

A NOS1 Variant Implicated in Cognitive Performance Influences Evoked Neural Responses During A High Density EEG Study of Early Visual Perception

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Abstract: *Background:* The nitric oxide synthase-1 gene (*NOS1*) has been implicated in mental disorders including schizophrenia and variation in cognition. The *NOS1* variant rs6490121 identified in a genome wide association study of schizophrenia has recently been associated with variation in general intelligence and working memory in both patients and healthy participants. Whether this variant is also associated with variation in early sensory processing remains unclear. *Methods:* We investigated differences in the P1 visual evoked potential in a high density EEG study of 54 healthy participants. Given both *NOS1*'s association with cognition and recent evidence that cognitive performance and P1 response are correlated, we investigated whether *NOS1*'s effect on P1 response was independent of its effects on cognition using CANTAB's spatial working memory (SWM) task. *Results:* We found that carriers of the previously identified risk "G" allele showed significantly lower P1 responses than non-carriers. We also found that while P1 response and SWM performance were correlated, *NOS1* continued to explain a significant proportion of variation in P1 response even when its effects on cognition were accounted for. *Conclusion:* The schizophrenia implicated *NOS1* variants rs6490121 influences visual sensory processing as measured by the P1 response, either as part of the gene's pleiotropic effects on multiple aspects of brain function, or because of a primary influence on sensory processing that mediates the effects already seen in higher cognitive processes. *Hum Brain Mapp* 33:1202–1211, 2012. © 2011 Wiley Periodicals, Inc.

Key words: visual evoked potentials; working memory; *NOS1* gene; schizophrenia

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INTRODUCTION

Nitric oxide (NO) is a highly reactive messenger molecule, which diffuses freely across membranes stimulating guanylyl cyclase and modifying protein structure with multiple roles in immune, cardiac, and neurological function. NO stimulates synthesis of cGMP and strongly influences glutamate neurotransmission via *N*-methyl-D-aspartate (NMDA) receptor interaction [Akyol et al., 2004; Brenman and Brecht, 1997]. NO is also involved in uptake, release and storage of other CNS neurotransmitters including acetylcholine, dopamine, noradrenaline, and GABA [Boehning and Snyder, 2003; Pepicelli et al., 2004]. Abnormal distribution of nitrinergic neurons in frontal and temporal lobes in schizophrenia (SZ) [Akbarian et al., 1996], increased NO metabolites in the serum of patients with SZ [Das et al., 1995; Taneli et al., 2004; Yilmaz et al., 2007], and postmortem increased *NOS1* messenger RNA in prefrontal cortex of patients [Baba et al., 2004] collectively suggest a functional role for NO in abnormal signaling. NO is produced by different nitric oxidase synthetase (NOS) enzymes including neuronal NOS and transported to different cellular compartments by adaptor proteins to minimize non-specific interactions. Neuronal nitric oxide synthase (nNOS) accounts for 90% of nitric oxide (NO) in the central nervous system, production of which is dynamically controlled both during development and in response to brain injury.

The nitric oxide synthase-1 gene (*NOS1*; OMIM 163731), encoding nNOS and mapping to 12q24, shows some evidence of association with risk for psychiatric disorders. In schizophrenia, *NOS1* falls within a region showing modest evidence of linkage to schizophrenia [Abkevich et al., 2003; Bailer et al., 2000, 2002; DeLisi et al., 2002]. Four of five published *NOS1* candidate gene association studies in schizophrenia suggest evidence of association [DeLisi et al., 2002; Fallin et al., 2005; Reif et al., 2006; Shinkai et al., 2002; Tang et al., 2008], the exception being Liou et al. [2003]. Molecular pathway analysis of structural variants implicated in SZ by Walsh et al. [2008], identified a significant excess of disrupted genes involving the NO signaling pathway. In their SZ genome-wide association study (GWAS), O'Donovan et al. [2008] identified a single-nucleotide polymorphism (SNP) at the *NOS1* locus (rs6490121) as being 1 of 12 SNPs with strong initial statistical evidence for association ($P = 9.82 \times 10^{-6}$). The same allele at this SNP was significantly associated in a replication sample of 1,664 cases and 3,541 controls of European ancestry but not in a sample of mixed European and Asian ancestry and not in subsequent schizophrenia GWAS. Three further replication studies have been reported for rs6490121, one reporting a positive association in an Asian sample [Cui et al., 2010] and two reporting negative associations in European and Asian samples, respectively [Okumura et al., 2009; Riley et al., 2009].

