Diagnostic and Symptom Quantification Procedures

Masters or Doctoral-level clinicians conducted diagnostic evaluations and clinical ratings. Diagnoses were confirmed by consensus conference. Among a random sample of 10 subjects, the level of inter-rater agreement for diagnosis was high, with kappa = 0.86. Symptoms were quantified with the Brief Psychiatric Rating Scale (BPRS), Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS). Inter-rater agreements as quantified by intraclass correlation were 0.93, 0.96 and 0.81 respectively, p < 0.05 for all. The total SANS and SAPS scores were used to quantify negative symptoms and psychosis severity respectively. Level of disorganization was quantified by adding the following sub-scores: Conceptual Disorganization, Mannerism and Posturing, and Disorientation from the BPRS; Attention from SANS; Formal Thought Disorder, and Bizarre Behaviour from SAPS (1).

Controlling for Movement

The following procedures were applied to minimize the effects of head movement on functional magnetic resonance imaging (fMRI) data. In the first-level fMRI regression matrix, we included three rotational (roll, pitch, and yaw) and three linear parameters (x, y and z) characterizing scan-to-scan head movement during scanning as covariates of non-interest. This procedure ensured that the regions identified by the between group contrasts were ones with significant differences in group activity after taking into account scan-to-scan movement. For the functional connectivity analyses, we controlled for movement in a manner analogous to what was done in the univariate analysis. We included each subject’s six total scan-to-scan movement parameters as covariates of non-interest.
movement parameters as covariates of non-interest in the statistical models comparing functional connectivity levels between groups.

The group means and standard deviations for the six movement parameters were: \( x_C = 0.0107 \pm 0.0089 \text{ mm/scan}; \) \( y_C = 0.0357 \pm 0.0260 \text{ mm/scan}; \) \( z_C = 0.0384 \pm 0.0466 \text{ mm/scan}; \) \( \text{roll}_C = 0.0005 \pm 0.0005 \text{ degrees/scan}; \) \( \text{pitch}_C = 0.0003 \pm 0.0002 \text{ degrees/scan}; \) \( \text{jaw}_C = 0.0002 \pm 0.0002 \text{ degrees/scan}; \) \( x_{SZ} = 0.0155 \pm 0.0086 \text{ mm/scan}; \) \( y_{SZ} = 0.0452 \pm 0.0294 \text{ mm/scan}; \) \( z_{SZ} = 0.0626 \pm 0.0531 \text{ mm/scan}; \) \( \text{roll}_{SZ} = 0.0009 \pm 0.0006 \text{ degrees/scan}; \) \( \text{pitch}_{SZ} = 0.0004 \pm 0.0002 \text{ degrees/scan}; \) \( \text{jaw}_{SZ} = 0.0003 \pm 0.0002 \text{ degrees/scan}. \)

**Beta-Series Functional Connectivity**

We utilized the beta series method to measure functional connectivity between regions of interest (ROIs) (2, 3). Whereas a traditional univariate analysis requires a total of three covariates to model the main stages of working memory (WM) (encoding, maintenance, and response) across all trials, a beta-series analysis entails modeling each task phases of every trial with unique hemodynamic response function-convolved covariates to generate beta estimates of every trial event. Thus, for subjects completing a total of 50 trials, 150 covariates were included in the generalized linear model. The resulting trial and task phase-specific beta values were sorted according to task phase, e.g. encoding, delay or response, to form a beta-series for each task stage. A voxel’s beta-series reflects its trial-to-trial variability. Regions whose beta-series are correlated are inferred to be functionally interacting. Correlation computations are performed separately on data from each individual subject and group means of these correlations were used for inferential testing. The magnitude of functional connectivity between two regions was quantified with the means of a Pearson’s correlation of their beta series. All correlation analyses were conducted in SPM5 with custom MATLAB (MathWorks, Natick, MA) scripts. While all trials were modeled, betas from only correct trials were included in
the correlation analysis. In this experiment, we focused on the response phase based on the fact that all the significant univariate results were restricted to this task phase.

**Localization of Cortical ROIs from Independent Scans for Functional Connectivity Analyses**

Identifying ROIs based on group differences in activity and analyzing the same scans in the functional connectivity analyses with these ROIs may contribute to either Type I or Type II error, depending on the combination of the ROIs in question. Since the prefrontal cortex (PFC) and substantia nigra (SN) regions shown in Figure 1 were identified on the basis that the former showed greater and the latter less activity in controls compared to patients, the pairing of these ROIs in a functional connectivity analysis could contribute to a Type I error in finding a group difference in PFC-SN connectivity. In the case of the PFC and caudate regions, the fact that they were identified on the basis that both showed lower activity in patients, their pairing could produce a bias against finding a true group difference in PFC-caudate connectivity. Consequently, we did not use the PFC region identified by the univariate contrast in the functional connectivity analyses. Instead, we utilized a PFC ROI that was localized from independent fMRI data so that we could run unbiased PFC-basal ganglia functional connectivity analyses. These independent fMRI data were generated from a 1-back face WM experiment, which all subjects completed. We also identified the fusiform face area (FFA), a visual cortical region specialized for processing faces, from this independent data so that we could determine the specificity of the PFC connectivity findings. These PFC and FFA ROIs have been designated with the prefix “Loc” to emphasize their independent localization (LocPFC and LocFFA, Figure S2). In the 1-back WM experiment, subjects viewed exemplars of four categories of visual objects, including faces, grouped in blocks. The contrast of face blocks vs. baseline constrained by the union of anatomic masks of the right inferior frontal gyrus and middle frontal gyrus identified LocPFC ROIs. The contrast of face blocks vs. all other visual
category blocks constrained by the union of parahippocampal, lingual and fusiform gyri identified the LocFFA ROI in the ventral visual cortex. Both contrasts were conducted within each group so that group-specific ROIs were identified. We derived group-specific ROIs to mitigate the possibility of group differences in location of peak activity affecting our results. We repeated our analyses using ROIs derived by combining all subjects into one group and we obtained essentially identical results. To verify that the LocPFC and LocFFA ROIs are engaged by the delayed-response WM task, we obtained trial-averaged blood oxygen level-dependent time series of these regions during this task. Both groups demonstrated robust and nearly identical patterns of task related activity, with no differences at any time point during the response period, \( p > 0.4 \), or the entire trial, \( p > 0.15 \), Figure S2.

