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Altered Cerebral Response During Cognitive Control: A Potential Indicator of Genetic Liability for Schizophrenia

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Aberrant activity in brain regions underlying various aspects of executive cognition has been reported in patients with schizophrenia and in their healthy relatives, suggesting an association with genetic liability. The aim of this study was to investigate brain responses to selective aspects of cognitive control in unaffected siblings who are at increased genetic risk of schizophrenia. Altogether, 65 non-affected siblings, 70 patients with schizophrenia spectrum disorders, and 235 normal controls participated in this study. Blood-oxygen-level-dependent functional magnetic resonance imaging was conducted while participants performed a cognitive control task ('flanker task') to identify brain activity and connectivity associated with response inhibition and conflict monitoring, and suppression. Behaviorally, similar to patients with schizophrenia, siblings were less accurate when inhibiting prepotent responses relative to normal controls. During response inhibition, again similar to patients with schizophrenia, siblings showed decreased activity in the anterior cingulate (ACC), along with increased functional coupling with the dorsolateral prefrontal cortex (PFC) when compared to normal controls. Our findings show altered ACC activity and PFC connectivity in unaffected siblings and patients with schizophrenia during response inhibition. These results suggest that such changes in the neural activity underlying aspects of cognitive control may represent a potential intermediate phenotype for the investigation of the genetic basis of schizophrenia.
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INTRODUCTION

Genetic studies using complex phenotypes such as clinical diagnosis or symptom dimensions have provided few and inconsistent results on the genetic variants associated with schizophrenia. An alternative approach is the use of intermediate phenotypes (Gottesman and Gould, 2003). They are narrower biological constructs that are closer to the effects of the risk genes than the diagnosis itself. They include biological markers that are associated with the disorder and are expressed more frequently in non-affected family members than in the general population (Gottesman *et al*, 2003; Tan *et al*, 2008). The idea that the effects of genes on neuroimaging-based susceptibility-related phenotypes have greater penetrance for the identification of genetic effects led to the development of the 'imaging genetics' approach (Weinberger *et al*, 2001).

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Cognitive function has emerged as an attractive intermediate phenotype for schizophrenia for multiple reasons, including its objective measurement, relative clinical stability during the course of illness, impact on disability, heritability, and link to genetic risk (Toulopoulou *et al*, 2010). Although cognitive symptoms are core features of schizophrenia (Goldberg and Weinberger, 1988), they represent a complex construct *per se*. Altered cognitive control has been frequently reported in this disorder (Carter *et al*, 2001; Goldberg *et al*, 1988). Cognitive control allows adaptive variation of thoughts and behavior to current goals based on contextual information, and includes multiple cognitive processes such as response inhibition, interference control, attention, and working memory (WM). Twin and family studies have suggested a genetic substrate for cognitive control (Swan and Carmelli, 2002). Interestingly, heritability of cognitive control capacity has also been associated with liability for schizophrenia. Unaffected siblings (SIBs) of patients with schizophrenia show impaired performance on those tasks that tap cognitive control functions such as WM, set-shifting, and response inhibition (Goldberg *et al*, 1995). Recent results from a large study that included first-degree relatives including co-twins by Toulopoulou *et al*, (2010) suggest that a significant

portion of the phenotypic correlation between schizophrenia and cognitive measures can be explained by shared genetic effects.

Neuroimaging studies show that a network of brain regions including lateral-PFC, dorsal anterior cingulate (ACC), and parietal cortex mediates cognitive control (Badre and Wagner, 2004; Blasi *et al*, 2006; Kerns *et al*, 2004). PFC and parietal cortex are implicated in the dynamic tuning of cognitive control via top-down modulation of attentional processes and via response suppression, whereas ACC is responsible for detection and direct or PFC-mediated suppression of cognitive conflict (see Mansouri *et al*, 2009 for a review), and response inhibition (Swick and Turken, 2002). Furthermore, lateral-PFC and ACC are both anatomically (Koski and Paus, 2000) and functionally connected (Badre *et al*, 2004), and interact in regulating cognitive control during higher cognitive demands (Medalla and Barbas, 2009), particularly during response inhibition (Stevens *et al*, 2009).

