Differential effects of DAAO on regional activation and functional connectivity in schizophrenia, bipolar disorder and controls

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Abstract
Recent studies have identified DAAO as a probable susceptibility gene for schizophrenia and bipolar disorder. However, little is known about how this gene affects brain function to increase vulnerability to these disorders. We examined the impact of DAAO genotype (rs3918346) on brain function in patients with schizophrenia, patients with bipolar I disorder and healthy controls. We tested the hypothesis that a variation in DAAO genotype would be associated with altered prefrontal function and altered functional connectivity in schizophrenia and bipolar disorder. We used functional magnetic resonance imaging to measure brain responses during a verbal fluency task in a total of 121 subjects comprising 40 patients with schizophrenia, 33 patients with bipolar disorder and 48 healthy volunteers. We then used statistical parametric mapping (SPM) and psycho-physiological interaction (PPI) analyses to estimate the main effects of diagnostic group, the main effect of genotype, and their interaction on brain activation and on functional connectivity. Inferences were made at p < 0.05, after correction for multiple comparisons across the whole brain. In the schizophrenia group relative to the control group, patients with one or two copies of the T allele showed lower deactivation in the left precuneus and greater activation in the right posterior cingulate gyrus than patients with two copies of the C allele. This diagnosis × genotype interaction was associated with differences in the functional connectivity of these two regions with other cortical and subcortical areas. In contrast, there were no significant effects of diagnosis or of genotype in comparisons involving bipolar patients. Our results suggest that genetic variation in DAAO has a significant impact on both regional activation and functional connectivity, and provide evidence for a diagnosis-dependent pattern of gene action.

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Introduction
Schizophrenia and bipolar disorder are severe psychiatric disorders with a significant genetic component (Owen et al., 2007; Craddock et al., 2006; Berrettini, 2003; Cardno et al., 2002). The identification of susceptibility genes has been problematic because of phenotypic complexity and a mode of transmission compatible with multiple loci. However, association studies have provided converging evidence in favour of a number of positional genes mapped to both schizophrenia and bipolar disorder linkage regions (Park et al., 2004; Lewis et al., 2003; Segurado et al., 2003); one such candidate is the d-Amino Acid Oxidase (DAAO) gene (D. Prata et al., 2008; D.P. Prata et al., 2008).

The DAAO gene is located at 12q23, a region linked to schizophrenia and bipolar disorder (Sklar, 2002). It was originally associated with schizophrenia by Chumakov et al. (2002) using Canadian samples. Further evidence of association comes from several replication studies (Schumacher et al., 2004; Liu et al., 2004; Kapoor et al., 2006; Corvin et al., 2007; Madeira et al., 2008; Bass et al., 2009). Recently, two genetic studies (D. Prata et al., 2008; D.P. Prata et al., 2008; Fallin et al., 2005) have also implicated the DAAO gene in bipolar disorder. First, Fallin et al. reported a significant association of this gene with bipolar I disorder but not with schizophrenia (Fallin et al., 2005). Prata et al. subsequently found significant association of a two-SNP risk haplotype for DAAO with bipolar disorder (D. Prata et al., 2008; D.P. Prata et al., 2008). Taken collectively, these studies suggest that DAAO may confer biological susceptibility to psychosis across the traditional Kraepelian dichotomy of schizophrenia and bipolar disorder (Cradock et al., 2007).

