Functional disconnectivity in subjects at high genetic risk of schizophrenia

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Schizophrenia is a highly heritable psychotic disorder. It has been suggested that deficits of the established state arise from abnormal interactions between brain regions. We sought to examine whether such connectivity abnormalities would be present in subjects at high genetic risk for the disorder. Functional connectivity analysis was carried out on functional MRI images from 21 controls and 69 high risk subjects performing the Hayling sentence completion task; 27 high risk subjects reported isolated psychotic symptoms, the remaining high risk subjects and controls did not. There were no significant differences in task performance between the groups. Based on previous findings we hypothesized: (i) state-related differences in connectivity between dorsolateral prefrontal cortex and lateral temporal lobe; (ii) genetically mediated reductions in a medial prefrontal-thalamic-cerebellar network; and (iii) increased prefrontal–parietal connectivity in high risk subjects (to a greater extent in those with isolated psychotic symptoms). Connectivity analysis was performed in two ways: with and without variance associated with task effects modelled and removed from the data. We did not find evidence to support our first hypothesis with either analysis method. However, consistent with hypothesis (ii), decreased connectivity between right medial prefrontal regions and contralateral cerebellum was found. This was only statistically significant in the analysis with task effects modelled and removed from the data. Finally, consistent with hypothesis (iii), increased connectivity between the left parietal and left prefrontal regions in high risk subjects was found in both analyses. These results, all in a situation uncontaminated by the effects of anti-psychotic medication, performance differences and prolonged illness, suggest there are abnormalities in functional connectivity over and above those attributable to task effects in high risk subjects. These connectivity abnormalities may underlie the diverse deficits seen in the established condition and the more subtle deficits seen in close relatives of those with the disorder.

Keywords: fMRI; schizophrenia; high risk; connectivity

Abbreviations: BA = Broadmann area; DLPFC = dorsolateral prefrontal cortex; EHRS = Edinburgh High Risk Study; fMRI = functional MRI; HRF = haemodynamic response function; IPL = inferior parietal lobule; MedFG = medial frontal gyrus; S/MTG = superior/middle temporal gyrus;

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Introduction

Schizophrenia is an incapacitating psychiatric disorder with a lifetime risk of ~1% worldwide. Because of the heritability of the condition, that risk is increased among relatives of affected individuals. Prospective study of individuals at high genetic risk allows the investigation of whether abnormalities seen in the established state predate the development of the illness and reflect genetic vulnerability, or whether they are associated with the manifestation of symptoms. The Edinburgh High Risk Study (EHRS) examines such individuals who are at high risk because they have two or more first- or second-degree relatives with schizophrenia (Johnstone et al., 2000). They are studied as they pass through the period of maximum risk of developing the disorder (i.e. ages 16–30 years). On the basis of the known frequency of
schizophrenia in individuals with this degree of heredity, 10–15% of the sample were predicted to develop schizophrenia by the age of 30 years (Johnstone et al., 2002). Previously, functional MRI (fMRI) in the EHRS subjects using a covert verbal initiation paradigm (the Hayling task) demonstrated localized deficits in prefrontal–thalamic–cerebellar activation in those at high risk compared with normal controls and demonstrated increased inferior parietal cortex activity, particularly but not exclusively in those with psychotic symptoms (Whalley et al., 2004).

Although it is generally accepted that schizophrenia is associated with neuronal deficits, it has not been possible to explain the complex features of the disorder on the basis of specific, localized, structural or functional abnormalities. This has led to the suggestion that the basis of the illness could lie in abnormal interactions, or disconnectivity, between a distributed network of brain regions (Gold and Weinberger, 1991; Friston and Frith, 1995). In neuroimaging, functional connectivity has been defined as cross correlations over time between spatially remote brain regions (Friston et al., 1993a,b). Early connectivity studies examined correlations between spontaneously active regions in normal subjects during the resting state (Lowe et al., 1998). Later studies used conventional task activation methods, for example by examining correlations between regional time courses over periods of task and baseline conditions. However if, for example, two regions are highly activated by a task, they will inevitably be seen to be co-active and hence viewed as functionally connected (Xiong et al., 1999). In this case, task related co-activity may therefore provide little more information than standard activation maps.

Several approaches have been undertaken to address these issues: by excluding rest or baseline conditions and examining correlations during ‘active’ periods only (see, for example, Just et al., 2004); or by the implementation of statistical methods to remove effects of task activation from the data (see Arfkanis et al., 2000). In the current study, we used a similar approach to the latter study by examining functional connectivity with variance associated with task effects removed. This allowed us to identify differences over and above those attributable to task related activation. For completeness, we also report functional connectivity analysis of the data with task effects remaining.