Although the role of *NOS1* in schizophrenia susceptibility is uncertain, more consistent evidence of association

with variation in cognitive function in both animal and human studies has been reported. In mouse models, *NOS1* knockouts have repeatedly been associated with variance in cognition [Kirchner et al., 2004; Weitzdoerfer et al., 2004]. Notably, phencyclidine hydrochloride-induced cognitive and behavioral deficits that model SZ symptoms (including pre-pulse inhibition, habituation of acoustic startle, latent inhibition, spatial learning, spatial reference memory, and working memory) can all be prevented by interfering with the production of NO [Johansson et al., 1997, 1998; Klamer et al., 2001, 2004a,b, 2005; Pålsson et al., 2007; Wass et al., 2006]. In patients with SZ, Reif et al. [2006] reported that two of four genetic markers tested at the *NOS1* locus were associated with variance in performance on measures of prefrontal function (the Continuous Performance Task, P300 peak amplitude, and response latency). We recently found that the risk "G" allele at the *NOS1* SNP rs6490121 identified by O'Donovan et al., is associated with significantly poorer performance in measures of both verbal intelligence and working memory in both patients with schizophrenia and healthy controls; findings which we replicated in independent samples of German patients and controls [Donohoe et al., 2009]. Based on this evidence, we concluded that *NOS1*'s association with SZ may reflect this gene's broader role in cognition.

A critical question for cognitive neuroscience regards how individual genes contribute to variation in cognitive function. Among several possibilities (e.g., impact on grey matter volume, white matter structure, white matter integrity), one hypothesis relevant to SZ is that genetic variants impact on cognitive ability via an influence on sensory level processing. In schizophrenia, observed deficits in sensory level processing [Butler et al., 2007; Foxe et al., 2001] are predicted to lower signal-to-noise ratio and increase the cognitive demands and errors made during cognitive task performance [Butler et al., 2007]. Javitt [2009] has suggested that deficits in encoding both auditory and visual information, as measured by sensory evoked potentials such as the P50, N1, P1, and the MMN may contribute to a variety of higher-level difficulties in SZ, including phonetic processing and facial recognition. Supporting this theory there is already evidence that at least one SZ candidate gene (DTNBP1) is associated both deficits in higher cognitive functions and deficits in early visual processing [Donohoe et al., 2007, 2008]. Whether this represents the "bottom up" effects of DTNBP1 on cognition, or multiple pleiotropic effects on sensory and cognitive processing [Donohoe et al., 2009] remains unclear.

In the present study we examined whether the *NOS1* SNP rs6490121, previously associated with variation in intelligence and working memory, was also associated with variation in early sensory processing as measured by the P1 component of the visual evoked potential (VEP). Specifically, we tested the hypothesis that the risk "G" allele at rs6490121 would be associated with decreased amplitude of the P1. We further sought to investigate

whether this response in turn predicted variation in cognitive ability, based on the SWM task employed in our previous neuropsychological study of *NOS1*. Empirical evidence that the amplitude of the visual P1 response can partially predict SWM response has recently been reported [Haenschel et al., 2009]. Finally, we sought to determine if the P1 response mediated the relationship between *NOS1* and SWM (a “bottom up” effect) or, alternatively, if the effects of *NOS1* on SWM had a “top down” effect on the P1 such that the relationship between *NOS1* and either the P1 or SWM disappeared after the effects of the other had been accounted for.

MATERIALS AND METHODS

Participants

Informed consent was obtained from 54 participants, Aged 18–60 who satisfied the criteria of having (a) no history of psychosis (based on clinical interview), (b) no history of head injury or loss of consciousness; (c) no history of drug or alcohol abuse, (d) Irish descent (Irish parents and grandparents on both sides), (e) no first degree relative with an Axis I Diagnosis (DSM-IV); and (f) no current cannabis abuse or history of drugs or alcohol abuse. None of the controls were on psychotropic medication at the time of testing. All participants had been included in our original neuropsychological study of *NOS1* [Donohoe et al., 2008] and represented those who, when re-contacted, were consenting and available to participate in an EEG assessment.