Studies in patients with schizophrenia (SCZs) have shown altered function of these regions during cognitive tasks (Callicott *et al*, 2003). In particular, SCZs show reduced activity in ACC during commission errors for stimuli that invoked strong conflict (Carter *et al*, 2001), conflict resolution (Carter *et al*, 2001), and inhibition of prepotent responses (Fallgatter *et al*, 2003). This functional alteration in cognitive control regions in the context of altered performance may be due to the subjects not engaging or not being able to engage in the task at hand (Ford *et al*, 2004). Similarly, previous studies have shown that SCZs show prefrontal hyperactivity when compared to healthy subjects (NCs) with similar behavioral performance, but SCZs who fail to sustain the prefrontal network, reflected as prefrontal hypoactivity, manifest lower accuracy (Callicott *et al*, 2003; Manoach *et al*, 2001). There are also reports of the lack of a relationship between behavioral performance and prefrontal responses associated with cognitive control (Minzenberg *et al*, 2009). Thus, the role of performance differences on brain correlates underlying cognitive control needs to be clarified. Importantly, the extent to which altered activity in ACC during cognitive control and response inhibition, in particular, can indicate that genetic liability for schizophrenia has yet to be determined.

In the current study, we used functional resonance imaging (fMRI) to elucidate the role of cortical responses associated with cognitive control on genetic risk for schizophrenia. We focused on brain activity related to response inhibition and interference suppression, two crucial processes that underlie cognitive control. The former is the ability to inhibit prepotent behavioral responses that are premature, inappropriate or incorrect; the latter requires the capability to detect and filter out irrelevant or conflicting information. We tested the hypothesis that individuals at risk for schizophrenia when challenged with demands on cognitive control, similar to SCZs, would also be impaired at the behavioral level as well as at the neural level, which will be reflected as decreased recruitment of ACC and altered functional coupling of ACC with lateral-PFC.

MATERIALS AND METHODS

Subjects

The sample consisted of 370 subjects: 65 SIBs, 70 SCZs, and 235 NCs (Table 1). Subjects were recruited nationwide as part of an ongoing family study of schizophrenia at the Clinical Brain Disorders Branch Sibling Study (Protocol 95-M-0150) at the NIH. All of the patients had a diagnosis of schizophrenia-spectrum disorder, and 77.1% of them met the DSM-IV-TR diagnostic criteria for schizophrenia. Exclusion criteria are detailed in Supplementary Materials. A minority of SIBs had a past lifetime history of a non-psychotic mental illness and/or substance abuse and/or

Table 1 Demographics of the Sample

	Controls	Siblings	Patients	Difference
N	235	65	70	
Male:female ratio	113:122	24:41	47:23	$\chi^2 = 13.00$; $P = 0.002$
Age (M ± SD, years)	31.8 ± 9.6	36.6 ± 10.4	31.8 ± 9.5	F (2367) = 6.425; $P = 0.002$
WRAT (M ± SD)	109.3 ± 8.1	105.5 ± 9.7	102.5 ± 9.4	F (2367) = 18.511; $P < 0.001$
Handedness	74.2 ± 50.4	76.5 ± 49.8	82.5 ± 45.0	F (2367) = 0.77; $P = 0.46$
Bipolar[a]	—	1	—	—
Major depression[a]	—	17	5	—
Substance[a] abuse/dependence	—	4	17	—
Other axis I disorders[a]	—	10	—	—
PANSS positive	—	—	14.2 ± 6.6	—
PANSS negative	—	—	19.0 ± 9.9	—
PANSS general psychopathology			30.2 ± 10.5	
Total PANSS score			60.14 ± 21.0	
Antipsychotic treatment (M ± SD, CPZ equivalents)	—	—	553.3 ± 607.7	—

Abbreviations: M, mean; PANSS, Positive and Negative Syndrome Scale; RT, reaction time; SD, standard deviation; WRAT, Wide Range Achievement Test.
[a]Past psychiatric history.

dependence (see Supplementary Materials), but none met the DSM-IV-TR criteria at the time of evaluation and only five of the SIBs were receiving psychotropic medicines. Although the prevalence of smoking was expectedly frequent in SCZs, SIBs and NCs included a similar minority of smokers (<10% in each group). All participants gave written informed consent, approved by the Institutional Review Board of the National Institute of Mental Health, to take part in the experiment.

