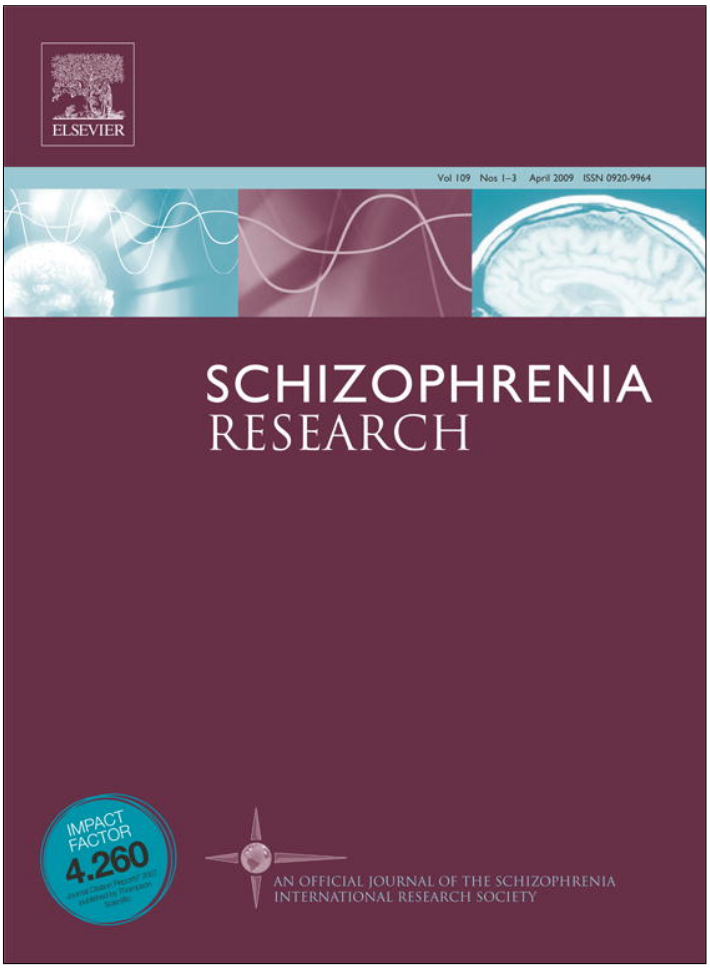

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Abnormal prefrontal cortical activity and connectivity during response selection in first episode psychosis, chronic schizophrenia, and unaffected siblings of individuals with schizophrenia

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ABSTRACT

The search for genes conferring liability for schizophrenia may be aided by the identification of endophenotypes. Response selection is a heritable cognitive function that is impaired in patients with schizophrenia and their unaffected siblings. The abnormalities in cerebral function that presumably underlie the deficit in patients and unaffected siblings remain to be elucidated. Cerebral neurophysiology during performance of a 4-choice reaction time (CRT) task in 25 patients with schizophrenia (15 medication free first episode (FEP) and 10 chronic patients), 32 controls, and 12 unaffected siblings of individuals with schizophrenia was investigated using fMRI. CRT was impaired in both medication free FEP and chronic patients with schizophrenia, and unaffected siblings. FEP patients, chronic patients, and unaffected siblings demonstrated greater BOLD response in the right dorsolateral prefrontal cortex (dlPFC) during CRT task blocks. The nature of the altered activation in the dlPFC was further examined using functional connectivity analysis. This revealed marked reductions in connectivity between the right dlPFC and multiple brain regions in both patient groups and, to a lesser degree, unaffected siblings. The magnitude of connectivity between right dlPFC and inferior parietal lobule correlated with task performance in the combined patient/unaffected siblings group, but not controls suggesting that the network of brain regions recruited to perform the task differed as a function of genetic liability for schizophrenia. The findings suggest that altered activity and connectivity of the right dlPFC appears to be related to genetic vulnerability for schizophrenia and may represent a potential endophenotype of the disorder.