Presentation

Participants were seated in a comfortable chair in a dimly lit room, 110 cm from the computer screen. Stimuli were presented with “Presentation” (version 14.2 Neurobehavioral Systems). For the P1 paradigm, which was divided into a series of 3-min blocks to allow resting periods, participants were presented with isolated-check images containing an 8×8 matrix of checks (7.3° wide by 7.3° tall at 64% contrast, 100 per block), and line drawings of two kinds of animals (5.2° wide by 3.6° tall; 40 per block) on a white background [Yeap et al., 2006]. The 64% contrast condition was chosen to stimulate both the magnocellular and parvocellular systems. Each image appeared for 60 ms with a variable inter-stimulus-interval (ISI) between 740 and 1,540 ms (randomly in steps of 200 ms) during which there was a blank white screen. The purpose of the target animal stimuli was to encourage participants to attend to the screen. Each block required participants to press the key-pad when they identified a target animal they were shown at the start of each block. Participants were directed to only respond to the target animal and refrain from responding to the non-target animal. Target and non-target animals were presented ran-

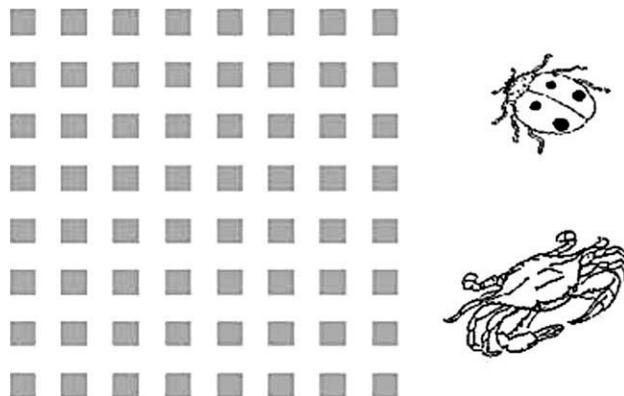


Figure 1.

The centrally presented visual stimuli used in each task. ERP waveforms were derived from the isolated check non-target stimulus whereas target discrimination was performed on the basis of infrequently presented animal line-drawings.

domly intermixed with the isolated-check stimuli, with both target and non-target animals appearing with equal probability. Each block contained a different animal pair (see Fig. 1) with each animal-pairing being somewhat similar to ensure the task was sufficiently challenging and to promote alertness. On average participants completed 9.62 blocks (SD 0.86).

Electrophysiological Data Acquisition

Continuous electroencephalographic (EEG) data were recorded to computer with the Biosemi Acquisition programme: ActiView/www.biosemi.com/. EEG was recorded using 128 scalp electrodes. Horizontal and vertical electro-oculograms were also recorded by means of electrodes placed at the left and right external canthi and an electrode below the left eye. Data were recorded continuously at a digitization rate of 512 Hz with an open pass-band. The Biosemi amplification system replaces the “ground” electrodes with two separate electrodes: common mode sense (CMS) active electrode and driven right leg (DRL) passive electrode (for more on the function of the CMS and DRL electrodes, see www.biosemi.com/faq/cms&drl.htm). For analysis and display purposes, data were subsequently filtered with a 0-phase-shift 40-Hz low-pass filter (48 dB/octave) after acquisition. No high pass filter was used. Only sweeps related to the isolated-check stimuli were included in the analysis.

Spatial Working Memory Assessment

All participants completed the SWM test from the Cambridge automated test battery (CANTAB Eclipse version, Cambridge Cognition, 2004) The touch screen computer task involves searching for “hidden” tokens in boxes whose number increases from trial to trial. Participants are

instructed to remember which box they visit as a token will never be hidden in the same box twice. An error is committed when a participant returns to a box location from which a token has already been recovered. The dependent variable was the total numbers of errors made. Participants also completed subtests from the Wechsler adult intelligence test (WAIS, 3rd edition) and the Wechsler Memory Scale (WMS, 3rd edition) to ensure that all participants' scored at or above the average range for IQ [see Donohoe et al., 2009].

Genetic Analysis

The SNP rs6490121 was genotyped using a Taqman[®] SNP genotyping assay on a 7900HT sequence detection system (Applied Biosystems). The call rate for the Taqman genotyping was 100% and samples were in Hardy-Weinberg equilibrium ($P > 0.05$). Along with these samples, a number of HapMap CEU DNA samples (www.hapmap.org) were genotyped for rs6490121 for quality control purposes and were all found to be concordant with available online HapMap data for this SNP. Only five participants were identified as GG genotype carriers (9.25% of sample). For statistical analyses, therefore, participants were grouped as GG carriers and AG genotype carriers ($n = 29$) versus AA genotype carriers ($n = 24$). Mean scores and standard deviations are also reported for each genotype group separately to provide evidence of allele dosage effects.