Task

All subjects performed a modified version of the flanker task (Blasi *et al*, 2006; see Supplementary Figure S1). Briefly, subjects saw a set of five symbols that included a central arrow pointing left or right, flanked by two pairs of symbols (arrows, boxes or X's), one on each side. This task included four experimental conditions: 'congruent', 'incongruent', 'neutral', and 'No-Go'. In all the conditions except 'No-Go', subjects were asked to indicate the direction of the central arrow by a button press as quickly and accurately as possible. In the 'congruent' condition, the central arrow was flanked by pairs of arrows orientated in the same direction. In the 'incongruent' condition, the flanking arrows were orientated in a direction opposite to the central arrow to evaluate interference monitoring and suppression. In the 'neutral' condition pairs of boxes flanked the central arrow. In the 'No-Go' condition two pairs of X's flanking the central arrow required subjects to withhold their motor response and served to evaluate response inhibition. Each trial was presented for 800 ms and a fixation crosshair (inter-trial-interval = 2200–5200 ms) was shown in between. A total of 145 pseudorandomized trials (No-Go/neutral/incongruent/congruent = 33/31/40/41) were presented. Performance was recorded through a fiber-optic response box, which allowed the measurement of correct responses and their reaction time (RT).

Image Acquisition

Blood-oxygen-level-dependent (BOLD)-fMRI was performed on a GE Signa3.0 Tesla magnet. A gradient echo BOLD-echo-planar imaging sequence was used to acquire 300 images. Each image consisted of 26 4-mm-thick axial slices, covering the entire cerebrum and most of the cerebellum (TR/TE = 2000/28 ms; FOV = 24 cm; matrix = 64 × 64; gap = 1 mm; flip-angle = 90).

Data Analysis

Demographics, behavioral data. One-way ANOVAs and χ^2 analyses were used to compare demographic data across diagnostic groups. General linear models (GLM) with repeated measures for task conditions and with age, gender, and premorbid-IQ as indexed by wide range achievement test (WRAT) served as covariates of no interest were used to evaluate performance differences across diagnostic groups. To test planned comparisons, linear t-contrasts were also computed. To exclude potential effects of current psychotropic treatment in SIBs, we re-run the behavioral analyses excluding the five SIBs on psychotropic drugs at the time of the data acquisition.

Imaging. Data were pre-processed and analyzed using Statistical Parametrical Mapping (SPM5; http://www.fil.ion.ucl.ac.uk, see Supplementary Materials). For each stimulus type, a stick function was convolved with a canonical hemodynamic response function at each voxel. Six subject-specific movement parameters obtained from the realignment procedure were included in the model as covariates of no interest, taking into account the effects of subject motion. All data sets underwent rigorous quality control check to exclude motion artifacts (>2 mm translation, <1.5 degrees rotation). We also included a regressor of no interest for incorrect and missed responses. For correct trials only, linear contrasts were computed producing voxel-wise t-statistical maps for interference monitoring and suppression (incongruent>congruent), and response inhibition (No-Go). The whole sample group maps of these contrasts that are orthogonal to the diagnosis were eventually used to mask random effects second-level analyses (mask size was 340 092 and 370 737 mm^3 for incongruent>congruent and No-Go, respectively). ANCOVAs with age, gender, WRAT, and reaction times (RTs, only for conditions requiring button press) as nuisance variables were used to identify significant differences in brain activation across the diagnostic groups. Pairwise diagnostic differences were tested with linear t-contrasts. In those brain regions where both SCZs and their SIBs had abnormal activity relative to NCs, the average cluster brain activations was extracted and pairwise compared using planned comparisons. To examine the cognitive control-dependent modulation of functional coupling of ACC with the rest of the brain, a psychophysiological interaction (PPI) analysis was performed. This analysis allows the evaluation of regional specific responses in terms of the interaction between the neural activity of different brain regions and an experimental condition. Based on our strong *a priori* hypothesis on the role of ACC in cognitive control we chose this region, as identified through WFU-pickatlas toolbox (http://fmri.wfubmc.edu/software/PickAtlas), as seed for the PPI. The first eigenvariate of individual time-courses was extracted from the seed, mean-centered, high-pass filtered, and deconvolved. A new GLM was then computed at each individual subject level using three regressors: a physiological regressor (the time course response from the seed), a psychological regressor (No-Go *vs* congruent for response inhibition, and incongruent *vs* congruent during interference monitoring and suppression, respectively), and a psychophysiological interaction term, calculated as the de-meaned scalar product of the physiological and psychological regressors. To identify differences in brain connectivity across diagnostic groups, individual PPI contrasts were entered into random effects ANCOVAs as for the activation analyses. To exclude potential effects of current psychotropic treatment in SIBs, we re-run the imaging analyses excluding the SIBs on psychotropic drugs at the time of the data acquisition. Furthermore, to exclude that group differences in neural activity and connectivity were unduly driven by differences in demographics and behavioral performance, we selected a subsample of 228 subjects matched also for age, WRAT, accuracy, and RT (Supplementary Table S1). Given the small number of female SCZs and male SIBs, we could not match for this variable; nevertheless, we added this variable in ANCOVAs