1. Introduction

Schizophrenia is a highly heritable disorder (Cardno and Gottesman, 2000; Shields and Gottesman, 1972). The search for liability genes for complex disorders such as schizophrenia may be aided by identifying endophenotypes (Gottesman and Gould, 2003). Endophenotypes are quantifiable markers of an illness that are, presumably, closer to the underlying biological causes and genetic basis of a disorder along the genotype–phenotype pathway and include biochemical, structural, and functional brain changes, including neuropsychological impairment (Braff et al., 2007; Turetsky et al., 2007; Cannon et al., 2002). To be useful an endophenotype must be: 1) associated with the illness; 2) heritable to some degree; 3) state independent in affected individuals (i.e. present regardless of the stage of the illness); and 4) observed in unaffected family members to a greater extent than the general population (Gottesman and Gould, 2003).

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[1] Location of work.

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Impaired response selection is one of several promising neuropsychological endophenotypes of schizophrenia (Nuechterlein, 1977; Krieger et al., 2001; Krieger et al., 2005; Pellizzer and Stephane, 2007). The prototypical response selection task, choice reaction time (CRT), requires subjects to execute one of several possible motor responses following presentation of a specific stimulus cue, the spatial location of a target for example. CRT is impaired in both first episode medication naïve and chronic patients and it has been hypothesized that the deficit relates to altered connectivity between brain regions involved in response selection (Krieger et al., 2001, 2005; Pellizzer and Stephane, 2007). More importantly from an endophenotype standpoint are findings from twin studies in controls and schizophrenia indicating that CRT is heritable, and, along with deficits in divided attention, working memory, and verbal learning, is more impaired in unaffected monozygotic than dizygotic twins discordant for schizophrenia (Cannon et al., 2000; Wright et al., 2001). Thus, in addition to being associated with the illness, impaired CRT meets several additional endophenotype criteria including: 1) heritability; 2) state-independence in patients; and 3) impairment in unaffected relatives of patients.

Surprisingly, the alterations in cerebral function associated with impaired CRT in patients with schizophrenia and their unaffected relatives have not been investigated; although many of the paradigms employed in imaging studies of cognition in schizophrenia rely heavily on response selection. For example, commonly used paradigms of procedural learning, working memory, and cognitive control require subjects to select from one of several motor responses following presentation of a specific stimulus cue (Zedkova et al., 2006; Callicott et al., 2003b; Becker et al., 2008). Typically, additional demands are placed on subjects by varying working memory load or perceptual/stimulus–response conflict. Consequently, examination of the neural correlates of response selection in many functional magnetic resonance imaging (fMRI) studies is often precluded by the fact that CRT, in one form or another, is used as a baseline control condition. Indeed, prior imaging studies of procedural learning and working memory employed a typical CRT task as the baseline condition (Reiss et al., 2006; Woodward et al., 2007; Zedkova et al., 2006; Callicott et al., 2003b,a). Failure to examine the neural correlates related to response selection may have important consequences for interpreting results related to other cognitive abilities given evidence that at least part of the behavioral deficits observed in executive functions and working memory in schizophrenia relates to impaired response selection (Krieger et al., 2001; Krieger et al., 2005). Reports of greater activation in the prefrontal cortex (PFC) during a CRT baseline condition, relative to the procedural learning condition, in patients and their unaffected siblings, but not control subjects, suggest that at least some of the changes observed in cerebral function during procedural learning may reflect alterations in the neural circuitry underlying the basic cognitive process of response selection (Zedkova et al., 2006; Woodward et al., 2007; Reiss et al., 2006).

In order to examine the neural correlates of CRT in schizophrenia and determine the extent to which abnormalities in brain function during CRT relate to genetic vulnerability for schizophrenia, we took advantage of the fact that our imaging investigations of procedural learning included a fixation period thereby allowing us to examine neural activity during a CRT task that served as the baseline condition in our prior studies. In addition to performing a novel imaging analysis, we further expanded upon our earlier investigations by including a group of medication-free first episode psychosis patients. The goals of this experiment were to: 1) replicate previous demonstrations of impaired CRT in first episode psychosis, chronic schizophrenia, and unaffected relatives of patients; 2) extend these findings by determining if impaired CRT performance in patients is associated with alterations in brain activation; and 3) determine if the exact same regional changes in brain function observed in patients are also present in unaffected relatives of patients.