ERP Analyses

ERP analyses were performed using BESA Software Version 5.2. Any EEG channels which were noisy or which were not connected properly during recording were identified and switched off for further analysis. Across participants, the average number of channels excluded in this manner from analysis was 9.92 ± 5.35 . The surrogate model [Berg and Scherg, 1994] was then used for further artifact correction. Artifact correction in the current study was based on a model [Berg and Scherg, 1994; Lins et al., 1993] of artifact topography (the averaged artifact) and a set of brain topographies (multiple dipoles). The result was an estimation of artifact activation based on the linear combination of brain and artifact activities. Corrected-epoch data were also inspected for other artifacts using the BESA artifact rejection interface [Berg and Scherg, 1994]. Grand averages were generated for each participant from the isolated-check stimuli only. Approximately 654 ± 241 sweeps per individual were averaged for the AA group and 669 ± 262 for the GG + AG group with an epoch of -200 to 1,000 msec. The average number of bad channels for the AA group was 8.41 and 11.15 for the GG + AG group. The P1 was defined as the area under the curve (versus the 0- μ V baseline) generated by the 64% contrast isolated-checkerboard stimuli within the post-stimu-

lus window of 70–110 msec spanning the P1 component. For the baseline correction, a baseline between -200 and 0 msec was set. A set of six symmetrical pairs of scalp sites were chosen over occipital scalp sites from which P1 amplitudes were extracted (Left hemisphere: P1/P3/P03; Right hemisphere: P4/P6/P04). These sites were chosen based on topographical analysis of the grand-average group data which revealed lateral-occipital topographies consistent with those previously reported in the literature [e.g., Foxe and Simpson, 2002], for left and right hemispheres, respectively.

For statistical analyses, P1 measures were submitted to analysis-of-covariance (ANCOVA) using SPSS Software (SPSS, Chicago, IL Version 16.0) with the NOS1 genotypes (AA versus GG + AG) as the between subject factor and P1 response (both averaged across all six target electrodes and for left lateral occipital and right lateral occipital regions taken separately) as the within-subjects factor, with age and gender entered as covariates of no interest in the analysis. To further investigate possible relationships between P1 and SWM in relation to NOS1, multiple regression analysis was performed, first to examine whether the P1 response predicted SWM performance, and second to examine whether any relationship observed between NOS1 and P1 performance was independent of variance in the P1 due to variance in SWM.

RESULTS

Demographic and Behavioral Differences Associated With NOS1

As this study was based on an opportunistic sample of consenting individuals who were still available following our original neuropsychological study, NOS1 genotype groups were not matched in advance for age, years in education or gender. No differences in age, education, or handedness were observed (age: AA = 24.8 [SD = 12.45], AG/GG = 29.1 [SD = 12.45], $t = 0.69$; $P = 0.49$; years in education: AA = 16.0 [SD = 2.1], AG/GG = 16.3 [SD = 2.2]; $t = 0.46$; $P = 0.64$; handedness: AA: 21/22 right handed; AG/GG: 28/30 right handed; $\chi^2 = 0.15$; $P = 0.69$). Differences in gender were observed (AA: 10/24 male, AG/GG: 21/29 male; $\chi^2 = 7.41$; $P = 0.006$). Consequently, gender was used as a covariate in all subsequent analysis of ERP components; age was also included as a covariate of no interest in the analyses.

To ensure comparability between GG + AG "risk" and the AA "non-risk" genotype groups in attending to the visual P1 eliciting stimulus (checkerboard) we examined the reaction time and accuracy with which both groups identified the animal line drawings dispersed between the checkerboard stimuli. The mean reaction-time for the AA group was 432.92 (± 37.85) and for the GG + AG group was 430.54 (± 42.17). The mean rate of correct responses for the AA group was 192.16 (± 7.89) and for the GG +

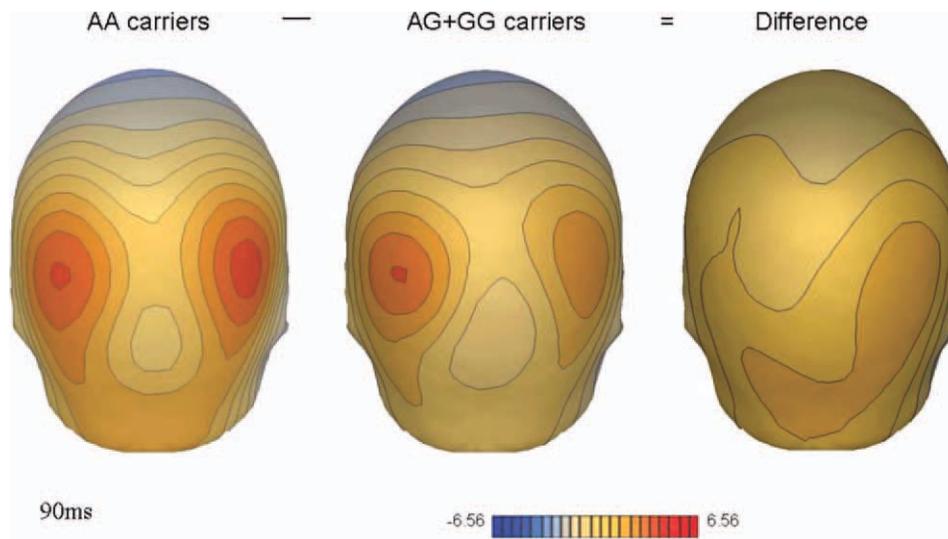


Figure 2.

