduced inhibition, presence of the schizophrenia risk allele thus might result in a *hyper*-active state of glutamatergic neurons in the prefrontal cortex by disinhibition.

Most interestingly, we were able to demonstrate that the schizophrenia risk allele is associated with fewer omission errors in the continuous performance test, i.e. improved sustained attention (Reif et al. 2006). This was paralleled by reduced P300 latencies suggesting an increased information-processing speed. To further explore the relationship between this genetic schizophrenia risk variant and prefrontal functioning in schizophrenia, we investigated patients suffering from chronic schizophrenia and controls by means of functional near-infrared spectroscopy (fNIRS) while performing two cognitive tasks known to be affected by disease (verbal fluency task, VFT, and the working-memory 2-back task). NIRS is an optical approach to measure brain activation as indicated by changes in blood oxygenation, i.e. based on the same haemodynamic response function assessed by functional magnetic resonance imaging (fMRI). Several studies showed the reliability (e.g. Plichta et al. 2007; Scheichmann et al. 2008) and the validity of fNIRS (correlation of fNIRS with fMRI signals; e.g. Huppert et al. 2006; for an overview see Steinbrink et al. 2006), as well as its suitability for application in psychiatric samples. We report on the largest schizophrenia cohort so far assessed by fNIRS, and also on the first fNIRS investigation during the 2-back task in schizophrenia. Since lateralization effects have been shown particularly for the VFT (Herrmann et al. 2006) and reduced or abnormal hemispheric lateralization has been reported for schizophrenic illnesses (Artiges et al. 2000; Weiss et al. 2004, 2006), schizotypy (Hori et al. 2008), and individuals genetically at risk for schizophrenia (Bhojraj et al. 2009), we considered possible lateralization effects throughout our functional imaging data, including the factor hemisphere in all corresponding analyses.

**Methods and materials**

**Subjects**

Altogether, 43 unrelated schizophrenia patients from the Lower Franconia area in Germany participated. All patients were recruited from the Department of Psychiatry, Psychosomatics and Psychotherapy,
University of Würzburg. The patients were suffering from schizophrenia according to ICD-10 criteria. None of the subjects remitted completely during the course of the disease and thus the sample consisted of patients suffering from chronic schizophrenia, i.e. severe cases. None of the subjects showed significant neurological comorbidity, mental retardation or other somatic disorders suggesting organic psychiatric disorder. Diagnoses were made by an extensive, semi-structured interview analogous to the AMDP interview (Arbeitsgemeinschaft, 2000) performed by an experienced psychiatrist. Furthermore, 44 controls from the same catchment area participated. Individuals were screened for a history of psychiatric disorders, with history for any Axis-I diagnosis or use of psychotropic medication being an exclusion criterion. In patients, there was no difference in the use of antidepressants, first- and second-generation antipsychotics, or benzodiazepines in AG/AA carriers compared to GG carriers (data not shown). Patients and controls were of Caucasian origin.

Four controls and five patients were excluded due to noisy fNIRS signals, two controls due to failed genotyping and 10 controls and 12 patients as a consequence of the genotype group matching for age, handedness and gender (Table 1), resulting in an experimental group of 28 controls and 26 patients. Diagnostic and genotype groups (AG/AA and GG) were comparable for age, gender, handedness, intelligence, and education level, except for diagnostic groups which differed significantly in education level and intelligence according to the Multiple choice vocabulary test (Mehrfachwahl-Wortschatztest – version B; Lehrl et al. 1995) (Table 2). Only four patients had an onset of illness > 40 yr indicating late-onset schizophrenia (Rajji et al. 2009), i.e. we investigated early-onset schizophrenia patients and our findings should not be affected by the influence of late-onset schizophrenia. Nonetheless, we inspected whether onset or duration of illness differed between the genotype groups and found no statistical difference (duration: \( t = 1.33, \text{d.f.} = 24, p = 0.197 \); onset: \( t = 1.07, \text{d.f.} = 24, p = 0.295 \)). Only individuals who gave written consent after oral as well as written explanation about the investigation were enrolled. The study was approved by the Ethics Committee of the University of Würzburg, and was in accordance with the Declaration of Helsinki.