2. Methods

2.1. Subjects

This study was approved by the institutional review boards of the University of Alberta Hospital and Alberta Hospital Edmonton. All subjects were provided a verbal and written description of the study prior to solicitation of written informed consent to participate. 75 right-handed subjects were initially recruited to participate in the study; however, 4 patients (1 FEP and 3 chronic patients) were excluded due to excessive head motion and 2 control subjects were excluded due to periods of sleep during scanning. The final sample consisted of 25 patients with schizophrenia (15 medication-free first episode psychosis (FEP) and 10 chronic patients), 32 healthy control subjects, and 12 unaffected siblings of patients with schizophrenia. Due to the fact that FEP patients were younger than chronic patients and the well established association between reaction time and age, controls were partitioned into two groups, denoted young adult (YA) and middle aged (MA), that were matched to the FEP and chronic patient sub-groups, respectively. Unaffected siblings were age-matched to the MA control group and all groups were matched on gender distribution and parental socio-economic status (Myers et al., 1965). 15 controls, 10 chronic patients, and 12 siblings were included in our prior reports (Woodward et al., 2007; Zedkova et al., 2006); however, this is a novel analysis of their data. Demographic data for the subjects is presented in Table 1. Complete details on subject characteristics and recruitment procedures are presented in the Supplemental material.

2.2. Behavioral paradigm and statistical analysis of behavioral data

The task has been described in detail previously (Woodward et al., 2007; Zedkova et al., 2006) and is virtually identical to CRT tasks used in prior behavioral and functional neuroimaging studies (Tuch et al., 2005; Schumacher et al., 2003). During scanning subjects were required to identify the location of a target that could appear in one of four boxes arranged horizontally by pressing one of four response keys on each trial. The outer and inner left stimulus locations corresponded to the middle and index finger of the left hand, and the inner and outer right locations corresponded to the index and middle finger of the right hand, respectively. Subjects were requested to respond as quickly and accurately as possible. Sixty trials comprised a block and there were two block conditions referred to as 'sequenced', during which the location of the target followed a 12-element sequence that repeated five times, and 'random', hereafter

Table 1
Demographic and clinical characteristics of healthy comparison subjects, patients with schizophrenia, and unaffected siblings.

	Controls				Patients with schizophrenia				Unaffected			Summary statistics				
													Contrasts			
Variable	YA Ctrl		MA Ctrl		FEP		Chronic		Siblings		Test	*p*-value	A	B	C	D
N	18		14		15		10		12							
Gender (m/f)	14/14		9/5		12/3		8/2		5/7		$x^2 = 6.46$	NS	–	–	–	–
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD						
Age [a]	22.5	3.3	32.4	10.9	22.5	3.3	33.5	7.5	36.9	13.3	$F(4,26.7) = 9.86$	<.001	NS	NS	NS	NS
Education	14.9	1.7	17.4	2.4	13.4	2.5	13.4	2.2	15.0	2.3	$F(4,64) = 7.47$	<.001	$p<.001$	$p<.05$	$p<.001$	$P<.01$
Parental SES [a]	2.6	0.5	2.6	0.5	2.5	0.7	3.0	0.5	3.2	0.8	$F(4,28.7) = 2.33$	NS	–	–	–	–
On set Age	–	–	–	–	22.5	3.2	21.7	6.3	–	–	$t(23) = 0.40$	NS	–	–	–	–
Duration of Illness [b]	–	–	–	–	0.4	0.3	11.5	6.4	–	–	$t(23) = 6.81$	<.001	–	–	–	–
PANSS Positive	–	–	–	–	19.0	4.6	14.7	3.7	–	–	$t(23) = 2.6$	<.05	–	–	–	–
PANSS Negative	–	–	–	–	18.9	4.0	10.7	2.4	–	–	$t(23) = 5.83$	<.001	–	–	–	–
PANSS General	–	–	–	–	38.5	6.8	26.1	4.2	–	–	$t(23) = 5.17$	<.001	–	–	–	–
GAF	–	–	–	–	45.4	12.2	50.6	15.9	–	–	$t(23) = 0.92$	NS	–	–	–	–