The current re-emergence of the disconnection hypothesis in schizophrenia has, in part, been prompted by evidence of a reversal in normal prefronto-temporal interactions from PET studies of chronic schizophrenic subjects during word generation tasks (Frith et al., 1995; Friston et al., 1996). Disrupted fronto-temporal connectivity has also been reported with fMRI (Lawrie et al., 2002; Shergill et al., 2003), and there are indications that there may be a disruption of anterior cingulate modulation of the interaction between these regions in the disorder (Fletcher et al., 1996, 1999). More recently, other regions have been implicated. For example, other studies have reported disruptions in subcortical and cerebellar connections to the prefrontal cortex in schizophrenia (Stephan et al., 2001; Schlösser et al., 2003) and between prefrontal and parietal regions (Kim et al., 2003). However, it is not known to what extent these abnormalities relate to the presence of symptoms and disabilities that characterize schizophrenia, to medication effects, or to the presence/degree of genetic vulnerability. Since the EHRS examines a large number of young adults at high risk of schizophrenia who were not suffering from psychotic illness when assessed nor receiving medication, we were able to investigate these issues in a situation uncontaminated by the effects of established illness or anti-psychotic medication.

In this study, we sought to test three specific hypotheses regarding altered functional connectivity in high risk subjects. By the term ‘trait-related’ effects, we refer to presumed genetically mediated differences occurring in all high risk subjects, regardless of the presence of any psychotic symptoms, on account of them all having close relatives with the established condition. The term ‘state-related’ effect is used here to refer to differences occurring only in those displaying some of the characteristic feature of the illness, i.e. those with isolated psychotic symptoms. It should also be noted that, at the time of the scan, none of the study participants met criteria for schizophrenia. Therefore, the term ‘state’ effects is used to describe partial phenotypic expression of some of the characteristic symptoms of the disorder, rather than full phenotypic expression of the disease.

The three hypotheses tested were:

(i) State-related abnormalities (decreases or increases) exist in dorsolateral prefrontal to superior/middle temporal gyrus connectivity in those at high risk with psychotic symptoms as reported in previous studies of established schizophrenia (Frith et al., 1995; Friston et al., 1996; Lawrie et al., 2002). Fronto-temporal disconnectivity was hypothesized to be a state-related rather than trait-related effect since it had previously been associated with psychotic symptoms (Lawrie et al., 2002) and had not been found in asymptomatic subjects at high genetic risk (Spence et al., 2000).

(ii) Trait-related reduced connectivity exists in a medial prefrontal-thalamus–cerebellar network.

(iii) Increased connectivity between dorsolateral prefrontal to inferior parietal lobule exists in high risk subjects, to a greater extent in those with psychotic symptoms.

Hypotheses (ii) and (iii) are based on our previous localization study in high risk subjects (Whalley et al., 2004).

Methods

Subject details

We report functional connectivity results from the first 100 subjects assessed as part of the EHRS from 1999 to mid-2002. Details of the sample and localization results for the Hayling task have been presented previously (Whalley et al., 2004). Participants were aged between 10–15% of the sample were predicted to develop schizophrenia by the age of 30 years (Johnstone et al., 2002). Previously, functional MRI (fMRI) in the EHRS subjects using a covert verbal initiation paradigm (the Hayling task) demonstrated localized deficits in prefrontal–thalamic–cerebellar activation in those at high risk compared with normal controls and demonstrated increased inferior parietal cortex activity, particularly but not exclusively in those with psychotic symptoms (Whalley et al., 2004). Although it is generally accepted that schizophrenia is associated with neuronal deficits, it has not been possible to explain the complex features of the disorder on the basis of specific, localized, structural or functional abnormalities. This has led to the suggestion that the basis of the illness could lie in abnormal interactions, or disconnectivity, between a distributed network of brain regions (Gold and Weinberger, 1991; Friston and Frith, 1995). In neuroimaging, functional connectivity has been defined as cross correlations over time between spatially remote brain regions (Friston et al., 1993a,b). Early connectivity studies examined correlations between spontaneously active regions in normal subjects during the resting state (Lowe et al., 1998). Later studies used conventional task activation methods, for example by examining correlations between regional time courses over periods of task and baseline conditions. However if, for example, two regions are highly activated by a task, they will inevitably be seen to be co-active and hence viewed as functionally connected (Xiong et al., 1999). In this case, task related co-activity may therefore provide little more information than standard activation maps.