Mapping of the difference topography associated with *NOS1* genotype. The grand averaged waveforms of each group were subtracted from one another to enable the difference effect to be illustrated.

AG group was $192.24 (\pm 12.95)$. The mean rate of incorrect responses to non-targets was $12.53 (\pm 6.66)$ for the AA group and $8.52 (\pm 4.24)$ for the GG + AG group. Between group differences on each of these metrics of performance were all non-significant (all P -values > 0.05). Collectively these data suggested that participants in both groups were equally engaged in the task, and given the high hit-rates, clearly focused their attention centrally toward the screen throughout each block presentation.

Differences in P1 VEP According to *NOS1* Genotype

Figure 2 shows the bilateral occipital distribution of the P1 in *NOS1* risk “GG + AG” and non-risk “AA” genotype groups. The map of the difference topography between these genotype groups (captured at maximal amplitude at 90ms) illustrates the reduction in P1 amplitude in the “GG + AA” group relative to the “non-risk” AA group. Figure 3 illustrates the individual P1 morphology for electrode sites included in the statistical analysis. At each site the “risk” GG + AG genotype group shows a reduced P1 response compared to the “non-risk” AA genotype group. Over the right lateral occipital region, where the P1 amplitude difference was maximal, the mean P1 amplitude was 147.25 ± 75.25 for the AA genotype group and 86.84 ± 52.42 for the GG + AG group. Figure 4 presents a scatterplot of P1 amplitudes measured at electrode sites included in the analyses (10/20 equivalents of: P1/P3/P03 and P4/P6/P04).

Reflecting these differences, a significant main effect of genotype group was observed, showing reduced P1 (measured as area under the curve) in the “risk” GG + AG genotypes

group compared to the “non-risk” AA genotypes group ($F(2,52) = 13.85$; $P = 0.001$). Differences associated with *NOS1* were found to be more robust over the right than the left hemiscalp [right: $F(3,52) = 16.73$, $P = 0.00016$]; [left: $F(3,52) = 3.14$, $P = 0.083$]. As mentioned the low frequency of GG carriers ($n = 5$) prevented a statistical analysis of GGvAGvAA groups separately. However, inspection of means and standard deviations across these groups suggested a gene dosage effect such that GG genotype individuals showed a less robust P1 evoked response than the AG group, who in turn showed a less robust P1 evoked response than the AA group for both hemiscalps (see Table I).

Group differences were also calculated for the N1 (97–185 msec) and P2 (160–300 msec). No significant differences were observed for either right or left hemisphere electrodes for these ERPs. Latency measures were also examined. The mean latency for AA carriers was 85.59 ± 12.32 and was 93.31 ± 12.92 for AG + GG carriers. These differences were not found to be significant [$F(1,50) = 3.30$, $P = 0.07$].

P1 VEP, *NOS1* Genotype, and SWM Performance

Given previous evidence of association between P1 and SWM performance, and evidence of association between *NOS1* and SWM in our previous study, we investigated whether P1 performance predicted SWM performance in the present study using regression analyses. For this analysis SWM task performance was entered as the dependent variable. Age and gender were entered on the first step of the equation as covariates of no interest and P1 performance (electrode sites for left and right hemiscalps averaged