The DAAO gene encodes the DAAO enzyme that oxidises d-serine, a co-agonist for the NMDA glutamate receptor (Boks et al., 2007). The...
NMDA receptor is known to play an important role in synaptic plasticity, neurodevelopment and excitotoxicity (Tuominen et al., 2005). It is characterised by two distinct sub-units known as NR1 and NR2: NR1 is a binding site for co-agonists glycine and D-serine while NR2 is the agonist binding site for glutamate. NR1 must be occupied for glutamate to be able to open the channel; this requires the availability of glycine and, to a greater extent, D-serine. Thus, selective degradation of D-serine by the DAAO enzyme results in reduced NMDA neurotransmission. There is growing evidence that NMDA neurotransmission is altered in psychosis (Verrall et al., 2010; Stone et al., 2009; Boks et al., 2007; Hashimoto et al., 2003; Coyle et al., 2003; Tanii et al., 1994; Javitt and Zukin, 1991). For example, D-serine levels are decreased in the cerebrospinal fluid and serum of patients with schizophrenia (Hashimoto et al., 2005) and administration of D-serine may reduce negative, positive and cognitive symptoms in schizophrenia (Heresco-Levy, 2005). It has therefore been proposed that increased activity of the DAAO enzyme may lead to increased degradation of D-serine in schizophrenia, resulting in relative hypoactivity of the DAAO enzyme, which may reduce NMDA neurotransmission. There is increased glutamine/glutamate concentration in the dorsolateral prefrontal cortex and cingulate gyrus in bipolar disorder (Michael et al., 2003; Dager et al., 2004).

While the effects of DAAO at molecular and cellular levels are emerging, little is known about this gene’s effects on the brain at macroscopic or systems levels. There have been only two previous studies investigating the association between DAAO genotype and cognitive function (Stefanis et al., 2007; Goldberg et al., 2006). Stefanis et al. (2007) found an effect of DAAO haplotype on spatial working memory in a population of 2243 male military conscripts. In contrast, in a sample of over 600 healthy controls, patients with schizophrenia and their nonpsychotic siblings, Goldberg et al. (2006) found no genotype or genotype × diagnosis interactions for any of the three DAAO SNPs used (MDAAO 5–7, using the nomenclature of Chumakov). To date, there have been no neuroimaging studies that investigated the impact of DAAO genotype on brain function in healthy controls or psychotic patients. Thus, little is known about how this gene affects brain function at a macroscopic or system level. Furthermore, it is unclear whether or not the impact of DAAO on brain function is expressed consistently across different diagnostic categories, given that recent studies on other candidate genes for schizophrenia and bipolar disorder have provided evidence for a disease-dependent pattern of gene action (Mechelli et al., 2008; D. Prata et al., 2008; D.P. Prata et al., 2008).

The aim of the present study was therefore to investigate the impact of genetic variation in DAAO (the rs3918346 polymorphism) on brain function in 3 diagnostic groups: healthy volunteers, patients with schizophrenia and patients with bipolar I disorder. We used functional magnetic resonance imaging (fMRI) to measure brain responses during a verbal fluency task that required subjects to generate and articulate words in response to letter cues. This task was chosen for two main reasons: first, it engages a distributed network of fronto-temporal cortical and sub-cortical brain regions that have been implicated in schizophrenia and bipolar disorder (Fu et al., 2005; Fu et al., 2002; Curtis et al., 2001); second, it taps into executive cognitive processes that are compromised in both schizophrenia and bipolar disorder (Daban et al., 2006; Kravariti et al., 2005; Krabbendam et al., 2005; Curtis et al., 2001). A “clustered” image acquisition sequence was used in order to record overt vocal responses in the absence of scanner noise (Fu et al., 2005, 2002) and then model correct and incorrect trials separately in the statistical analysis.

Our first hypothesis was that DAAO genotype would have a measurable impact on activation during the verbal fluency task. While there is evidence for DAAO’s action in the prefrontal cortex (Verrall et al., 2010; Hashimoto et al., 2009; Verrall et al., 2007), this gene is thought to be expressed throughout the cortex; we therefore tested for its effects in the whole brain using an appropriate correction for multiple comparisons. Our second hypothesis was that the impact of DAAO on the prefrontal function would vary across the three diagnostic groups as revealed by significant genotype × diagnosis interactions.

Materials and methods

Some of the functional neuroimaging data examined in the present investigation have been included in previous studies which investigated brain dysfunction in psychosis (Fu et al., 2005) or the impact of other candidate genes (Mechelli et al., 2008; D. Prata et al., 2008; D.P. Prata et al., 2008; Prata et al., 2009a, 2009b).