as a nuisance variable to account for this difference and also ran further confirmatory analyses in a gender-matched subgroup (see Supplementary Materials). For both the task activation and PPI ANCOVA analyses, a statistical threshold of $P<0.05$ corrected for multiple comparisons with family-wise error small volume-correction (FWE-SVC) was used to identify significant differences within anatomical regions of interest (ROI) associated with task effects (Blasi *et al*, 2006). ROIs were created using WFU-pickatlas and comprised the following *a priori* regions: ACC (BA24/32), and lateral-PFC (BA9/10/44/45/46/47). A single mask including ACC and bilateral PFC was used to perform FWE-SVC for activation responses, whereas a bilateral PFC ROI was used for the ACC-PPI connectivity analyses. All coordinates are reported in MNI system.

Linear correlations between the first eigenvariate of the signal of the clusters showing the effect of diagnosis on brain responses (activity and connectivity) associated with response inhibition and accuracy were performed in each diagnosis group separately. In SCZs, correlations between brain responses and treatment variables (chlorpromazine equivalents) were analyzed. To correct for potential dependence of behavioral (accuracy and RT) and neural responses (ACC activation and PPI in lateral-PFC) to the flanker task within members of the same family, we also performed confirmatory one-way ANOVAs on behavioral and neural measures across all diagnostic groups, and between SIBs and SCZs with the robust variance correction as applied in Stata10.0 (see Supplementary Materials) as in Rasetti *et al*, 2011. This type of correction estimate uses a robust covariance matrix to estimate the standard error by taking into account within-cluster (family) correlation (data not independent within-groups but dependent across group clusters, ie families). This estimate is then used for adjusting appropriately neural and behavioral responses results for within-family correlations.

RESULTS

Behavioral Results

Accuracy. There was a main effect of diagnosis (Figure 1): SCZs had significantly lower accuracy compared to SIBs and NCs ($P<0.0001$). There was a trend towards significance for task condition [$F(3,1092)=2.1988$; $P=0.087$)], with the lowest performance on the No-Go condition ($P<0.0001$). Additionally, accuracy during the incongruent condition was significantly lower relative to the congruent ($P<0.0001$). There was also an interaction of diagnosis-by-task condition with SIBs ($P<0.02$) and SCZs ($P<0.00001$) having worse accuracy during response inhibition but not during interference monitoring (*t*-contrast incongruent > congruent by groups, $P=0.8$) compared to NCs. Robust-variance corrected analyses confirmed the effect of diagnosis on accuracy during No-Go ($P<0.001$), but not in the other task conditions ($P>0.1$).