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16–25 years when first recruited in the initial phase of the EHRs (1994–1999) and had two or more first- or second-degree relatives with schizophrenia. Out of the first 100 subjects, six declined or were unable to participate in scanning. Two further subjects were excluded due to minor vascular malformations, which became apparent at the time of the scan, and an additional two subjects were excluded due to excessive movement (for more details, see Whalley et al., 2004). In total therefore, 90 subjects (21 normal controls and 69 high risk subjects) provided usable fMRI scans. The control group consisted of healthy volunteers who had no family history of psychotic illness. All subjects provided written informed consent and the study was approved by the Psychiatry and Clinical Psychology subcommittee of the Lothian Research Ethics committee.

On detailed interview (the Present State Examination; Wing et al., 1974) at the time of the scan, none of the EHRs subjects or controls met diagnostic criteria for any psychotic disorder. Twenty-seven high risk subjects reported isolated transient or partial psychotic symptoms; the remainder of the high risk group and all of the controls reported no psychotic symptoms. None of the subjects were on anti-psychotic medication, seeking treatment or indeed saw themselves as unwell. Group details have been presented previously (Whalley et al., 2004).

**Scanning procedure**

Imaging was carried out at the Brain Imaging Research Centre for Scotland (Edinburgh, Scotland, UK) on a GE 1.5 T Signa scanner (GE Medical, Milwaukee, WI, USA). A high resolution structural scan was acquired using a 3D T1-weighted sequence [inversion recovery preparation with an inversion time (TI) of 600 ms]. Functional data was acquired using an echoplanar imaging (EPI) sequence.

The Hayling task was acquired using the following parameters: axial orientation TR (repetition time)/TE (echo time) = 4000/40 ms; matrix 64 × 128; field of view (FOV) 22 × 44 cm²; 38 slices; 5 mm slice thickness; no gap. A total of 204 volumes were acquired. The first four volumes of each acquisition were discarded. Visual stimuli were presented using a screen (IFIS, MRI Devices, Waukesha, WI, USA) placed in the bore of the magnet; corrective lenses were used where necessary. Analysis was carried out using SPM99 (Wellcome Department of Cognitive Neurology and collaborators, Institute of Neurology, University College London, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). Data were first realigned to correct for head movement, normalized to the standard EPI template and smoothed with a $6 \times 6 \times 6$ mm³ full-width at half maximum (FWHM) Gaussian filter.

**Experimental details**

Details of the Hayling task have been presented previously (Whalley et al., 2004). Briefly, subjects were shown sentences with the last word missing and were asked to think of an appropriate word to complete the sentence (without speaking the word), and then press a button when they had done so. Sentences were presented in blocks of fixed difficulty, of which there were four levels. The rest condition consisted of viewing a screen of white circles on a black background. The order of the blocks was pseudo-random and each block was repeated four times (different sentences were used for each sentence block). Immediately after scanning, subjects were given the same sequence of sentences on paper and requested to complete each sentence with the word they first thought of in the scanner. ‘Word appropriateness’ scores were determined from the word frequency list of sentence completion norms (Bloom and Fischler, 1980). For a number of subjects (six controls, 13 high risk subjects), no reaction time measures were recorded in the scanner. Since these subjects presented activation patterns consistent with performance of the task and since they indicated at debriefing that they had indeed performed the task in the scanner, they were included in the analysis.

**Functional connectivity analysis**

### Seed selection

To address our three specific hypotheses, voxels were identified in the bilateral dorsolateral prefrontal cortex (DLPFC), superior/middle temporal gyrus (S/MTG), medial frontal gyrus (MedFG), thalamus and inferior parietal lobule (IPL). Seeds located in the DLPFC and S/MTG were selected based on regions involved in the task, with reference to seed regions which had been used previously (Lawrie et al., 2002) and in accordance with the standard Talairach and Tournoux atlas (Talairach and Tournoux, 1988). Voxels in bilateral MedFG, thalamus and IPL were selected in accordance with our previous group difference findings in these subjects (Whalley et al., 2004). Coordinates for the seed regions are detailed in Table 1.

**First level analysis**

The functional connectivity analysis methods have been published previously (Deary et al., 2004). In summary, maps of cross-correlation coefficients were computed by measuring the correlation (in time) between each ‘seed’ brain region (a small sphere of 6 mm radius) and all the other brain voxels. Before computing the cross-correlations, the voxel time courses were filtered in time (high pass filter: cut off period of 400 s; low pass filter: convolution with a Gaussian with 4 s FWHM) and corrected for global signal fluctuations by global scaling of the images. Maps at this stage are referred to as ‘time filtered only’ and correspond to the data in which variance associated with task effects remained.