Subjects

A total of 121 subjects were investigated, including 48 healthy volunteers, 40 patients with schizophrenia and 33 patients with bipolar I disorder. All participants were native English speakers and gave written informed consent in accordance with protocols approved by the Local and Multicentre Research Ethics Committee (LREC, MREC). Healthy volunteers were recruited through local advertisement and had no family history of psychiatric illness as assessed using the FIGS (Family Interview for Genetic Studies). Patients with schizophrenia and bipolar disorder were recruited through the South London and Maudsley NHS Trust or other NHS Mental Health Trusts and met relevant DSM-IV criteria, determined after a detailed clinical interview and systematic review of the medical records. Full-scale IQ was assessed using the WAIS-III (Wechsler Adult Intelligence Scale–III; Wechsler, 1997), the WAIS-R (Wechsler Adult Intelligence Scale–R; Wechsler, 1981), the WASI-FSIQ-4 (Wechsler Abbreviated Scale of Intelligence; Wechsler, 1999) or the Quick Test (Ammons and Ammons, 1962). The WAIS-III correlates highly both with the WAIS-R (93.8%; Wechsler, 1997) and with the WASI-FSIQ-4 (92%; Wechsler, 1999). The Quick test has also been shown to yield comparable results to WAIS (Quick Test, 78 ± 7 and WAIS, 83 ± 6 in schizophrenia; Frith et al., 1991). The proportion of subjects assessed with each method was matched between genotype groups.

All participants were genotyped for the DAAO SNP rs3918346 (see Materials and methods below) with C and T being the two alleles. Demographic data (including age, full-scale IQ, years of education, handedness, gender and ethnicity) and clinical data (including positive and negative symptoms, duration of illness and antipsychotic medication) for each diagnostic group and for each genotypic group are reported in Table 1 and described in supplementary materials (S1).

Genotyping

Genomic DNA was extracted from blood or cheek swabs by SGDP (Social Genetic and Developmental Psychiatry) laboratory technicians following standard methodology (Freeman et al., 2003). Genotyping of the DAAO rs3918346 single nucleotide polymorphism (SNP), was performed blind to status under contract by KBioscience (Herts, UK; http://www.kbioscience.co.uk/) using a competitive allele specific PCR system (CASP). This single nucleotide polymorphism (SNP) was chosen because it was the single marker most frequently associated with schizophrenia and bipolar disorder in previous studies either individually or in haplotype form (D. Prata et al., 2008; D.P. Prata et al., 2008; Corvin et al., 2007; Wood et al., 2007; Schumacher et al., 2004). The genotype counts retrieved were: 29 CC, 16 CT and 3 TT in the bipolar disorder group. The genotyping results of our sample were in Hardy Weinberg equilibrium ($X^2 = 0.34; p = 0.6291$).
Verbal fluency task and image acquisition

The task and image acquisition was performed as described before (Fu et al., 2002), see S2 for further details. In brief, during a “generation” condition, subjects were visually presented with a series of letters and required to overtly articulate a word beginning with the presented letter. This condition was contrasted with a “repetition” condition, in which subjects were presented with the word “rest” and were required to say rest out loud. Task difficulty was manipulated by presenting separate sets of “easy” and “hard” letters. Verbal responses were recorded allowing the identification of “incorrect” trials, in which the subject did not generate any response or generated repetitions, derivatives, or grammatical variations of a previous word.

fMRI data analysis

The analysis of the fMRI data was performed using Statistical Parametric Mapping (SPM5) software (Friston, 2003), running under Matlab 6.5. A 3 × 2 × 2 full-factorial ANOVA was used in which diagnostic category and genotype were modelled as between-subject factors and task difficulty was modelled as a within subject factor. See S3 for details. Statistical inferences were made in SPM5 using a statistical threshold of p < 0.05 after family-wise error (FWE) correction for multiple comparisons across the whole brain and an extent threshold of 5 voxels.