Reaction time. There was a main effect of diagnosis [$F(2,364)=4.8058$, $P=0.00871$]: SIBs ($P=0.04$) as well as SCZs ($P<0.0001$) were slower relative to NCs. There was also a main effect of task condition [$F(2,728)=6.8929$, $P=0.001$] with incongruent being the slowest condition

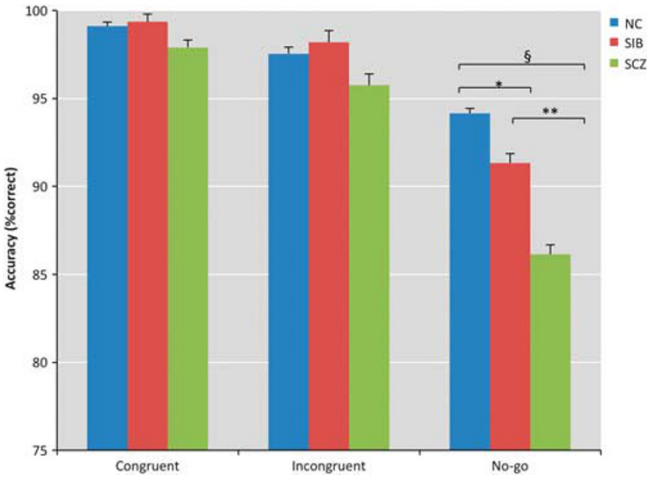

Figure 1 Condition by diagnosis interaction ($F(6, 1092)=9.5455$, $P<0001$] showing lower performance in unaffected siblings ($^{*}P=0.02$) and patients with schizophrenia ($^{\S}P=0.00001$) during response inhibition relative to normal controls. Moreover, diagnosis-modulated task performance ($F(2, 364)=10.971$, $P=0.00002$) with patients having the worst accuracy. Accuracy is indicated as mean percent correct. Error bars indicate SE of the mean. $^{**}P=0.0005$.

($P<0.05$). Interestingly, there was no significant diagnosis-by-task condition interaction (Supplementary Figure S2). Robust-variance corrected analyses confirmed the effect of diagnosis on RT for congruent and incongruent ($P<0.005$).

All the behavioral results were similar and statistically significant after excluding SIBs on psychotropic drugs (data not shown).

Imaging Results

Effect of task conditions. During response inhibition, participants showed predominantly right-lateralized activation in the following brain regions: dorsolateral-PFC (DLPFC, BA9/46), ventrolateral-PFC (VLPFC, BA44/47), supplementary motor area (BA6), ACC (BA32), insula, caudate, thalamus, precuneus (BA7/40), and occipital regions. Interference monitoring and suppression was associated with greater activity in a network of brain regions including parietal cortex (BA7/40), VLPFC (BA44/45/47), insula (BA13), DLPFC (BA9/46), ACC (BA24/32), caudate, putamen, and thalamus.

Effect of diagnosis. Response inhibition: SCZs and SIBs showed decreased activation in ACC during 'No-Go' trials (BA24/32; $xyz=0,15,36$; $k=70$; $Z=4.17$, $P=0.00002$, FWE-SVC = 0.02; Figure 2a). Planned comparison analyses confirmed decreased activation in the ACC of SIBs (Figure 2b; $P=0.007$; FWE-SVC = 0.001) when compared only with NCs, but showed no significant difference between SIBs and SCZs ($P>0.2$). This decreased engagement is not an effect of more frequent errors in SCZs and SIBs across all the trials, as these analyses were based only on correct trials.

Interference monitoring and suppression: SCZs showed decreased activation in ACC (BA24), bilateral DLPFC (BA9), right supplementary motor area (BA6), bilateral caudate, and thalamus (Supplementary Figure S4). None of these