To remove cross correlations purely induced by task related effects, the task conditions were modelled with standard block effects and convolved with the canonical haemodynamical response function in SPM99. This model, which also included the six regressors for the estimated head movement, was fitted to the time filtered data and the residuals of this procedure were used to compute the cross-correlations maps. These maps are referred to a ‘model filtered’ and correspond to the data where variance associated with task effects has been removed.

Since the distribution of cross-correlation values are not normally distributed, both the time filtered only and model filtered functional connectivity maps were transformed using the r-to-z Fisher transform to permit further statistical analysis. The mean and standard deviation of each corrected map were then estimated and each map was rescaled to zero mean and unity standard deviation (Lowe et al., 1998; Hampson et al., 2002; Deary et al., 2004). Corrected maps were

**Table I Coordinates of seed regions**

<table>
<thead>
<tr>
<th>Seed region</th>
<th>Coordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLPFC, BA9/46: left/right</td>
<td>±(40, 32, 21)</td>
</tr>
<tr>
<td>S/MTG, BA21: left/right</td>
<td>±(53, -44, 6)</td>
</tr>
<tr>
<td>MedFG, BA10/32: left/right</td>
<td>±(14, 47, 0)</td>
</tr>
<tr>
<td>Thalamus: left/right</td>
<td>±(8, -13, 6)</td>
</tr>
<tr>
<td>IPL, BA40/7: left/right</td>
<td>±(42, -48, 48)</td>
</tr>
</tbody>
</table>

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Finally smoothed with a Gaussian kernel of $6 \times 6 \times 6 \text{ mm}^3$. After the processing steps, these values are now in arbitrary units where positive values refer to positive correlations, zero refers to no observed correlation, and negative values refer to negative correlations.

**Second level analysis**

Within group analysis was performed for each of the seeds using one sample t-tests examining both positive and negative correlations. For the controls and high risk with psychotic symptom groups, a threshold of $P < 0.05$ (corrected for multiple comparisons) minimum cluster size of $k = 20$ voxels was used. For the high risk without psychotic symptoms group, since this was a larger study group and hence the size of the clusters tended to be greater, a more stringent threshold was used: $P < 0.001$ (corrected for multiple comparisons) minimum cluster size of $k = 20$ voxels. For each of the three groups, only those results with $Z$ scores $>5$ are reported.

Analysis of group differences was performed for each of the seeds in the study using ANCOVA (analysis of covariance). Differences in activation due to genetic ‘trait’ effects were initially examined by comparing controls versus all high risk subjects. Symptomatic ‘state’ effects were initially examined by comparing the non-symptomatic groups (controls plus high risk without psychotic symptoms) versus high risk subjects with psychotic symptoms. This analysis structure was chosen to simplify the reporting of results and to minimize the number of group comparisons. The contrast maps were first thresholded at $P < 0.01$ (uncorrected for multiple comparison) and minimum cluster size $k = 50$. Regions were considered significant at $P < 0.05$ cluster level, corrected for multiple comparisons.

To further clarify any potential genetically mediated or symptom related findings, post hoc group comparisons were conducted (as in Whalley et al., 2004). The criteria we followed for differences to be identified as potential trait-specific effects were that similar differences should be found between (a) controls versus high risk with symptoms and (b) controls versus high risk without symptoms but not between (c) high risk with symptoms versus high risk without symptoms. Criteria for any potential state-specific effects were that similar differences should be found between (a) high risk with symptoms versus controls and (b) high risk with symptoms versus high risk without symptoms but not between (c) high risk without symptoms versus controls. Inclusive masks based on the main analysis clusters were used to determine unambiguously if similar differences were found in the more detailed group comparisons. These values are detailed in the respective results tables. All $P$ values quoted in the text and tables are at the corrected cluster level. Coordinates were converted from MNI (Montreal Neurological Institute) to Talairach coordinates using a non-linear transformation (MRC Cognition and Brain Sciences Unit, University of Cambridge, Cambridge, UK; http://www.mrc-cbu.cam.ac.uk/Imaging/Common/).

**Results**

**Demographic and performance details**

There were no significant differences in mean age, gender, mean National Adult Reading Test intelligence quotient (NART IQ) or handedness between the groups (see Whalley et al., 2004). Behavioural measures, described previously by Whalley et al. (2004) indicated that subjects were performing the tasks appropriately in the scanner. There were no significant differences in terms of performance between the groups.