Functional connectivity analysis

To establish putative alterations in functional integration that were underlying genotype-related differences in regional activation, we used the psycho-physiological interaction (PPI) method (Friston et al., 1997). PPI is a well established analytical technique based on multiple regression that allows the investigation of the functional coupling between regions (“functional connectivity”) as a function of one or more experimental manipulations of interest. In brief, for each region of interest (defined as spheres with a 6 mm radius) the time series was extracted from each participant using the “VOI extraction” tool in SPM software; the choice of regions of interest were based on voxels which showed a significant interaction between genotype and diagnostic category in the standard analysis of regional responses. For each region of interest, the time series from each subject was multiplied by a vector which contrasted verbal fluency vs baseline. This resulted in a “PPI vector” which encoded the interaction between the activation of a region of interest and the experimental context (verbal fluency vs baseline). A correlation analysis was then performed between this PPI vector and the rest of the brain in a subject specific fashion; finally, the subject-specific contrast images were entered into an ANOVA to make inference at the second level. This procedure allowed the identification of alterations in functional integration underlying the interactions between genotype and diagnostic category detected in the standard analysis of regional responses. Statistical inferences were made in SPM5 using a statistical threshold of p < 0.05 after family-wise error (FWE) correction for multiple comparisons across the whole brain and an extent threshold of 5 voxels.

Results

Performance

The effects of diagnostic group, genotype, task difficulty and their interaction on the accuracy of verbal responses during scanning (Table 1) were tested using a repeated measures ANOVA in SPSS (Statistical Package for Social Sciences version 15.0). The number of errors significantly differed as a function of diagnostic group (F = 4.621; df = 2; p = 0.012). Post hoc t-tests revealed that patients with schizophrenia made significantly more errors than healthy volunteers (F = 11.108; df = 1; p = 0.001). Patients with bipolar disorder made an intermediate number of errors and did not significantly differ (p = 0.05) from either healthy volunteers or patients with schizophrenia. The number of errors did not differ (p = 0.05) as a function of genotype, irrespective of whether the 3 diagnostic groups were considered separately or in combination. The

Table 1

Demographics and behavioural performance in control (C), schizophrenic (S) and bipolar (BD) samples with CC genotype and CT or TT genotype respectively. Degrees of freedom (df), F or χ² test value and p-value are reported for comparisons between diagnostic groups that reached significance at p < 0.05. The Scale for the Assessment of Positive Symptoms (SAPS) and the Scale for the Assessment of Negative Symptoms (SANS) measure symptoms experienced within the month prior to the interview. The asterisk (*) indicates a significant difference between the CC genotype and the CT and TT genotypes combined within schizophrenic sample for CPZ rate (F = 8.229; df = 1; p = 0.007) and within the bipolar sample for scores on the Beck Depression Inventory (BDI; F = 5.371; df = 1; p = 0.027) and on Altman Self-Rated Mania Scale (ASRM; F = 4.275; df = 1; p = 0.47).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>C</th>
<th>S</th>
<th>BD</th>
<th>Group comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>CC</td>
<td>CT/TT</td>
<td>CC</td>
<td>CT/TT</td>
</tr>
<tr>
<td>N. of subjects</td>
<td>29</td>
<td>19</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Age: mean (sd)</td>
<td>34.5 (10.5)</td>
<td>33.5 (10.9)</td>
<td>34.5 (12.6)</td>
<td>35.8 (10.8)</td>
</tr>
<tr>
<td>IQ: mean (sd)</td>
<td>115.2</td>
<td>115.8</td>
<td>98.4</td>
<td>97.6</td>
</tr>
<tr>
<td>(12.7)</td>
<td>(8.9)</td>
<td>(15.0)</td>
<td>(15.6)</td>
<td>(15.7)</td>
</tr>
<tr>
<td>Gender: male/female</td>
<td>15/14</td>
<td>9/10</td>
<td>15/3</td>
<td>18/4</td>
</tr>
<tr>
<td>Ethnicity: cauc/black/carib/mixed</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Handedness: right/left/mixed</td>
<td>27/2</td>
<td>16/1</td>
<td>15/3</td>
<td>21/1</td>
</tr>
<tr>
<td>Years of education: mean (sd)</td>
<td>14.5 (2.3)</td>
<td>15.9 (3.1)</td>
<td>14.2 (2.3)</td>
<td>14.0 (2.2)</td>
</tr>
<tr>
<td>CPZ rate: mean (sd)</td>
<td>781.3</td>
<td>(410.9)*</td>
<td>397.7</td>
<td>(432.5)*</td>
</tr>
<tr>
<td>Years of antipsychotic medication: mean (sd)</td>
<td>14.1 (11.1)</td>
<td>10.7 (8.0)</td>
<td>13.6 (10.7)</td>
<td>9.1 (5.5)</td>
</tr>
<tr>
<td>SAPS: mean (sd)</td>
<td>9.61 (8.87)</td>
<td>5.86 (4.90)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SANS: mean (sd)</td>
<td>9.72 (5.37)</td>
<td>7.05 (3.86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDI: mean (sd)</td>
<td>6.21 (5.58)*</td>
<td>12.79 (10.56)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ASRM [mean (sd)]</td>
<td>2.58 (2.46)*</td>
<td>4.57 (3.08)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. of errors: mean (sd)</td>
<td>10.62 (7.64)</td>
<td>7.52 (4.98)</td>
<td>12.61 (5.30)</td>
<td>15.63 (7.75)</td>
</tr>
<tr>
<td>N. of easy errors: mean (sd)</td>
<td>4.28 (3.56)</td>
<td>1.78 (2.30)</td>
<td>4.67 (3.09)</td>
<td>6.59 (4.76)</td>
</tr>
<tr>
<td>N. of hard errors: mean (sd)</td>
<td>6.34 (4.54)</td>
<td>5.73 (3.28)</td>
<td>7.94 (3.32)</td>
<td>9.05 (4.59)</td>
</tr>
</tbody>
</table>
main effect of task difficulty was significant, with the “hard” condition associated with a greater number of errors than the “easy” condition (F = 54.174; df = 1 p < 0.001). Finally, there were no 2-way or 3-way interactions between diagnostic group, genotype and task difficulty (p > 0.05).