Parental SES unavailable for 2 subjects. Planned Contrasts: A = Schizophrenia vs. Controls, B = FEB vs. YA Ctrls, C = Chronic Patients vs. MA Ctrls, D = Siblings vs. MA Ctrls.
Abbreviations: FEP: First Episode Psychosis; GAF: Global Assessment of Function; MA: Middle Age; NS: Not Significant; PANSS: Positive and Negative Syndrome Scale; YA: Young Adult.
[a] Welch test used to compare group means.
[b] Duration of illness in years.

referred to as 'CRT blocks', during which the location of the stimulus appeared pseudorandomly. The CRT condition is the focus of the current behavioral and imaging analyses. Subjects completed two scanning runs, each consisting of 3 sequenced and 3 random blocks alternating in a blocked AB manner, with each block separated by an 18 s fixation point rest period.

Individual subject median reaction times (RTs), excluding errors, and accuracy were the dependent variables for the analysis of behavioral data. Behavioral data were analyzed in two steps. First, all patients and controls were entered into a univariate ANOVA and a-priori planned contrasts comparing patients to controls, FEP patients to YA controls, and chronic patients to MA controls were performed. These contrasts were carried out to determine if there was a general diagnosis effect on performance and if both FEP and chronic patient sub-groups differed from their respective age-matched controls on RT and accuracy. Following that, a post-hoc independent groups *t*-test comparing unaffected siblings to MA controls was carried out to determine if the same behavioral changes observed in patients was present in unaffected siblings. This post-hoc contrast was one-tailed with directionality of the contrast determined by the results obtained from the patients vs. controls contrast. In cases when the assumption of equality of variances was violated, based on a significant Levene's test at alpha<.05, the groups were compared using the Welch test (Welch, 1947, 1951).

2.3. fMRI data analysis

Complete details regarding the acquisition and pre-processing of fMRI data are presented in the Supplementary Material and in prior publications (Woodward et al., 2007; Zedkova et al., 2006). Statistical analyses of fMRI data proceeded by modeling the functional time course data at each voxel as a boxcar function, convolved with a gamma function to account for lag in the hemodynamic response, with CRT blocks and fixation periods entered as predictors in a random effects general linear model (GLM). Statistical parametric maps (SPMs) comparing CRT blocks to fixation were created for each group to identify the regions that demonstrated a significant BOLD response to the CRT condition. Imaging data were analyzed in a similar manner as the behavioral data. First, to determine if patients differed from controls in their BOLD response to the CRT task, all schizophrenia patients and controls were entered into a random effects GLM analysis. The SPM generated from this contrast was thresholded at the whole-brain cluster level corrected alpha = .05 for voxel-wise alpha = .005 using the procedure described by Forman et al. (1995), as implemented in Brainvoyager QX (Goebel et al., 2006). The magnitude of the BOLD response during CRT blocks, defined as predictor beta weights, was extracted from the significant clusters identified in the schizophrenia vs. controls contrast from all subjects, including unaffected siblings, and subjected to a univariate ANOVA that included planned contrasts comparing FEP patients to YA controls and chronic patients to MA controls, and a post-hoc independent groups *t*-test comparing unaffected siblings to MA controls to determine if the same BOLD changes observed in patients was present in unaffected siblings. This post-hoc contrast was one-tailed with directionality of the contrast determined by the results obtained from the patients vs. controls contrast. As with the analysis of behavioral data, the groups were compared using the Welch test when the region-of-interest BOLD changes violated the assumption of homogeneity of variance.