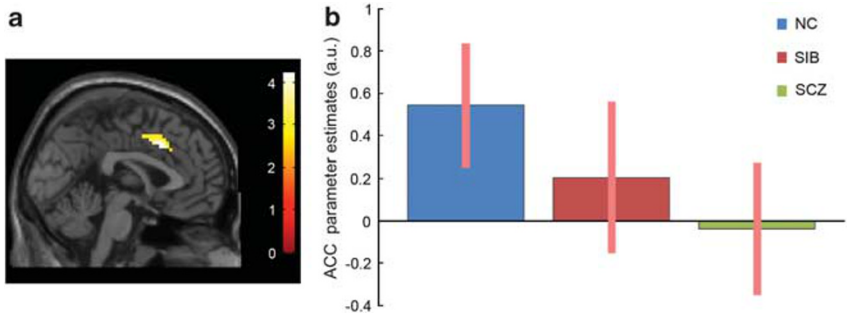

Figure 2 Subjects at risk for schizophrenia have decreased anterior cingulate cortex (ACC) activation during response inhibition. Siblings ($P<0.01$) and patients with schizophrenia ($P<0.0001$) showed decreased dorsal ACC ($x=0, y=15, z=36$) relative to normal controls. (a) Thresholded statistical map of the effect of diagnosis (normal controls > siblings > patients) is overlaid on sagittal sections ($x=0$) of T1 MNI template ($P=0.005$). Color bar indicates *T*-values. (b) Mean parameter estimate values extracted from the peak voxel—expressed in arbitrary units (a.u.). Error bars indicate 90% confidence interval of the parameter estimates at the peak voxel. MNI, Montreal Neurological Institute.

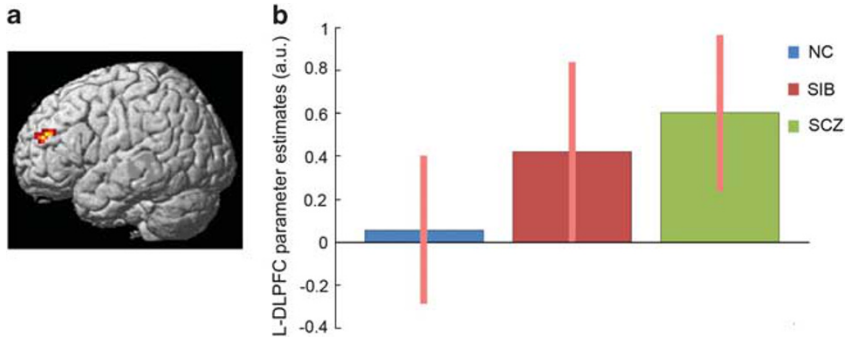

Figure 3 Subjects at risk for schizophrenia have increased anterior cingulate-prefrontal connectivity during response inhibition. Siblings ($P=0.02$) and patients with schizophrenia ($P<0.0001$) showed increased functional coupling between dorsal anterior cingulate and left dorsal prefrontal cortex (L-DLPFC, $x=-39, y=48, z=24$) relative to normal controls. (a) Thresholded statistical map is rendered on T1 MNI template ($P=0.005$). (b) Parameter estimate values extracted from significant cluster—expressed in arbitrary units (a.u.). Error bars indicate SE of the mean. MNI, Montreal Neurological Institute.

clusters, however, showed a significant difference between NCs and SIBs.

The effects of diagnosis on task-related response associate with response inhibition, and interference monitoring and suppression were confirmed in the matched subsample (see Supplementary Figure S6).

PPI: During response inhibition, there was increased PPI between ACC and a network of brain regions that included bilateral VLPFC, right DLPFC, posterior parietal (BA7), thalamus, and putamen (see Supplementary Figure S5) relative to the rest of the brain. Both SCZs and SIBs showed marginally significant greater PPI during response inhibition in the left lateral-PFC (BA10/46; $xyz=-39,48,24$, $k=6$, $Z=3.46$, $P=0.00027$, FWE-SVC $=0.09$; Figure 3) when compared to NCs. Planned comparison analyses indicated increased PPI of ACC with this region in SIBs ($P=0.02$) when compared with NCs. A further analysis in the matched subsample confirmed the significance of this comparison ($P=0.003$, FWE-SVC $=0.03$; see Supplementary Figure S7). Robust-variance corrected analyses confirmed the effect of diagnosis on No-Go activation and PPI during response inhibition ($P=0.001$).