**Localization data**

Activation maps for these subjects performing the Hayling task have been presented previously (Whalley et al., 2004). For sentence completion versus rest all groups demonstrated activation in regions commonly associated with self generated word production tasks, i.e. dorsolateral and medial prefrontal regions and superior/middle temporal gyrus (Frith et al., 1995; Nathaniel-James et al., 1997; Lawrie et al., 2002). In addition, the high risk with psychotic symptoms group demonstrated increased activation of the left inferior parietal lobule. The effects of task difficulty were also consistent with similar studies reporting involvement of lateral and medial prefrontal regions (Lawrie et al., 2002; Nathaniel-James and Frith, 2002). On group comparison for this contrast, the high risk subjects demonstrated reduced activation of medial prefrontal, thalamic and cerebellar regions in response to increasing task difficulty.

**Functional connectivity: within groups**

Most seed regions demonstrated connectivity to contralateral homologous regions. Additional connections are detailed below. Due to space restrictions, only the within group connectivity patterns pertaining specifically to the outlined hypothesized networks are described. Full details are available as supplementary online material/available on request. The terms ‘high risk with or without symptoms’ refers specifically to psychotic symptoms.

**Time filtered only data**

**Prefrontal-temporal connectivity**

All three groups showed negative correlations between the seeds located in bilateral DLPFC and contralateral lateral temporal regions. For the seed located in the left MTG, negative correlations were observed with right middle frontal gyrus [Brodmann Area (BA) 8 and 10 for the controls, and BA6 for the high risk groups]. For the right MTG seed, negative correlations were seen with the left middle frontal gyrus in the high risk groups only (BA8 and 10).

**Medial prefrontal-thalamic-cerebellar connectivity**

For the seeds centred on the left MedFG, negative correlations were observed only in the high risk groups with bilateral cerebellar regions and the thalamus (this formed part of a larger cluster centred in the midline cerebellum in the high risk with psychotic symptoms group). This pattern of connectivity was not seen in the controls. For the right MedFG seed, negative correlations were seen in all three groups with bilateral cerebellar regions. Negative correlations with thalamic regions were, however, only seen in the high risk without symptoms group at the chosen significance levels.
Non-significant negative correlations with the thalamus were apparent in the high risk with psychotic symptoms group ($Z$ score = 4.49), but were not evident in the control group. Thalamic seeds demonstrated negative correlations with bilateral medial frontal gyrus (BA10) in the high risk groups only.

**Prefrontal-parietal connectivity**

At the chosen levels of significance, seeds centred on the bilateral DLPFC demonstrated positive connectivity with ipsilateral parietal regions in the high risk groups but not in the control subjects. For the seed located in the left IPL, a large cluster centred on the seed location extended to regions of the left lateral prefrontal lobe in both of the high risk groups, which was not seen in the control group. A similar pattern was seen for the seed located in the right IPL, where positive correlations were seen in both high risk groups with right lateral prefrontal regions (this formed part of a larger cluster centred on the seed location for the high risk with symptoms group, but was a separate cluster in the high risk without symptoms group). This pattern was not seen in the controls.

**Model filtered data**

**Prefrontal-temporal connectivity**

For the seed located in the left DLPFC, negative correlations were seen across all three groups with contralateral lateral temporal regions. For the right DLPFC seed, negative correlations with contralateral lateral temporal regions were observed only in the high risk without symptoms group. Additional negative correlations were also seen in this group between ipsilateral dorsolateral prefrontal regions and lateral temporal lobe. For the seed located in left MTG, negative correlations were demonstrated with medial/middle prefrontal region BA10 and orbitofrontal region BA11 in the high risk groups only. For the right sided seed in the MTG, no negative correlations were seen with lateral prefrontal regions in any of the groups at the chosen significance levels.

**Prefrontal-parietal connectivity**

As for the time filtered data, both high risk groups demonstrated positive correlations between the seed located in the left DLPFC and the left IPL. This formed part of a larger cluster centred on the seed location for the high risk without symptoms group. This pattern of connectivity was not seen in the control group (Fig. 1). For the right-sided DLPFC seed, positive correlations were seen across all three groups with right posterior parietal regions. The left IPL seed showed positive correlations with left inferior frontal gyrus across all three groups (Fig. 2). This figure demonstrates that the extent of the region in the lateral prefrontal region was smaller in the control group than in the high risk groups. For the right IPL seed, positive correlations were seen with right lateral prefrontal regions across all three groups.