3. Results

3.1. Behavioral data

Mean RT and accuracy for each group are presented in Fig. 1. Behavioral data for one MA control subject was lost due to experimenter error. Age was positively correlated with RT ($r = .54$, $p<.001$) across all subjects, supporting our decision to match FEP, chronic patients, and unaffected siblings to age matched control sub-groups. The ANOVA comparing RT across patient and control groups was significant ($F(3,52) = 5.46$, $p<.005$) as were the planned contrasts comparing schizophrenia patients to controls ($p<.005$), FEP patients to YA controls ($p<.05$), and chronic patients to MA controls

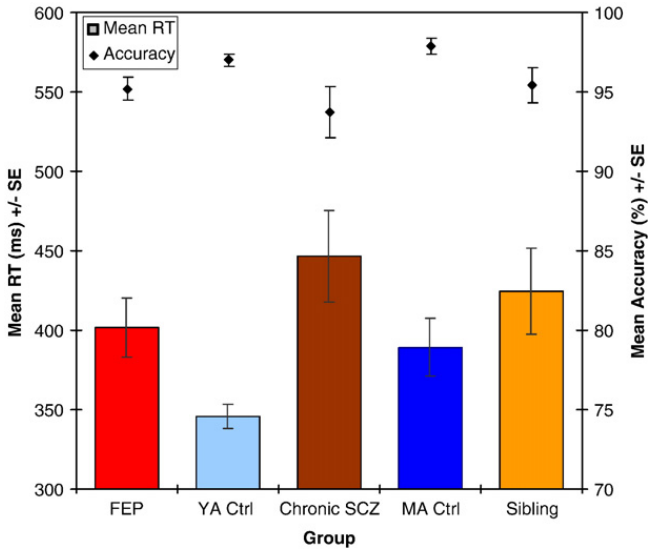

Fig. 1. Choice reaction time in patients with schizophrenia, unaffected siblings, and healthy comparison subjects.

($p<.05$) due to the fact that patients demonstrated longer RTs. With the exception of the chronic patients vs. MA controls contrast, which remained significant at the trend level ($p<.08$), the results where unchanged when education was entered as a covariate. Levene's test revealed that the assumption of homogeneity was violated for CRT accuracy ($F(3,52)=6.34$, $p<.005$). Welch's test revealed a main effect of group for accuracy ($F(3,23.9)=4.34$, $p<.05$). Patients made more errors than controls ($p<.005$), and this was true for both FEP and chronic patient sub-groups, relative to their age matched control sub-groups ($p<.05$; respectively). The difference in accuracy between chronic patients and MA controls remained significant after covarying for education ($p<.05$); however, the contrast between FEP and YA control did not ($p<.21$). To determine if the deficits in CRT varied between patient groups, effect sizes (ESs) for RT and accuracy were created for each patient group, relative to their respective control groups, and compared. The effect sizes for RT and accuracy did not differ between patient groups ($t(22.8)=1.26$, $p<.27$ and $t(23)=1.46$, $p<.16$, respectively) indicating that the degree of impairment on the CRT task, relative to their age-matched control groups, was equivalent in both patient groups. Post-hoc independent groups t-tests comparing unaffected siblings to MA controls indicated that unaffected siblings demonstrated equivalent RTs ($t(24)=1.08$, $p<.15$), but made more errors ($t(15.6)=2.04$, $p<.05$). The difference in accuracy between unaffected siblings and MA controls remained significant at the trend level after covarying for education ($p<.08$). It should be noted that although statistical differences in accuracy were observed, accuracy was above 90% for all groups. Exploratory correlations did not reveal associations between RT and education or parental SES ($r=-.12$ and $r=.20$, respectively). Accuracy was not correlated with age or parental SES.