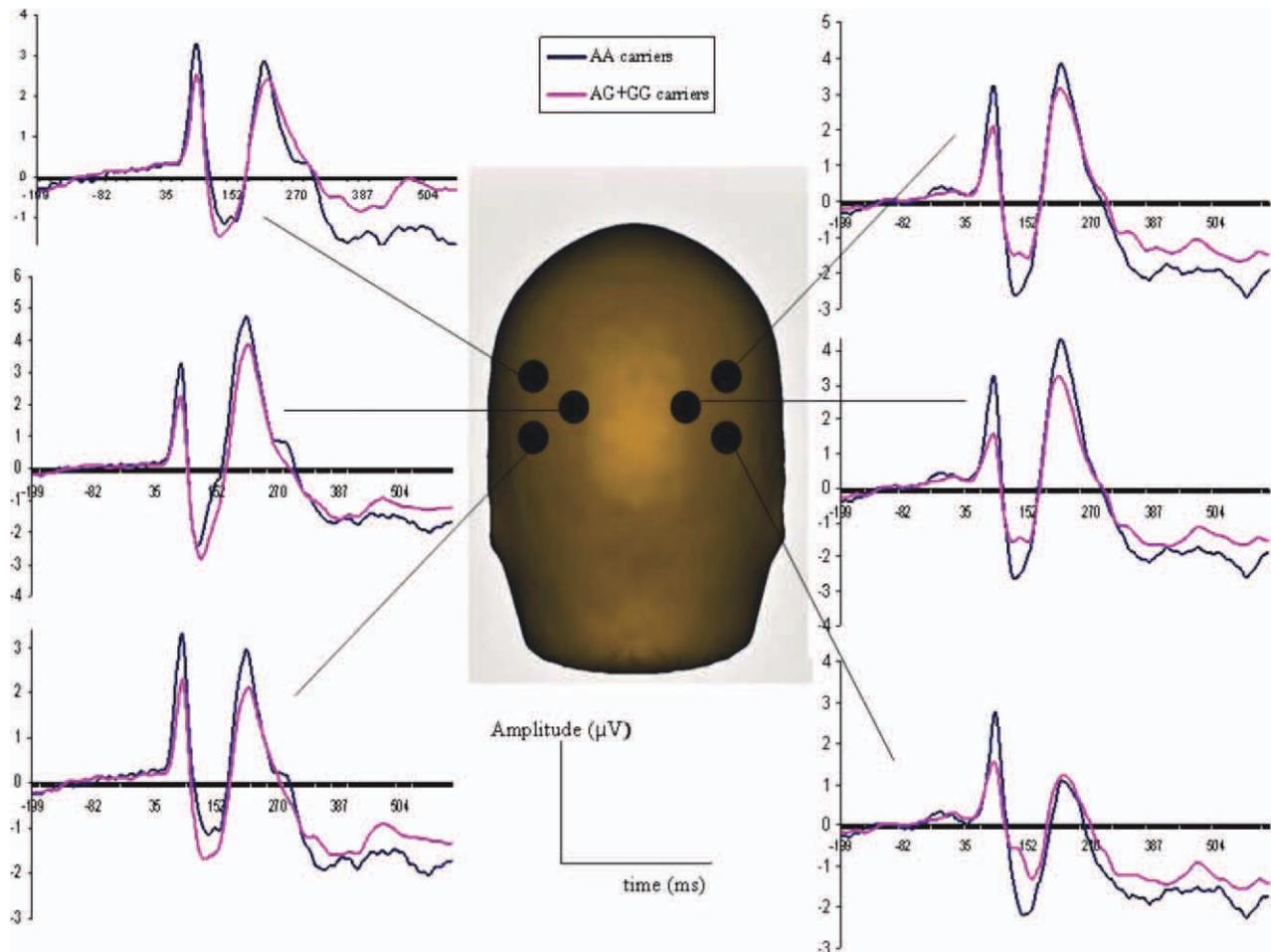


Figure 3.

ERP morphology across the scalp for both groups illustrating responses from electrodes at occipital scalp regions. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

together) was entered on the second step as the independent variable of interest. After the effects of age and gender were accounted for, P1 response explained a further 12.9% of variance in SWM performance (F change (1,40) = 7.74, $P = 0.009$).

NOS1 Effects on Sensory and Cognitive Processing: Top Down Versus Bottom Up Influences

We next determined, using a multiple regression analysis, whether *NOS1*'s observed influence on the P1 response might be accounted for by the previously observed influence of *NOS1* on SWM performance. To do this the P1 response was entered as the dependent variable, SWM performance as the independent variable in the first step of the analysis, followed by *NOS1* as the independent vari-

able in the second step. P1 response was again measured in terms of the area under the curve, based on the electrode site in which differences between *NOS1* risk carriers and non-carriers were maximal (i.e., right occipital electrodes P4/P6/P04). We reasoned that if the effects of *NOS1* on the P1 response were being mediated by SWM, *NOS1*'s effects on the P1 response would become non-significant once the variance attributable to SWM was accounted for. Instead, we found that even after accounting for the effects of SWM performance on the P1 response (which accounted for 26% of the variance in P1 response), *NOS1* independently explained a further 9% of variation in P1 response. ($R^2 = 0.35$; $F(1,40) = 5.04$, $P = 0.03$). This suggested that at least some of the effects of *NOS1* on P1 performance are independent of *NOS1*'s previously reported influence on SWM. We also intended to examine whether *NOS1*'s influence on SWM performance was mediated by P1 response. Unfortunately, we were prevented from