**Functional connectivity: between groups**

For all analyses, only those results that fulfilled the more strict criteria for trait- or state-related effects are presented. Results for the time filtered only data are presented in Table 2. Regarding the first hypothesis of state-related increases or decreases in connectivity between DLPFC and MTG, this deficit was not seen in our high risk subjects with symptoms. For the second hypothesis of trait-related reductions in connectivity in the medial prefrontal–thalamic–cerebellar...
network, there was evidence of reduced connectivity between the left MedFG and the left cerebellum, although this did not meet criteria for statistical significance ($P = 0.108$). For hypothesis (iii), however, there was evidence for a statistically significant increased connectivity in the high risk subjects between the left IPL and the left inferior frontal gyrus (Fig. 3). Consistent with the within group maps described above, this figure indicates that both high risk groups presented higher positive values than the control group. This increased connectivity was only found in the trait contrast, i.e. high risk subjects versus the controls. Based on our previous findings, it was anticipated that this inter-regional connectivity may be increased to a greater degree in those with symptoms. Closer inspection of this prefrontal—parietal connectivity indicated significant increases across the three groups. Using a contrast weighting of $\{-3 1 2\}$ representing increases (across groups: controls, high risk without, and high risk with psychotic symptoms), this connectivity was found to be lowest in the controls, higher in those at high risk without symptoms and highest in those at high risk with symptoms ($P < 0.001$; $Z$ score = 5.08; $x = -44$, $y = 24$, $z = 17$; BA45), suggesting the presence of additional state components associated with this finding.

Results for the data with the variance associated with task effects removed, the ‘model filtered’ data, are presented in Table 3. As in the previous analysis, we found no evidence for disrupted connectivity between prefrontal and temporal regions in the high risk subjects. Regarding the second hypothesis, significant trait-related reductions in connectivity were found between the right MedFG and the left cerebellum, and at a trend level to the right cerebellum (Fig. 4A and C). Consistent with the within group maps, these indicated that both high risk groups demonstrated larger negative connectivity values between the right medial frontal region and cerebellum than the controls. There were also indications of trait-related reductions in connectivity between the left
Table 2  Functional connectivity: time filtered only data

<table>
<thead>
<tr>
<th>Seed location</th>
<th>P value (Z score)</th>
<th>Peak height coordinates</th>
<th>Region of connectivity difference</th>
<th>Post hoc P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypothesis (i): state-related differences in prefrontal–temporal connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State contrast: no symptoms &gt; symptoms: n/s</td>
<td>L MedFG, BA10/32</td>
<td>0.108</td>
<td>([-26 - 55 -14])</td>
<td>L cerebellum</td>
</tr>
<tr>
<td>State contrast: no symptoms &lt; symptoms: n/s</td>
<td>(-14 -47 0)</td>
<td>(3.80)</td>
<td>([-38 -74 -15])</td>
<td>L cerebellum</td>
</tr>
<tr>
<td><strong>Hypothesis (ii): trait-related decreases in medial prefrontal–thalamic–cerebellar connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trait contrast: controls &gt; high risk:</td>
<td>L MedFG, BA10/32</td>
<td>0.108</td>
<td>([-36 -63 -12])</td>
<td>L temporal: fusiform g, BA19</td>
</tr>
<tr>
<td>Trait contrast: controls &lt; high risk:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L IPL, BA40/7</td>
<td>0.001</td>
<td>([-44 24 18])</td>
<td>L frontal: inferior frontal g, BA 9/45</td>
<td></td>
</tr>
<tr>
<td>(-42 -48 48)</td>
<td>(3.87)</td>
<td>([-39 14 44])</td>
<td>L frontal: middle frontal g, BA6</td>
<td></td>
</tr>
<tr>
<td>([-43 18 24])</td>
<td>([-36 18 24])</td>
<td>L frontal: inferior/mid frontal g,</td>
<td></td>
<td></td>
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</tbody>
</table>

Only significant differences relating to specific hypotheses are reported. c = controls; hr_n = high risk without psychotic symptoms; hr_p = high risk with psychotic symptoms. P values pertain to each cluster reported. Each cluster comprises several maxima corresponding to different regions within the cluster. Since these give an indication of the extent of the cluster, all three are detailed in the table. n/s = not significant.