Genotyping

rs41279104 was determined as described previously (Reif et al. 2006) by standard polymerase chain reaction. The following primers were used to amplify a region of 150 bp: 5’-CTGACTGCCCCCTTGTCTCCTC-3’ and 5’-GGGACTGGGGTTAATTGAC-3’. PCR product was digested with Fnu4HI (New England Biolabs, USA) at 37°C. Fragments were visualized on an agarose gel stained with ethidium bromide.

**VFT**

All participants performed the letter (phonological) version of the VFT and a weekday control task as described in our previous publications (e.g. Ehils et al. 2007; Schecklmann et al. 2007). For the fluency task condition, participants were instructed to name as many nouns as possible beginning with a certain letter (A, F, S); for the control task, they had to name the weekdays. Both tasks were conducted alternately in a block-wise fashion. Each task comprised three 30-s active-task periods that were separated by 30-s resting blocks, during which participants were instructed
to sit still. Additionally, a 10-s baseline period preceded the first segment. The investigator recorded the number of correctly generated words and weekdays.

2-back test

All subjects performed two versions of a letter n-back task (cf. Ehlis et al. 2008). For the 1-back condition [low working-memory (WM) load], they were instructed to press a response button, whenever a letter presented on a screen was identical to the preceding letter. For the 2-back condition (high WM load), they had to respond whenever a letter was identical to the one two trials before. The tasks were performed alternately in a block-wise fashion and separated by 30-s resting segments during which participants were instructed to sit still. Letters were presented in pseudorandomized order with a presentation time of 300 ms and an interstimulus-interval of 1700 ms. Both task conditions were conducted three times each, resulting in three 30-s task segments for both paradigms. A 10-s baseline period preceded the first task. For both tasks, a total of 12 target trials appeared. The number and reaction times (RTs) of correct responses were analysed.

fNIRS

fNIRS measurements were conducted with the ETG-4000 Optical Topography System (Hitachi Medical Co., Japan) using two 22-channel arrays of optodes, covering frontal areas on the left and right side of the head (12 cm each, inter-optode distance 30 mm). The arrays were localized by placing the penultimate optode of the bottom row onto T3/T4 on the left and right side of the head, respectively, and orienting the array towards Fp1/Fp2 (according to the International 10/20 system for electrode placement). The fNIRS probe set therefore covered lateral parts of the fronto-temporal cortex (Fig. 1). Both arrays consisted of eight light emitters and seven photo-detectors. The laser diodes emitted light of two wavelengths, modulated at a particular frequency for each wavelength and channel (695 ± 20 nm and 830 ± 20 nm; optical intensity 2.0 mW per wavelength). Signals were measured simultaneously with a sampling rate of 10 Hz, and analysed and transformed according to their wavelength and location using a modified Beer–Lambert equation for highly scattering media. This resulted in estimated changes in oxy-haemoglobin (O₂Hb) and deoxy-haemoglobin (HHb) concentration for every fNIRS channel.

Table 2. Descriptive data of the analysed groups

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<th>Patients</th>
<th>Controls</th>
<th>Analysis of variance (d.f. = 1, 50)</th>
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<td>AG/AA (n = 9)</td>
<td>GG (n = 17)</td>
<td>AG/AA (n = 12)</td>
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<td>Age (yr)</td>
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<td>Gender (female/male)</td>
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a According to the Multiple choice vocabulary test [Mehrfachwahl-Wortschatztest – version B (MWT-B); Lehrl et al. 1995].
Data analysis and statistics

Slow changes in the fNIRS signal unrelated to functional stimulation were removed by a linear fitting procedure, using a pre-stimulus baseline (the mean across 10 s before the active-task period) and a post-stimulus baseline (the mean across the last 10 s of the 20-s rest segment). This procedure is recommended for removing long-lasting physiological effects unrelated to brain activity due to functional stimulation (see Ehlis et al. 2009). According to the time intervals of the linear fitting procedure for the three repetitions of each task condition, data were averaged according to the specific task condition (2-back, 1-back, VFT, weekday naming) channel-wise for each participant.