Functional MRI

Effects of generation vs. baseline

A distributed network of regions including the left inferior frontal gyrus, left insula and left superior temporal gyrus were more activated during generation than baseline (Table 2). Conversely, the left precuneus, bilateral angular gyrus, bilateral anterior cingulate, right medial frontal gyrus, bilateral insula and right postcentral gyrus expressed increased activation during baseline relative to generation (Table 2).

Main effect of diagnostic group

Controls expressed greater activation (i.e. generation > baseline) relative to patients with schizophrenia in the left posterior cingulate gyrus (x = 30, y = -48, z = 14; Z-score = 5.08, p = 0.001 after FWE correction for multiple comparisons). Conversely, no regions expressed greater activation in patients with schizophrenia relative to controls. There were no significant differences between bipolar patients and controls and between bipolar and schizophrenic patients.

Main effects of genotype

There was a significant effect of genotype in the precuneus bilaterally (right: x = 14, y = -50, z = 12; Z-score = 3.10; p = 0.001 after FWE correction; left: x = -14, y = -54, z = 16; Z-score = 4.52; p = 0.014 after FWE correction). Exploration of the parameter estimates revealed that this region expressed greater deactivation during generation relative to baseline in CC homozygotes than those with one or two copies of the T allele (Fig. 1).

Effects of interaction of diagnostic group × genotype

The interaction between diagnostic group and genotype was examined by contrasting the effect of genotype in one diagnostic group against the same effect in another diagnostic group within our full-factorial ANOVA. This revealed two regions which expressed a significant diagnosis × genotype interaction at p < 0.05 after FWE correction for multiple comparisons. In these regions, the significant interaction contrast was “CC > CT & TT in healthy volunteers” patients with schizophrenia”. One region to show a significant diagnosis × genotype interaction was the left precuneus (x = -14, y = -56, z = 26, Z-score = 4.79, p = 0.004 after FWE correction; see Fig. 2), which also expressed deactivation during generation relative to baseline across different diagnostic and genotypic groups. Exploration of the parameter estimates revealed that, in this region, deactivation during generation relative to baseline was less pronounced in schizophrenic patients with one or two copies of the T allele than those without it (x = -16, y = -58, z = 28; Z-score = 4.55; p = 0.012 after FWE correction); in contrast the opposite trend was expressed amongst healthy volunteers (x = -14, y = -52, z = 26; Z-score = 2.77, p < 0.01 uncorrected). The other region to show a significant diagnosis × genotype interaction were the right posterior cingulate gyrus (x = 20, y = -54, z = 12; Z-score = 4.42, p = 0.021 after FWE). In this region, there was activation during generation in patients with schizophrenia who had at least one copy of the T allele while there was deactivation in those without it (x = 16, y = -52, z = 12; Z-score = 5.11, p = 0.001 after FWE); in contrast no such effect of DAAO genotype was expressed in healthy volunteers. There were no diagnostic group × genotype interactions which involved the group of bipolar patients.