**Discussion**

We have examined functional connectivity in a large group of anti-psychotic naive individuals at high genetic risk of schizophrenia. Regarding the within group maps connectivity was generally demonstrated between the seed region and homologous region in the contralateral hemisphere. This is consistent with other studies of functional connectivity in normal subjects (Bowen et al, 1995; Stein et al, 2000; Koch et al, 2002). In addition, these maps indicated that there were prefrontal–cingulate connectivity hubs in the frontal lobes. We believe that this connectivity was not found in the present study. Unlike previous findings in the established state using a similar version of the verbal initiation task (Lawrie et al, 2002), disconnectivity between lateral prefrontal to lateral temporal disconnection was not found in the present study. Unlike previous findings in the established state using a similar version of the verbal initiation task (Lawrie et al, 2002), disconnectivity between lateral prefrontal to lateral temporal disconnection was not found in the present study. Unlike previous findings in the established state using a similar version of the verbal initiation task (Lawrie et al, 2002), disconnectivity between lateral prefrontal to lateral temporal disconnection was not found in the present study.
### Table 3  Functional connectivity: model filtered data

<table>
<thead>
<tr>
<th>Seed location</th>
<th>P value (Z score)</th>
<th>Peak height coordinates</th>
<th>Region of connectivity difference</th>
<th>Post hoc P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypothesis (i): state-related differences in prefrontal–temporal connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State contrast: no symptoms &gt; symptoms: n/s</td>
<td></td>
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<tr>
<td>State contrast: no symptoms &lt; symptoms: n/s</td>
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<tr>
<td><strong>Hypothesis (ii): trait-related decreases in medial prefrontal-thalamic–cerebellar connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trait contrast: controls &gt; high risk:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R MedFG, BA10/32 (14 47 12)</td>
<td>&lt;0.001 (4.28)</td>
<td>–30 –51 –16</td>
<td>L cerebellum: anterior lobe</td>
<td>&lt;0.001; &lt;0.001; n/s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–16 –54 –16</td>
<td>L cerebellum: anterior lobe</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>–6 –66 –16</td>
<td>L cerebellum: posterior lobe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.081 (3.18)</td>
<td>30 –58 –18</td>
<td>R cerebellum: anterior lobe</td>
<td>0.007; 0.023; n/s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 –65 –26</td>
<td>R cerebellum: anterior lobe</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>32 –56 –5</td>
<td>R temporal fusiform g, BA 37</td>
<td></td>
</tr>
<tr>
<td>L thalamus (–8 –13 6)</td>
<td>0.056 (4.65)</td>
<td>34 26 –24</td>
<td>R frontal: inferior frontal g, BA11</td>
<td>0.003; 0.004; n/s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 18 –16</td>
<td>R frontal: inferior frontal g, BA47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 10 –11</td>
<td>R frontal: inferior frontal g</td>
<td></td>
</tr>
<tr>
<td><strong>Hypothesis (iii): state- or trait-related increases in prefrontal–parietal connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State contrast: no symptoms &lt; symptoms: n/s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trait contrast: controls &lt; high risk:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L IPL, BA40/7</td>
<td>0.008</td>
<td>–50 30 14</td>
<td>L frontal: inferior frontal g, BA9/46</td>
<td>0.001; 0.007; n/s</td>
</tr>
<tr>
<td>(–42 –48 48)</td>
<td>(3.75)</td>
<td>–45 27 17</td>
<td>L frontal: inferior frontal g, BA45</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>–38 32 –3</td>
<td>L frontal: inferior frontal g</td>
<td></td>
</tr>
</tbody>
</table>

Only significant differences relating to specific hypotheses are reported. c = controls; hr_n = high risk without psychotic symptoms; hr_p = high risk with psychotic symptoms. P values pertain to each cluster reported. Each cluster comprises several maxima corresponding to different regions within the cluster. Since these give an indication of the extent of the cluster, all three are detailed in the table. n/s = not significant.
study. This is, however, consistent with the evidence from the within group maps where all groups demonstrated the normal inverse relationship between activity in the dorsolateral prefrontal cortex and lateral temporal lobe. Since none of our high risk subjects met criteria for schizophrenia, this finding may indicate that such disconnectivity is found only in those with the established condition. Indeed, there is evidence to suggest that lateral prefrontal to lateral temporal disconnectivity may be associated specifically with the presence of active symptomatology in the established state and, in
reported reduced effective connectivity between the left lateral prefrontal and right cerebellar regions in medicated subjects in the established state (Schlösser et al., 2003). That the involvement of the lateral prefrontal regions reported here occurred in un-medicated high risk individuals indicates that this is not an effect attributable to anti-psychotic medication. The study by Schlösser and colleagues also reported increased connectivity from the thalamus to left lateral prefrontal regions, which was considered to compensate for the decreased prefrontal–cerebellar connectivity. Such increases in prefrontal–thalamic connectivity were not seen here. Unlike the present study, however, the subjects in the report by Schlösser and colleagues exhibited significant performance differences between groups, which may account for this discrepancy. Perhaps it is the case that a compensatory increase in thalamic input to prefrontal cortex only becomes apparent when significant performance deficits emerge.