For statistical analyses ‘mean activation values’ of O$_2$Hb and HHb were calculated for the 2-back and 1-back versions of the WM task, as well as for the letter fluency and the control task weekday naming in the VFT. Activation segments were defined with respect to the beginning and end of the 30-s stimulation (task) periods. Mean values of the activation periods were then contrasted between active tasks (VFT and 2-back) and corresponding control tasks (weekday naming and 1-back) via t tests. We defined regions of interest (ROIs) as channels showing significant contrasts simultaneously for both O$_2$Hb and HHb within the group of healthy controls (Schecklmann et al. 2008). Active channels of the patient group were solely found within these ROIs (cf. Fig. 1). For further analyses, we calculated mean differences between active and control tasks for the channels of the ROIs, in order to eliminate unspecific activation (e.g. resulting from simple psychomotor processes) and focus on core processes involved in the executive functions of WM and verbal fluency.

Fig. 1. T-maps of brain activation (mean amplitude during task activation contrasted against control tasks).
Despite our definition of task-relevant areas (ROIs) based on signal changes of both O$_2$Hb and HHb, all subsequent (within- and between-subject) analyses were separately conducted for both NIRS parameters. We did this for several reasons: first, even though in functional NIRS data neuronal activation is generally reflected by an increase of O$_2$Hb with a corresponding decrease in HHb concentration, both parameters differ regarding the stability of this finding, sometimes with paradoxical signal changes for HHb, but not O$_2$Hb concentrations (Hoshi et al. 2001; Yamamoto & Kato, 2002). Moreover, while O$_2$Hb has a better signal-to-noise ratio and has been suggested to be the most sensitive indicator of changes in rCBF in functional NIRS measurement (Hoshi, 2007), HHb appears to be more spatially localized, i.e. HHb findings seem to show a more pronounced regional specificity (see Hirth et al. 1996). Finally, inconsistent findings have been reported regarding the correlation of fMRI blood oxygen level-dependent (BOLD) signal and NIRS signal changes. However, several studies found a stronger correlation between HHb and BOLD signal changes (Huppert et al. 2006; Toronov et al. 2001, 2003), while other studies report the strongest fNIRS–BOLD correlations for O$_2$Hb (Strangman et al. 2002), so the relative validity of the two NIRS signals has yet to be determined. Based on these different strengths and weaknesses of both NIRS parameters, we decided to define the ROIs based on signal changes of both O$_2$Hb and HHb to obtain NIRS channels that are considered to be associated with task-related activity with the highest probability. According to the afore-mentioned suggestions, we decided to separately analyse and report concentration changes of O$_2$Hb and HHb, which is also in line with the general convention in fNIRS literature.

Furthermore, we conducted 2 × 2 ANOVAs (diagnosis: patients vs. controls; genotype: AG/AA vs. GG) for behavioural data, and 2 × 2 × 2 ANOVAs for fNIRS data (additional factor ‘hemisphere’: left vs. right). Two-tailed $t$ tests for independent samples and additional ANOVAs were used for post-hoc analyses. In an additional exploratory analysis, we correlated behavioural data of the WM task and VFT using parametric (Pearson) or non-parametric (Spearman) measures as appropriate. Similarly, behavioural data of both tasks were correlated with task-related changes in O$_2$Hb and HHb. Data analyses and statistical calculations were performed with MatLab 6.5 (The MathWorks Inc., USA) and SPSS 15.0 (SPSS Inc., USA). All reported statistical values are uncorrected $p$ values.

**Results**

ROIs during N-back comprised nine channels in the left (channels 1, 2, 3, 4, 6, 7, 8, 11, 12) and eight channels in the right (channels 1, 2, 3, 4, 6, 7, 8, 12) probe set; for the VFT, ROIs consisted of 11 channels on the left (channels 2, 3, 4, 6, 7, 8, 9, 11, 12, 13, 17) and six channels on the right (channels 2, 3, 4, 6, 7, 8) side of the measurement array. These channels (Fig. 1) showing simultaneous increases of O$_2$Hb and decreases of HHb in the control sample are located over fronto-temporal areas, in accordance with previous findings (Ehls et al. 2008; Scheckmann et al. 2007).