Effects of functional connectivity

The aim of the PPI analysis was to examine the task-dependent changes in functional connectivity which mediated the diagnosis × genotype interactions reported above. We therefore performed two PPI analyses which focussed on the left precuneus and the right posterior cingulate respectively.

For the first region of interest, the left precuneus, the PPI analysis revealed that the diagnosis × genotype interaction on regional activation was mediated by significant changes in functional connectivity (p < 0.05 after FWE correction) between this region and a distributed network including left and right precuneus, left putamen, right posterior cingulate gyrus, left caudate and right angular gyrus (Fig. 3A and Table 3). Here the significant interaction contrast was “CT and TT > CC in healthy volunteers” patients with schizophrenia”; exploration of the parameter estimates indicated that less pronounced deactivation during generation relative to baseline amongst schizophrenic patients with one or two copies of the T allele was associated with weaker functional connectivity.

Finally, for the second region of interest, the right posterior cingulate gyrus, we found that the diagnosis × genotype interaction on regional activation was mediated by significant changes in functional connectivity (p < 0.05 after FWE correction) between this region and two areas: the right precuneus and the left insula (Fig. 3B and Table 3). Here the significant interaction contrast was “CC > CT and TT in healthy volunteers” patients with schizophrenia”; exploration of the parameter estimates indicated that greater activation during generation relative to baseline amongst schizophrenic patients with one or two copies of the T allele was associated with stronger functional connectivity.

Possible confounding variables

We considered the possibility that the above diagnosis × genotype interactions could be explained by inter-subject variability in age, gender, handedness, IQ, years of education and ethnicity. We therefore repeated the statistical analysis modelling of these factors as covariates of no interest; however this did not alter any of the results. We also considered the possibility that our results could be driven by the significant difference in anti-psychotic medication between schizophrenic patients with and without the T allele. When medication variables including dose, type (first vs. second generation) and duration of antipsychotic treatment were entered into the statistical analysis as covariates of no interest, they did not change.
the foci of maximal significance or reduce the associated Z-scores. We also performed a correlation analysis to test for a possible association between medication dose and brain activation in the three regions which expressed a significant diagnosis×genotype interaction. We found no evidence for a significant correlation between dose of antipsychotic medication and brain activation in these regions (p > 0.05), suggesting that the diagnosis×genotype interactions were unlikely to be explained by medication effects. Likewise, the inclusion of dose of lithium medication as covariate of no interest did not affect the results and the amount of activation in the left precuneus and the right posterior cingulate gyrus was not related to this variable (p < 0.05 uncorrected).

Discussion

We examined the effect of DAAO genotype on brain function during a verbal fluency task which engages brain regions and cognitive processes that are impaired in both schizophrenia and bipolar disorder (Daban et al., 2006; Krabbendam et al., 2005; Fu et al., 2002, 2005; Curtis et al., 2001). Independent of diagnostic group, the left precuneus showed greater deactivation during task performance relative to baseline in CC homozygotes than those with one or two copies of the T allele (p < 0.05 after FWE correction). In addition, a significant genotype×diagnostic group interaction was found in the left precuneus and the right posterior cingulate gyrus. These findings
are unlikely to be attributable to differences in age, gender, handedness, ethnicity or years of education, since these factors were modelled in the statistical analysis as covariates of no interest.