As hypothesized, increased connectivity between left dorsolateral prefrontal and inferior parietal regions was found in high risk subjects in both analyses. This was reflected in the within group maps as larger positive correlations between these regions in the high risk subjects. The expected state-related increases in prefrontal–parietal connectivity specifically in those with psychotic symptoms were not found, however, but increases across groups indicated an interaction between trait and state effects. Activity in these two regions has been reported to be inter-dependent, and they are strongly connected anatomically (Petrides and Pandya, 1984). Increased prefronto–parietal connectivity is also consistent with our previous functional imaging finding in high risk individuals where activation in the parietal cortex was elevated (particularly, but not exclusively, in those with psychotic symptoms (Whalley et al., 2004)). Since there were no differences in performance between the groups, this was interpreted as compensatory, and the increased connectivity seen here may represent neural processes associated with this mechanism. This suggestion is consistent with another functional imaging study in normal controls that reported increased connectivity between inferior frontal and parietal regions in response to increasing working memory load and consequently increased demand on executive resources (Honey et al., 2002), and with another study suggesting that increases in parietal cortex activation in schizophrenic subjects acts as compensatory mechanism for prefrontal cortex dysfunction in a working memory task (Quintana et al., 2003). An earlier study also reported connectivity between these structures in normal controls during a semantic decision task and suggested this fronto-parietal connection may be important in mediating subvocal articulatory rehearsal in working memory or self-monitoring tasks (Bullmore et al., 2000; see also Becker et al., 1999). Disruption in the neural integrity of the arcuate fasciculus (the white matter tract connecting these regions) as measured with diffusion tensor imaging has also been reported in established schizophrenia (Burns et al., 2003). The causal sequence of events within this parietal–prefrontal network in the high risk subjects is impossible to determine.

Fig. 5 Example of activation over time after model filtering. The model filtered activation time course (in arbitrary units over seconds) is shown from the left IPL lobe seed, on the low constraint condition of the task, for high risk subjects with psychotic symptoms (blue), high risk without (green) and controls (red), with the ‘ideal’ time course in light blue. The figure demonstrates a similarly successful modelling out of activity across all three groups.
with functional connectivity approaches, but effective connectivity techniques may be able to provide more insight into this abnormal pattern of connectivity.

There are two main methodological issues to consider regarding the current study. First, the connectivity analysis was conducted with and without the variance associated with task effects removed from the data. Addressing task associated variance is a relatively novel approach to examining functional connectivity. In this study, this was conducted primarily to identify differences that were not simply task activation specific and those that were over and above those attributable to the localization data. It has also been suggested that examining connectivity that is not modulated by transient activity may be more related to underlying anatomical connectivity (Koch et al., 2002). We may have anticipated finding more group differences in the data with variance associated with task effects remaining, but this was not the case. One possible explanation is that, in the time filtered only data, the large fluctuations associated with task effects were masking more modest connectivity differences, and the removal of variance associated with these task effects allowed more subtle group differences to be detected, as in the abnormal prefrontal–(thalamic)–cerebellar connectivity.

Secondly, as discussed by Calhoun et al. (2004), it should be appreciated that connectivity data with variance associated with task effects removed could represent truly task-independent effects or could reflect effects which are still modulated to some degree by the task. Regardless, it is unlikely the current data is equivalent to examining subjects in the resting state, since at rest mental activity is completely unconstrained. In effect, we have identified functional disconnectivities in subjects at high risk of schizophrenia which are over and above those attributable to the large transient changes associated with task related activation and which cannot be attributed to other potential confounders such as task performance differences between groups, medication or disease effects.

In summary, we found no evidence for state-related fronto-temporal disconnectivity in high risk subjects. However, these findings do support the hypothesis of genetically mediated reduced connectivity in medial prefrontal–(thalamic)–cerebellar circuits and trait/state related increased connectivity between prefrontal–parietal regions. Since these are found in subjects at enhanced risk, are not confounded by antipsychotic medication, by the effects of the illness or by performance differences between groups, these connectivity abnormalities may represent the neurophysiological basis of the diverse deficits seen in the established state and in individuals at high risk of schizophrenia (Byrne et al., 2003).

Acknowledgements

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References


Bloom PA, Fischler I. Completion norms for 329 sentence contexts. Memory Cogn 1980; 8: 631–42.


Ford JM, Mathalon DH, Whitfield S, Faustman WO, Roth WT. Reduced communication between frontal and temporal lobes during talking in schizophrenia. Biol Psychiatry 2002; 51: 485–92.