For verbal fluency, we found significant diagnosis main effects for the number of nouns generated during the fluency task ($F = 10.587$, d.f. = 1, 50, $p = 0.002$), the control task ($F = 5.884$, d.f. = 1, 50, $p = 0.019$), and for O$_2$Hb ($F = 16.226$, d.f. = 1, 50, $p < 0.001$) and HHb ($F = 21.419$, d.f. = 1, 50, $p < 0.001$), indicating diminished verbal fluency functioning in schizophrenia (due to reduced performance and brain activity). For HHb, we furthermore found a statistical trend for a hemisphere × genotype interaction effect ($F = 3.498$, d.f. = 1, 50, $p = 0.067$) and a significant hemisphere × diagnosis × genotype interaction ($F = 7.272$, d.f. = 1, 50, $p = 0.010$). O$_2$Hb showed a hemisphere × genotype interaction with a statistical trend ($F = 3.529$, d.f. = 1, 50, $p = 0.066$). Post-hoc analyses for the hemisphere × genotype effects for HHb and O$_2$Hb revealed no significant results. Post-hoc tests for the three-way interaction (group × hemisphere × genotype) showed that the hemisphere × genotype interaction effect reached statistical significance only for patients ($F = 7.675$, d.f. = 1, 24, $p = 0.011$): within the group of schizophrenia patients, carriers of the AG/AA genotype showed higher deactivation (increase of HHb) over the right compared to the left hemisphere, they also showed a more pronounced deactivation within the right hemisphere in contrast to carriers of the GG genotype (Fig. 2b). Exploratorily, we conducted the same post-hoc analyses for O$_2$Hb and found again a borderline significant hemisphere × genotype interaction effect only for patients ($F = 4.255$, d.f. = 1, 24, $p = 0.051$), indicating higher right hemispheric deactivation (decrease of O$_2$Hb) for AG/AA genotype carriers (Fig. 2a).

For the WM task, we found significant diagnosis main effects for the RTs of correct answers during 2-back ($F = 43.249$, d.f. = 1, 50, $p < 0.001$). Furthermore, during the 1-back test we found significant diagnosis main effects for the proportion of correct answers ($F = 4.435$, d.f. = 1, 50, $p = 0.040$) and RTs for these correct answers ($F = 27.901$, d.f. = 1, 50, $p < 0.001$), as
well as for O$_2$Hb ($F = 11.728, \text{d.f.} = 1, 50, p = 0.001$) and HHb ($F = 6.559, \text{d.f.} = 1, 50, p = 0.014$), indicating diminished WM functioning in schizophrenia (reduced performance and brain activity). For RTs during 2-back, the main effect of genotype ($F = 4.240, \text{d.f.} = 1, 50, p = 0.045$) and the interaction effect of diagnosis $\times$ genotype ($F = 5.787, \text{d.f.} = 1, 50, p = 0.020$) were significant. Post-hoc tests revealed overall longer RTs for rs41279104 AG/AA carriers ($628.86 \pm 222.80$ ms) compared to GG carriers ($579.90 \pm 132.87$ ms). Regarding the significant interaction, this slowing of RTs in carriers of the A allele was only statistically present (as a trend) in the group of patients ($t = 2.06, \text{d.f.} = 11, p = 0.065$, see Fig. 2c), whereas controls showed equally long RTs for both genotype groups ($t = 0.36, \text{d.f.} = 26, p = 0.72$).

We calculated ANOVAs similar to the analyses reported above; first, with intelligence quotient as covariate; second, with level of education as covariate, as our groups were not matched for intelligence and level of education. Third, we performed the original ANOVAs with the originally collected sample, as we eliminated 17 patients and 16 controls from our original sample for reasons of comparability for age and gender. These ANOVAs produced results comparable to the findings reported above, i.e. significant results of the above reported analyses remained significant or showed at least statistical trends.

For the VFT, we did not find any significant correlations between behavioural measures and functional NIRS data. For the n-back task, the number of correct responses during 2-back was significantly correlated with changes in O$_2$Hb (left ROI: $r = -0.370, p = 0.052$; right ROI: $r = -0.390, p = 0.040$) as well as HHb (right ROI: $r = 0.353, p = 0.065$) in controls; similarly, in the group of patients the number of correct 2-back responses was significantly correlated with changes in HHb within the right ROI ($r = 0.407, p = 0.039$). Additionally in patients, the RT during 1-back was significantly correlated with changes in HHb (left ROI: $r = -0.446, p = 0.022$; right ROI: $r = -0.458, p = 0.019$).