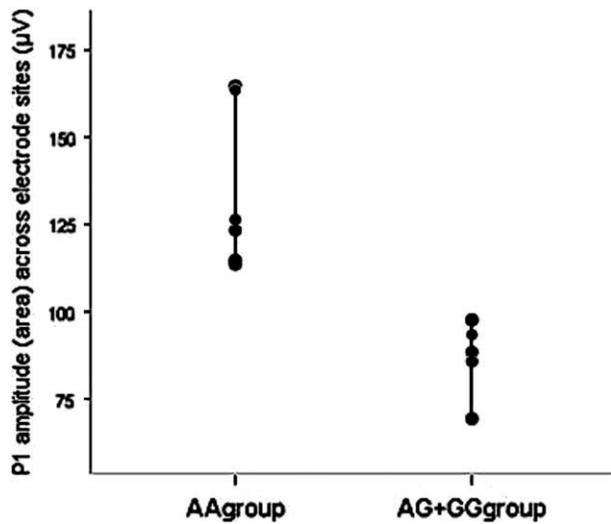


Figure 4.

Scatterplot of P1 amplitudes (area under the curve) across electrodes used in statistical analysis.

doing so due to insufficient power to detect association between *NOS1* and SWM performance in the restricted EEG sample ($n = 54$ versus the overall neuropsychological sample of $n = 160$) and so this analysis could not be undertaken.

DISCUSSION

We have previously reported evidence that the risk “G” allele at the SZ GWAS identified *NOS1* variant rs6490121 was associated with poorer performance in SWM and verbal IQ in independent samples of both SZ patients and healthy controls. Following up these findings, the present study investigated whether the same *NOS1* variant was also associated with poorer performance in sensory level processing as measured by the P1 visual evoked potential in a sample of healthy participants. Consistent with our hypothesis, we observed that the associated risk allele at rs6490121 was associated with a significantly reduced P1 response bilaterally. No differences in N1 or P2 response associated with *NOS1* were observed.

As an endophenotypic measure, the P1 has the major advantage of being relatively easy to measure quickly and accurately. The large differences between healthy controls and both patients and their first-degree relatives suggest this component is heritable [Donohoe et al., 2007, 2008; Haenschel et al., 2009; Yeap et al., 2006; Walters and Owen]. As a largely automatic response, it is not as susceptible to the same motivational factors or fluctuations in clinical state as later cognitive components such as the P300. However, this is not to say that the P1 is not cognitively penetrable. Although early stages of perceptual processing (from as early as 50–100 msec post-stimulus)

serve an important role in “spotlighting” of relevant information for later processing, these early processing stages (from 70 msec onward) appear to be reciprocally modulated by higher processing areas [Martinez et al., 1999].

It is interesting to speculate about the twin effects of *NOS1* on (in our larger sample) SWM, and (in the present study), the P1 response. These associations may reflect the reciprocal relationship between early sensory and higher cognitive function, particularly for visual information. On one hand, deficits in “capturing” visual information are likely to increase difficulties in efficiently maintaining and updating that information “online” during SWM tasks. Conversely, an inability to maintain context during later stages of processing leads to difficulties focusing on relevant information during earlier stages of visual processing. A relationship between the P1 response and SWM performance has been empirically demonstrated previously [Haenschel et al., 2007] and, in the present study, we were able to replicate this evidence: the P1 response significantly predicted SWM task performance in our participants.

For the first time (to our knowledge) we were able to partly test whether the genetic effects on either of these stages of processing (early visual sensory versus SWM) were being mediated by the other. Although insufficient power prevented us from determining whether *NOS1*’s effects on SWM were mediated by P1 performance, we were able to reject the hypothesis that *NOS1*’s effect on the P1 was being mediated in a “top-down” fashion by *NOS1*’s influence on SWM performance. In a multiple regression analysis, while SWM significantly predicted variance in the P1 response, *NOS1* continued to explain a significant amount of variance in the P1 response even after the variance associated with SWM was accounted for. We interpret these data as suggesting that *NOS1* has a direct influence on visual sensory processing as measured by the P1 response, either because of pleiotropic effects of this gene on multiple aspects of brain function, or because of a primary influence on sensory processing that mediate the effects already seen in higher cognitive processes. This evidence supports the increasingly popular theory that some deficits in cognitive processing may result at least in part from sensory level processing deficits [Javitt, 2009], but require testing in a larger sample to confirm the effect of the P1 response as mediating the influence of *NOS1* on

TABLE I. Differences in P1 response according to genotype group (measured as the area under the curve) for left hemisphere electrode sites, right hemisphere electrode sites, and averaged across electrode sites