3.2. fMRI data

As shown in the online Supplementary Fig. 1, CRT was associated with widespread BOLD responses in regions of the frontal, parietal, and occipital lobe in all groups. As shown in Table 2 and Fig. 2, direct comparison between patients and controls revealed that patients demonstrated greater BOLD response during CRT blocks compared to controls in two areas; right middle frontal gyrus corresponding to Brodmann's area (BA) 9 and right SMA corresponding to BA 6. Relaxing the voxel-wise statistical threshold to $p=.01$, while maintaining the same minimum cluster size, revealed additional areas in which patients demonstrated greater BOLD response than controls including a region of the left middle frontal gyrus homotopic to the cluster identified in the right hemisphere. Controls did not demonstrate greater activity in any region compared to patients at either the stringent or more liberal thresholds.

CRT predictor beta-weights were extracted from the two significant clusters identified in the patients vs. controls

Table 2
Differences in BOLD response and functional connectivity during choice reaction time in healthy comparison subjects and patients with schizophrenia.

		Talairach coordinates				
Contrast	Brain region	*X*	*Y*	*Z*	*t* score	Size(mm^3)
Schizophrenia>Controls						
$p=.005$, $k=20$	R. middle frontal gyrus (BA 9)*	38	36	33	4.23	1863
	R. Supplementary Motor Area	4	15	43	3.52	540
$P=.01$, $k=20$						
	L. middle frontal gyrus (BA 9)	−37	33	29	3.19	945
	L. Postcentral gyrus (BA 3)	−37	−21	45	3.46	864
	L. middle frontal gyrus (BA 6)	−43	1	42	3.12	675
	R. precentral gyrus (BA 4)	44	−18	41	3.38	1053
	R. middle occipital gyrus (BA 18)	15	−89	15	3.34	702
	R. lingual gyrus (BA 18)	21	−79	−8	3.26	567
Functional connectivity analysis						
Controls>Schizophrenia						
Positively correlated with seed region in controls	L. middle frontal gyrus (BA 8)	−38	24	39	3.88	1512
	R. middle frontal gyrus (BA 9)	38	33	37	4.33	2970
	R. inferior parietal lobule (BA 40)	53	−32	32	3.48	810
Negatively correlated with seed region in controls	L. medial frontal gyrus (BA 9)	−3	49	26	3.64	1161
	L. middle frontal gyrus (BA 10)	−43	45	4	3.64	1377

*Seed region for functional connectivity analysis.
Abbreviations: L: left; R: right; BA: Brodmann's Area; k: cluster size in voxels (1 voxel = 27 mm^3).

contrast and compared across all groups. The results of this analysis are depicted in Fig. 2C and D. For the cluster identified in the right prefrontal cortex corresponding to BA 9, the planned contrasts comparing FEP patients to YA controls and chronic patients to MA controls were significant ($p<.05$ and $p<.005$; respectively). These results remained unchanged when education was entered as a covariate. The post-hoc contrast comparing siblings to MA controls revealed that unaffected siblings also demonstrated greater activity in this region ($t(24)=1.93$, $p<.05$) and this contrast remained significant when education was entered as a covariate in a linear regression analysis ($t(23)=1.73$, $p<.05$). For the cluster identified in the right SMA, the planned contrasts comparing FEP and chronic patients to their respective age matched control sub-groups were significant ($p<.05$ and $p<.005$; respectively) and remained unchanged when education was entered as a covariate. However, the post-hoc contrast comparing siblings to MA controls was not ($t(24)=0.40$, $p<.35$). As with the behavioral data, we calculated ESs for the difference in BOLD response between each patient group and their respective control group and compared ESs between patient groups. This analysis revealed that the relative over-activation of the right BA 9 cluster observed in patients was greater in chronic patients compared to FEP (ES = 1.71 vs. 0.76; $t(23)=2.19$, $p<.04$). Thus, although both patient groups demonstrated greater activation in right BA 9 compared to their respective control groups, the relative degree of over-activation was greater in chronic patients. There was no difference between patient groups in the relative degree of activation in the right SMA cluster ($t(23)=0.64$, $p<.53$). Exploratory correlations revealed a significant positive association between activity in the right SMA cluster and RT ($r=.24$, $p<.05$) indicating that greater activity in this region corresponded to longer RTs. BOLD response in right BA 9 and SMA did not correlate with any demographic variable.