All the imaging results were similar and statistically significant after excluding SIBs on psychotropic drugs (data not shown). We did not find any effect of diagnosis on the context-dependent coupling of ACC during interference monitoring and suppression. PPI of ACC was weakly but positively correlated ($r=0.12$, $P=0.05$) with No-Go accuracy in NCs as well as in SIBs ($r=0.25$, $P=0.04$ after removing one outlier). We did not find other brain response–behavior correlations in any diagnosis group. Treatment variables were not significantly associated with brain responses in SCZs.

DISCUSSION

The present study investigated whether brain responses underlying cognitive control, particularly those germane to ACC, are associated with genetic liability for schizophrenia in a cohort of SIBs. Behaviorally, SIBs showed decreased accuracy relative to NCs during response inhibition. Similar to SCZs, SIBs also showed decreased dorsal ACC activation during this task in comparison to NCs, and this physiological difference occurred when they were not making errors. Furthermore, both SIBs and SCZs showed altered context-related modulation of functional connectivity, as measured by the PPI, between ACC and lateral-PFC during response inhibition relative to NCs.

In our study, SIBs showed overall decreased accuracy during response inhibition relative to NCs. Most studies have identified impaired response inhibition in SCZs during a Stop-task (Badcock *et al*, 2002; Enticott *et al*, 2006), although negative findings have also been reported (Rubia

et al, 2001). A recent study using masked negative priming has reported that voluntary but not unconscious response inhibition is impaired in schizophrenia (Huddy *et al*, 2009) suggesting, together with another study based on a Stop-task (Badcock *et al*, 2002), that impairments in response inhibition are not due to slower processing speed *per se*. Decreased performance on neuropsychological tests that tap into attentional control functions has been reported in unaffected relatives of patients with schizophrenia (Cannon *et al*, 1994; Goldberg *et al*, 1995). More specifically, Groom *et al*, (2008) found a longer latency of response inhibition on the Hayling Sentence Completion Task in both siblings and patients. Notably, we observed similar behavioral impairments during response inhibition in unaffected siblings also in the context of similar performance on the other task conditions, thus suggesting a possible role of this measure in genetic liability to schizophrenia (see below).

Most importantly, our study identified altered neural correlates of response inhibition processing in unaffected siblings of patients with schizophrenia. Converging evidence from multi-modal neuroimaging studies indicates altered ACC function in siblings of patients with schizophrenia. Most studies show decreased ACC activation in siblings on a variety of executive cognition tasks during fMRI (Callicott *et al*, 2003; Filbey *et al*, 2008; Sepede *et al*, 2010; Whalley *et al*, 2004), although some studies have reported increased ACC activity (Thermenos *et al*, 2004) or no difference (Becker *et al*, 2008; MacDonald *et al*, 2006; Vink *et al*, 2006; Zandbelt *et al*, 2011). We found decreased activation in ACC specifically during response inhibition in unaffected siblings relative to normal controls. These findings are consistent with previous studies in patients with schizophrenia (Arce *et al*, 2006; Ford *et al*, 2004; Kaladjian *et al*, 2007; Rubia *et al*, 2001) and in their siblings (Blackwood *et al*, 1999). Notably, to exclude that the present physiologic results were affected by behavioral differences, we analyzed only correct trials and confirmed these results in a sample that was matched for task performances. Thus, our data suggest that the abnormal ACC engagement most likely relates to the neural strategy for performing response inhibition, and not to its success or failure. Moreover, whereas our sample of SIBs included a minority of individuals with a past history of psychiatric treatment for nonpsychotic disorders, none were in continuing treatment and the groups also did not differ in smoking frequency, suggesting that obvious secondary confounders are not likely explanations for the results.