**Discussion**

In this paper we investigated the influence of a functional promoter polymorphism of the NOS1 gene on prefrontal functioning in schizophrenia. To this end, patients as well as controls executed two well-established cognitive tasks while functional NIRS was recorded. As expected, patients suffering from schizophrenia performed worse indicating deficits in prefrontal functioning. This is in line with the ‘hypofrontality hypothesis of schizophrenia’, suggesting impairment of the prefrontal cortex in schizophrenia. While this has been established by fMRI, featuring a relatively small number of patients, the method of fNIRS has not been employed frequently and this is the first study examining both paradigms in a rather large sample. As fNIRS has many advantages over fMRI (e.g. ecological validity, cost-effectiveness, and less susceptibility towards movement artifacts), it might constitute an attractive alternative to fMRI.

Compared to the control group, the sample of schizophrenia patients displayed reduced activation of the lateral prefrontal cortex as shown by decreased blood oxygenation during both tasks (cf. Fig. 1 and diagnosis effects of ANOVAs). This is in line with behavioural deficits reported above and previous neuroimaging studies demonstrating diminished activation of the frontal cortex in schizophrenia during processes of WM and executive control, including VFTs (for a previous VFT-NIRS study conducted at our laboratory see Ehlis et al. 2007). Moreover, they indicate an insufficient recruitment of frontal lobe
structures that are usually found to be involved in tasks of executive control. The latter interpretation is in line with the hypofrontality concept of schizophrenia (Ingvar & Franzen, 1974) and studies reporting altered prefrontal activation in schizophrenia even when behavioural performance was matched (Barbalat et al. 2009). It should be noted that the lack of a correlation between prefrontal cortex activation as assessed by our NIRS data and VFT performance measures does not contradict this general interpretation. In contrast to the process WM, which is a relatively distinct neuropsychological function, several different cognitive components contribute to word production in general (Levelt, 1999) with a distributed set of brain regions underlying the different aspects of word comprehension and generation (Price, 1998). More specifically, VFT-like tasks have been shown to activate a broad network of brain structures including (but not limited to) the lateral prefrontal cortex, the middle and inferior temporal gyri as well as striatal and/or extra-striatal areas (e.g. Friedman et al. 1998). Therefore, a significant part of the variance underlying the overt VFT performance might be attributed to brain regions outside our measurement area, possibly accounting for the non-significant correlation between VFT performance measures (i.e. the number of generated words) and lateral prefrontal cortex activation, even though both dorsolateral prefrontal cortex activation and fluency performance were significantly compromised in our sample of schizophrenia patients.

However, the molecular mechanisms of disturbed prefrontal functioning in schizophrenia are elusive. Several genes such as COMT (Egan et al. 2001) and GRM3 (Egan et al. 2004; Tan et al. 2007) have been suggested to influence prefrontal brain functions. Here we have focused on a functional promoter variant of NOS1; this gene has repeatedly been suggested to be associated with schizophrenia (Cui et al. 2010; Donohoe et al. 2009; Fallin et al. 2005; Reif et al. 2006; Tang et al. 2008), and specifically the promoter region appears to drive this association. In the present study, we have further investigated this variant and found that – within the group of patients – the risk allele resulted in higher right-hemispheric deactivation during the VFT (increases in HHb, and decreases in O₂Hb, indicating reduced brain activation) and slower RT in the 2-back test, while no changes in brain activation or behaviour as function of genotype were observed in controls.

Why is the AG/AA genotype of the rs41279104 associated with performance deficits during WM but altered cortical activation during verbal fluency?, and why are cortical alterations restricted to the right hemisphere? First of all, the n-back task and the VFT differ regarding the specific structures and functions involved. In contrast to the VFT, the letter n-back task requires only limited language functions. Moreover, verbal fluency performance involves the search for semantic or memory associations. Retrieval of this kind of memory seems to depend on correct functioning of the hippocampus (Kircher et al. 2008). We reported a higher expression of the NOS1 exon 1c in the hippocampus in contrast to the frontal cortex (Reif et al. 2006), i.e. possible genotype effects might be accentuated in the hippocampus. This might explain the effect of the genotype on brain activity during verbal fluency.