3.2.1. Functional connectivity

The altered right BA 9 activity observed in patients and unaffected siblings was investigated further using functional connectivity analysis. Functional connectivity was measured

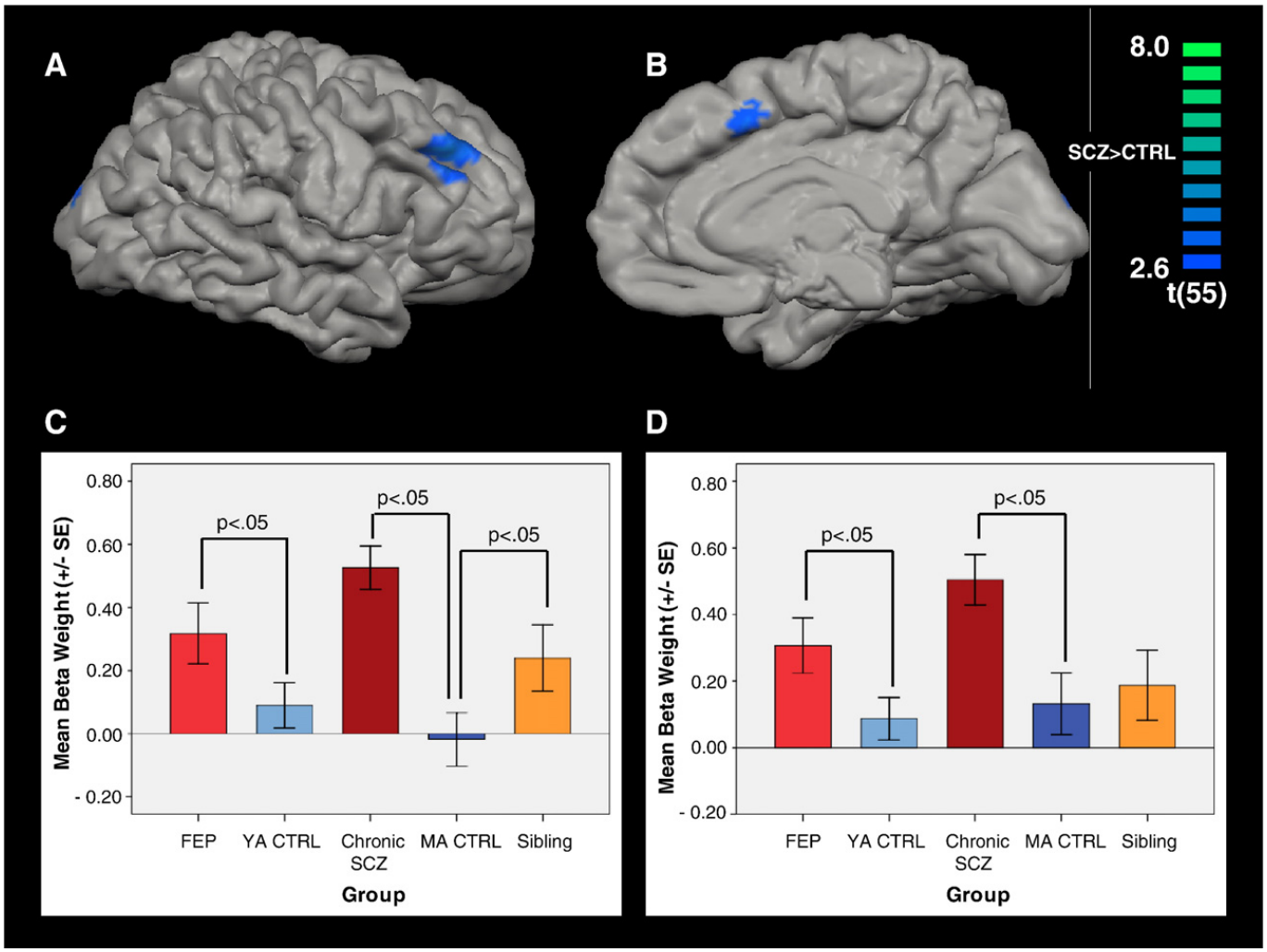

Fig. 2. Differences in brain activity between patients with schizophrenia, unaffected siblings, and healthy comparison subjects during choice reaction time (CRT) task. Compared to controls, patients demonstrated greater activity during CRT blocks in right BA 9 (A) and right SMA (B). Activity in right BA 9, in terms of task predictor beta weights, was greater in both patient sub-groups and unaffected relatives compared to their respective control groups (C). First Episode Psychosis (FEP) and chronic patients, but not unaffected siblings, demonstrated greater activity in the right SMA compared to their respective control groups (D). Note: activity depicted in graphs C and D was extracted from the two clusters (right BA 9 and SMA) that showed a significant difference between patients and controls at the whole brain corrected threshold $p<.05$.

by extracting the time series data from the right BA 9 cluster to create additional regressors for the random effects general linear model described above, after orthogonalizing the seed region time series with respect to estimated motion and global nuisance signals. This was done by calculating the point-wise product of seed time series with the task predictors (Friston et al., 1997), allowing connectivity during CRT blocks to be examined directly. Consistent with the between group analyses described for the block design analysis, connectivity maps were created for each group and these connectivity maps were initially compared between patients and controls. The SPM generated from the schizophrenia vs. control contrast was thresholded at the same whole-brain corrected cluster-wise alpha = .05 at voxel-wise alpha = .005 used in the univariate analysis. Predictor beta-weights for the seed region regressor were extracted from the clusters identified in the patient vs. controls contrast and subjected to planned contrasts comparing patient sub-groups to their respective control groups, and post-hoc contrasts comparing unaffected siblings to MA controls.