**Methodological Factors Affecting the Detectability of SN Hyperactivity in Schizophrenia**

There are two major methodological factors impacting the detectability of SN hyperactivity in schizophrenia, which may explain, in part, why this finding has not been observed in prior fMRI studies. The first factor relates to the apparent task phase specificity of this hyperactivity. We observed statistically significant hyperactivity only during the response phase of WM. Most prior fMRI studies in schizophrenia did not employ event-related designs and instead they utilized block designs. A recent meta-analysis revealed that of 41 studies examining executive function in schizophrenia using fMRI, only 8 were conducted using event-related designs (4). Consequently, hyperactivity of the SN may have been undetected because prior studies largely employed analytic methods less sensitive to detecting task phase specific activity.

The second major factor relates to the scale of spatial smoothing of fMRI scans. The vast majority of fMRI studies in schizophrenia have applied standard spatial smoothing protocols, calling for smoothing kernels with full width half maximal or equivalent dimensions of 8 mm or larger. These protocols are optimized for the detection of larger cortical activations but
large smoothing kernels can strongly obscure smaller activations occurring in subcortical regions. The impact of smoothing kernel size on SN hyperactivity detectability is illustrated in Figure S1. We see that the activation peaks in the SN region in a group contrast map produced from 8 mm smoothed images are markedly diminished compared to the peaks in a contrast map generated from 2 mm smoothed images. These results vividly illustrate the possibility that SN hyperactivity in schizophrenia may be present in other data sets but that optimal image smoothing may be required to uncover this hyperactivity. All of the eight event-related fMRI studies of executive function in schizophrenia identified in the meta-analysis (4) used an 8 mm smoothing kernel.

**Table S1.** List of psychotropic medications taken by schizophrenia subjects.

<table>
<thead>
<tr>
<th>Medications</th>
<th>n</th>
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<tbody>
<tr>
<td>Atypical Antipsychotics</td>
<td>17</td>
</tr>
<tr>
<td>Typical Antipsychotics</td>
<td>1</td>
</tr>
<tr>
<td>Selective Serotonin Reuptake Inhibitors</td>
<td>7</td>
</tr>
<tr>
<td>Mood Stabilizers</td>
<td>3</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>2</td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>1</td>
</tr>
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Figure S1. The impact of smoothing kernel scale on SN hyperactivity detectability. Unthresholded between-group t-contrast maps (C > SZ and SZ > C maps superimposed on each other) derived from echo planar imaging processed with two different smoothing kernel sizes. (A) Contrast maps generated from images smoothed with a 2 mm kernel, show discrete regions of SZ hyperactivity (red clusters indicated by white arrows) in and around the SN which are surrounded by regions of C > SZ (blue) activity. The maximal color-coding of the t-values has been set to ± 2.5 for illustration purposes and these values are different than those used in Figure 1. (B) Smoothing with a larger 8 mm kernel resulted in decreased magnitude of both SZ > C and C > SZ activity in the midbrain. (C) Left panel: SZ > C contrast maps derived from 8 mm smoothed images thresholded at $t = 2.5$ revealed no voxels with t-values greater than 2.5. Right panel: Only when the threshold applied to the SZ > C map is lowered to $t = .75$ does any region showing SZ > C activity is revealed. C, control; L, left; R, right; SN, substantia nigra; SZ, schizophrenia.
**Figure S2. Cortical ROIs localized from independent fMRI scans.** Cortical ROIs (LocPFC and LocFFA) involved in face WM were localized using independent fMRI scans to avoid biasing functional connectivity results. The surface rendering of group specific cortical ROIs, LocPFC (A) and LocFFA (C); healthy control (blue) and SZ (red) groups, with regions in purple representing areas of overlap between these group ROIs. (B and D) Trial averaged BOLD time series from the LocPFC and LocFFA showing robust activity in these regions during the delayed-response WM task for both groups. Red and blue lines represent SZ and C groups respectively. Error bars indicate SEM. BOLD, blood oxygen level-dependent; C, control; L, left; LocFFA, localized fusiform face area; LocPFC, localized prefrontal cortex; R, right; ROI, region of interest; SZ, schizophrenia; WM, working memory.
Supplemental References