Regarding the lateralization of the NOS1 exon 1c effect with a right-hemispheric deactivation in AG/AA carriers, schizophrenia patients have been reported to show a diminished hemispheric asymmetry for tasks usually lateralized to the left side of the brain, with indications for an additional recruitment of the right hemisphere especially during language tasks such as the VFT (Hori et al. 2008; Sommer et al. 2001; Weiss et al. 2004, 2006). In our sample, patients showed overall diminished prefrontal functions (performance and brain activity), and right-hemispheric compensation mechanisms seemed to fail especially for the AG/AA genotype, i.e. the risk genotype. In contrast, the genotype effect during WM was restricted to a relatively global measure of performance (RT) with no apparent correlate in cortical brain activation. This indicates that – while the genotype effect on brain activation during verbal fluency might reflect a failed laterality compensation effect perhaps modulated by hippocampus functioning – the AG/AA genotype effect on WM performance represents a more general effect not specifically related to the frontal lobe aspect of the WM task.

The risk allele of rs41279104 results in about a 30–50% decrease of exon 1c expression (Cui et al. 2010; Saur et al. 2004), probably paralleled by a proportional up-regulation of NOS1 exon 1f (Saur et al. 2004). As exon 1c is the predominant isoform in the frontal cortex, this will either result in decreased total NOS1 expression or in a change in alternative first exon expression patterns. As Cui and co-workers assessed total NOS protein levels, and found that the amount of NOS-I protein is reduced by 50% in risk allele carriers (Cui et al. 2010), a decrease in total NOS1 expression seems to take place. Nevertheless, both scenarios will probably result in compromised prefrontal NO signalling which has already been implicated in cognitive deficits in schizophrenia. Inhibition of NOS resulted in normalization of PCP-induced WM deficits (Wass et al.
et al. (2009). As there is now solid evidence that NOS1 is associated with schizophrenia (Cui et al. 2009), which might also provide a rationale for the finding that activation of prefrontal GABA(B) receptors synergistically with NOS inhibition prevented PCP-induced PPI disruption. Intriguingly, malformation of these neurons was demonstrated in schizophrenia (Fejgin et al. 2009) further reinforcing the notion of disturbed prefrontal NO neurotransmission playing a role in schizophrenia.

In line with the data from animal studies outlined above, several studies have reported an impact of genetic variation in NOS1 on cognitive measures. Following our previous study (Reif et al. 2006) demonstrating that rs41279104 and a functional NOS1 promoter repeat coincide with changes in prefrontal functioning and disease severity, respectively, another group was able to demonstrate in two large independent samples that an intronic polymorphism in NOS1 (rs6490121), which was shown to be associated with schizophrenia by means of a genome-wide association study (O’Donovan et al. 2008) also affects cognition, i.e. the schizophrenia risk variant was associated with worse performance in verbal IQ and WM, in both schizophrenia and healthy subjects (Donohoe et al. 2009). As there is now solid evidence that NOS1 is associated with schizophrenia (Cui et al. 2010; Fallin et al. 2005; O’Donovan et al. 2008; Reif et al. 2006), the data presented here suggest that genetic alterations of the NO system may be connected to this disorder by moderating cognitive deficits probably due to malactivation of the prefrontal cortex as evidenced by fNIRS. However, the precise neural correlates remain elusive, and in this respect replication is key as there still remains the possibility of false-positive findings which are easily obtained due to multiple testing in this kind of study. However, strict correction for multiple testing would render the results non-significant due to the small effect sizes and the relatively small number of subjects available for neuroimaging studies. Nevertheless, we are confident of not reporting a chance finding due to the consistency of the data. However, further examination of the nitrinergic prefrontal neurocircuitry in schizophrenia is clearly warranted to corroborate our findings and to clarify the role of NO in schizophrenia and cognition.

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Statement of Interest

None.

References