The precuneus and posterior cingulate gyrus are part of the “resting state” or “default” network that often shows a decrease in activation during task performance relative to baseline (Vanhaudenhuyse et al., 2010; Mechelli et al., 2008; McKiernan et al., 2006; 2003; Binder et al., 1999; Shulman et al., 1997; McGuire et al., 1996). This network is thought to be implicated in ongoing internally generated processes that occur during any low level baseline condition. The latter are disrupted when the subject has to then engage in a more demanding experimental task (McKiernan et al., 2006; 2003; Binder et al., 1999), and task-related deactivation within the default network may reflect a reallocation of cognitive resources (McKiernan et al., 2006; 2003; Binder et al., 1999). Furthermore, in previous studies, this network was functionally altered in patients with schizophrenia relative to healthy controls (Kim et al., 2009; Whitfield-Gabrieli et al., 2009). One possible interpretation of our finding is that the active task might have produced differential interference with ongoing internally generated processes in schizophrenic patients as a function of genotype.

In order to characterise the task-dependent changes in functional integration which underlay the interactions between DAAO genotype and diagnosis, we performed two PPI analyses. These revealed that the diagnosis-dependent effect of genotype in the left precuneus was mediated by differential functional connectivity with a distributed cortical and sub-cortical network, while the diagnosis-dependent effect of genotype in the right posterior cingulate gyrus was mediated by differential functional connectivity with the right precuneus and the left insula. Thus, the present study indicates that the impact of DAAO genotype on brain function is not limited to regional responses but is also evident in the functional integration between regions; this is consistent with previous reports that genetic vulnerability to psychosis is associated with altered functional integration (Whalley et al., 2005; Whitfield-Gabrieli et al., 2009).

The genotype × diagnostic group interactions in the left precuneus and the right posterior cingulate gyrus were driven by effects of DAAO genotype which were evident in patients with schizophrenia but not in healthy controls. This pattern of results is consistent with previous reports of disease-specific effects of candidate genes for psychosis on brain structure and function (Addington et al., 2007; Frodl et al., 2004; [Fig. 3]. A. Results of the PPI analysis with the left precuneus as region of interest; the plot indicates that less pronounced deactivation during generation relative to baseline in schizophrenic patients with one or two copies of the T allele was associated with weaker functional connectivity. B. Results of the PPI analysis with the right posterior cingulate gyrus as region of interest; the plot indicates that greater activation during generation relative to baseline in schizophrenic patients with one or two copies of the T allele was associated with stronger functional connectivity.

<table>
<thead>
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<th>Table 3</th>
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Results of the psycho-physiological interaction (PPI) analyses. Coordinates and Z-scores (in brackets) refer to regions which showed task-dependent changes in functional connectivity (FC) which mediated the diagnosis-genotype interactions in regional activation (p < 0.05 after FWE correction).

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Coordinates (Z-score)</th>
</tr>
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<tbody>
<tr>
<td>FC with left precuneus</td>
<td></td>
</tr>
<tr>
<td>Left precuneus</td>
<td>−22</td>
</tr>
<tr>
<td>Right precuneus</td>
<td>10</td>
</tr>
<tr>
<td>Left putamen</td>
<td>−28</td>
</tr>
<tr>
<td>Right cingulate gyrus</td>
<td>8</td>
</tr>
<tr>
<td>Left caudate</td>
<td>−6</td>
</tr>
<tr>
<td></td>
<td>−18</td>
</tr>
<tr>
<td></td>
<td>−12</td>
</tr>
<tr>
<td>Right angular gyrus</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>44</td>
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<td>FC with right posterior cingulate gyrus</td>
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<td>Right precuneus</td>
<td>6</td>
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<tr>
<td>Left insula</td>
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</tbody>
</table>
The mechanisms that lead to effects of DAAO genotype being evident in patients with schizophrenia but not in healthy controls are unclear. One possibility is that the effects of variation in a given gene depend on the genetic context (Weinberger, 2010). Patients with schizophrenia are likely to carry a larger number of risk genes than healthy controls; these risk genes may interact with the DAAO gene such that its expression is evident in patients but not in controls. An alternative explanation is that the effect of a gene may vary with differences in environmental exposure (Caspi and Moffitt, 2006); which may also differ between patients and controls. A final possibility is that the effects of a given gene may interact with the effects of schizophrenia and bipolar disorder on the function of the brain.