Interestingly, siblings of patients also have functional alterations in PFC (Callicott *et al*, 2003; Thermenos *et al*, 2004) and in PFC connectivity (Rasetti *et al*, 2011), and we demonstrated alterations in ACC-PFC coupling during more demanding conditions of cognitive control. ACC is functionally connected with DLPFC within a fronto-cingulate-parietal network (Wang *et al*, 2010) that supports cognitive control (Stevens *et al*, 2009). Recently, Brazdil reported hierarchically organized intrinsic effective connectivity within an ACC-PFC circuit, so that ACC modulates DLPFC during attentional tasks (Brazdil *et al*, 2007). Other reports have suggested a bidirectional connectivity between these two regions as being critical for optimal attentional control (Wang *et al*, 2010). Increased connectivity between PFC and ACC found in our patient and sibling samples could reflect a neural processing strategy to compensate for suboptimal function of these two regions as suggested by other studies in patients with schizophrenia and aging (Sambataro *et al*, 2009, 2012). This may account for the correct performance observed during the analyzed trials. Indeed, we did find a weak positive correlation between ACC-PFC connectivity and better performance during response inhibition in normal controls as well as in the siblings. The lack of a significant PFC-ACC connectivity–performance correlation in patients may be suggestive of failed compensation due to reduced PFC efficiency (Callicott *et al*, 2003).

ACC-PFC coupling is crucial to enhance signal relative to noise and filter out irrelevant information. Primate studies have shown that the ACC projection neurons innervate excitatory pyramidal neurons in PFC responsible for response selection via large boutons with inhibitory neurons that come into play at high cognitive task demands (Medalla *et al*, 2009). At low cognitive loads, pyramidal neurons in BA9 are modulated only via PFC pathways. When cognitive demands become higher, ACC neurons enhance cognitive control both by, (1) suppressing excessive PFC noise through large boutons projecting on calbindin inhibitory neurons, and (2) reversing decisions via greater PFC activity of previous signals as well as enhancing new signals through activity of large boutons (Medalla *et al*, 2009). Therefore, decreased ACC function might result in suppressed activity of PFC inhibitory interneurons, and consequently decreased cognitive control during challenging cognitive tasks (Medalla *et al*, 2009). Alternatively, decreased ACC activation and increased coupling within the PFC-ACC network may suggest that siblings, similarly to patients with schizophrenia, are not optimally engaged in the task and examine each trial on a trial-by-trial basis, and possibly have diminished prepotent response prior to the No-Go trials when compared to normal controls (Ford *et al*, 2004; Kaladjian *et al*, 2007). Unfortunately, there was no way we could assess the prepotent response behaviorally with the task paradigm we used.

In our study, altered ACC activity and connectivity were not unduly driven by group differences in task performance, as we only included the brain responses related to correct trials in the imaging analyses. Furthermore, we were able to replicate these results in a subsample matched for performance and demographic variables across diagnostic groups, thus excluding any biasing effects.

Of note, we did not find altered interference processing in unaffected siblings. Previous studies did not identify altered ACC response in healthy first-degree relatives, but decreased lateral-PFC response together with poor behavioral responses during conflict tasks including the Stroop-task (Becker *et al*, 2008; MacDonald *et al*, 2006). Performance differences across diagnostic groups, the presence of psychiatric disorders in relatives, and age heterogeneity due to the inclusion of first-degree relatives including offspring, parents, and siblings may explain differences between our findings and those of these earlier reports. Furthermore, the Stroop paradigm elicits a stronger conflict relative to Eriksen's flanker congruency task (see above). These tasks differ critically in the underlying processing demands and sensitivity to conflict. In the Stroop-task,

interference consists of color-naming mismatch (Stroop effect) that is different from the flanker task where conflict is based on location (flanker incongruency effect). In the Stroop-task, conflict between different categories translates into engagement of additional neural processes which require greater effort to adjust responses to contextual information (Lehle and Hubner, 2008).

The candidate intermediate phenotypes reported would be expected to be present in high-risk individuals. We could not test this possibility as the average age of the unaffected siblings in our sample was beyond the typical age of risk for developing schizophrenia. Future studies with siblings of a younger age could help inform this issue.

In conclusion, the present study shows that unaffected siblings of patients with schizophrenia evince altered brain function during response inhibition. Our results suggest that impaired response inhibition, which is associated with altered function of an ACC-PFC network, is at the least familiar and may reflect genetic risk for schizophrenia and as such may be an intermediate phenotype for genetic studies of schizophrenia. Further work addressing the heritability of this phenotype is warranted.

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DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)