	GG ($n = 5$)	AG ($n = 25$)	AA ($n = 22$)
P1 Left hemisphere	65.77 (55.46)	97.70 (60.98)	118.64 (59.56)
P1 Right hemisphere	78.53 (51.24)	88.47 (52.45)	150.04 (76.02)
P1 Both hemispheres combined	72.15 (50.44)	91.58 (44.96)	134.34 (52.33)

cognition. A limitation of our findings concerned the observed gender and sex differences between genotype groups. Although these differences were co-varied for in the analysis and did not appear to influence the significance of our results, replication of these findings in more gender and sex matched genotype groups will enable a better assessment of the contribution of these variables. In the current study, the GG groups were grouped together as the frequency of the GG genotype group was too low. Future replication studies could also include a sample where AG and GG groups are better individually represented.

NOS1: Molecular Mechanism and Functional Implications

The implicated SNP (rs6490121) has no obvious functional effect and may reflect a proxy association with 1 or more other causal genetic variants in SZ. Based on HapMap CEU data, rs6490121 is not in high linkage disequilibrium (LD; $r^2 > 0.80$) with any other common SNP at this locus. *NOS1* is characterized by complex transcriptional regulation. We previously investigated whether the cognitive effects of this *NOS1* variant could be explained by the dinucleotide variable-number tandem repeat located in the core promoter region of Exon 1f, the short arm of which is associated with electrophysiological measures of attentional control [Reif et al., 2009] and which is in partial LD with this SNP [$D' = 0.70$, $r^2 = 0.26$; Donohoe et al., 2009]. However, we failed to find evidence that this variant explained variation in cognition in our samples. Similarly, to explore potential mechanisms by which NO could exert an effect on cognitive processes, we previously screened experimentally validated protein-protein interactions of *NOS1* using the protein-protein interaction databases and identified 19 confirmed human binary interactions, including SZ relevant susceptibility genes involved in presynaptic synaptogenesis (*NOS1AP* and syntrophin [*SNTA1*] [OMIM 601017]) and postsynaptically through the Postsynaptic Density 95 (*PSD95*). Elements of *PSD95* signaling cascades have been targeted in SZ genetic association studies including erbB4/neuregulin signaling and the N-methyl-D-aspartate receptor complex, which is involved in long-term potentiation, memory, and learning. Of these, we have investigated the neuropsychological effects of the *NOS1AP* SNP implicated in SZ risk (rs12742393) and found no evidence of association with variation in cognition (data available on request). We have as yet to explore the influence of this or other interacting genes on the visual evoked potentials reported here.

Since its original identification as a common genetic variant associated with SZ risk by [O'Donovan et al., 2008] none of the subsequent genome wide association studies of SZ have identified *NOS1* rs6490121 as achieving genome wide level significance [Stefansson et al., 2008; The International Schizophrenia Consortium, 2009; Walsh

et al., 2008]. We have previously suggested that *NOS1* may be a modifier gene that influences cognitive ability without having a direct influence on disease risk. The present data suggest an even broader role for *NOS1* in information processing, impacting early sensory as well as later cognitive function. This broad influence on information processing is consistent with the known biology of *NOS1*, including negative feedback on N-methyl-D-aspartate (NMDA) receptor function and inhibition of synaptic reuptake of dopamine. This position at the crossroads of two mutually regulating messenger systems, and its ubiquitous expression throughout the brain, together make a discrete influence on only one level of information processing unlikely. A wider role for genetic variants influencing NMDA at the levels of both SWM and P1 response has already been reported in the case of Dysbindin-1 [DTNBP1; Donohoe et al., 2007, 2008].

CONCLUSION

As originally conceived, the use of cognitive and EEG measures as “intermediate” or “endo”-phenotypes was proposed as a strategy for reducing the genetic complexity of broader clinical phenotypes that would allow greater power for identifying genes of small effects [Gottesman and Gould, 2003]. Since then, several EEG studies have focused on confirming the effects of variants already associated with increased disease risk on individual brain systems for the purposes of characterizing the effects on these variants on individual aspects of brain function. Such an approach may be helpful in elucidating gene-disease pathways [Walters and Owen] and, eventually, therapeutic targets. However, there is currently little evidence that the genetic architecture of cognition is much less complex than that of disease phenotypes. Thus, cognitive neuroscience studies of psychiatric disease associated variants, in which information processing is disrupted, is likely to have an equally valuable role in elucidating the molecular biology of information processing in the general population. Evidence of *NOS1*'s role in early visual processing presented here is therefore likely to be relevant not just to schizophrenia pathophysiology, but to understanding the molecular basis of visual processing more generally.

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