It should be noted that, although a number of genetic studies have associated the T allele for DAAO rs3918346 with increased risk of schizophrenia and bipolar disorder relative to the C allele, the results have not always been consistent as to which of these alleles confers the higher risk (Liu et al., 2004; Schumacher et al., 2004; Fallin et al., 2005; Yamada et al., 2005; Liu et al., 2006; Shinkai et al., 2007; Li and He, 2007; Wood et al., 2007; Corvin et al., 2007; Ohnuna et al., 2009; Bass et al., 2009); this could be due to false positive results or reflect allelic heterogeneity (i.e. different alleles in the same marker being associated with disease) (Moskvina and O’Donovan, 2007). In the present investigation, we have therefore avoided reference to the terms low or high risk, and have referred to the alleles instead. Assuming that DAAO rs3918346 does moderate the risk of schizophrenia and bipolar disorder, it remains to be established whether or not the diagnosis-dependent effects identified in this study lie upon the pathway between genes and clinical phenotype (Owen, 2010).

This is an important question, since imaging endophenotypes may mediate the increased risk conferred by genes but also reflect gene effects which do not necessarily result in increased risk, consistent with the notion of pleiotropy (Owen, 2010).

In this study all significant DAAO effects were from the contrast between patients with schizophrenia and healthy controls with no significant effects in the bipolar patients. However, the direct comparison between schizophrenic and bipolar patients was not significant and, furthermore, exploration of the parameter estimates indicated that the bipolar patients consistently showed intermediate values between schizophrenic patients and controls. This suggests that there may be effects of DAAO in the bipolar population that were not detected due to the relatively small sample size. Alternatively, the effects of DAAO on brain function may be more pronounced in schizophrenia than bipolar disorder due to systematic differences in the disease as well as the higher risk (Liu et al., 2004; Schumacher et al., 2004; Fallin et al., 2005; Yamada et al., 2005; Liu et al., 2006; Shinkai et al., 2007; Li and He, 2007; Wood et al., 2007; Corvin et al., 2007; Ohnuna et al., 2009; Bass et al., 2009); this could be due to false positive results or reflect allelic heterogeneity (i.e. different alleles in the same marker being associated with disease) (Moskvina and O’Donovan, 2007). In the present investigation, we have therefore avoided reference to the terms low or high risk, and have referred to the alleles instead. Assuming that DAAO rs3918346 does moderate the risk of schizophrenia and bipolar disorder, it remains to be established whether or not the diagnosis-dependent effects identified in this study lie upon the pathway between genes and clinical phenotype (Owen, 2010).

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The present investigation has a number of limitations which require careful consideration. Firstly, within the DAAO gene, several SNPs have been identified by genetic association in addition to rs3918346, and there is no agreement as to which is the marker with the most significant effect on disease-risk; at present a functional polymorphism with plausible causality is yet to be identified. Secondly, genotypic groups were not balanced for dose of antipsychotic medication within the schizophrenic sample. However we did not detect any significant correlation between regional activation and chlorpromazine equivalences. Thirdly, individuals with two copies of the T allele constitute only a small fraction of the population and in the present investigation were collapsed with the group of individuals with CT genotype. This means that our data cannot reveal whether the action of the T allele on brain function is best described by a dominant or an additive model.

In conclusion, we have shown for the first time that DAAO genotype has a measurable impact on brain function in healthy volunteers, and in patients with schizophrenia and bipolar disorder. Our findings also suggest that the effects of DAAO genotype differ between patients with schizophrenia and healthy volunteers. As DAAO genotype is thought to influence glutamate neurotransmission, this disease-dependent pattern of gene action provides indirect support for the hypothesis that glutamate dysfunction contributes to the pathophysiology of schizophrenia (Olmey, 1995). A more comprehensive understanding of how variation in DAAO affects brain function will require investigation of interactions with other candidate genes and with environmental risk factors.

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