Functional connectivity maps generated using the right BA 9 cluster as a seed region are presented in the online Supplementary Fig. 2. Overall, right BA 9 was positively correlated with adjacent right dorsolateral cortical regions and the homotopic region in the contralateral hemisphere, sensorimotor cortical regions, SMA, inferior parietal lobule, and posterior middle temporal gyrus, and inversely correlated with the medial prefrontal cortex, posterior cingulate/precuneus, and middle temporal gyrus. Comparison between controls and schizophrenia patients revealed differences in functional connectivity in five clusters that including regions in the bilateral middle frontal gyrus, right inferior parietal lobule, and left medial frontal gyrus (see Table 2 and online Supplementary Fig. 3). In each case, controls demonstrated greater connectivity with the seed region than patients. With the exception of the left middle frontal gyrus region, for which only FEP, but not chronic patients, differed from controls, both patient groups demonstrated less connectivity compared to their respective control sub-groups. There were no differences between patient groups with respect to the relative degree of changes in functional connectivity. Post-hoc contrasts revealed that unaffected siblings demonstrated less connectivity between the seed region and left middle frontal gyrus BA 10 ($t(24) = 2.02$; $p < .05$) and left BA 8 at the trend significance level ($t(24) = 1.66$, $p < .06$). The results for the unaffected siblings should be considered exploratory given that five contrasts were performed and no Type I error correction was applied. Functional connectivity results are summarized in Fig. 3. It is also noteworthy that, in contrast to controls, the degree to which left BA 9 and left BA 10 correlated with the seed region did not differ significantly from zero in both patients and unaffected siblings.

Exploratory correlations revealed significant associations between CRT task performance and seed region connectivity with left BA 8, right BA 9, and right BA 40 (r ranging from −.24 to −.38 for RT and .25 to .35 for accuracy) indicating that less

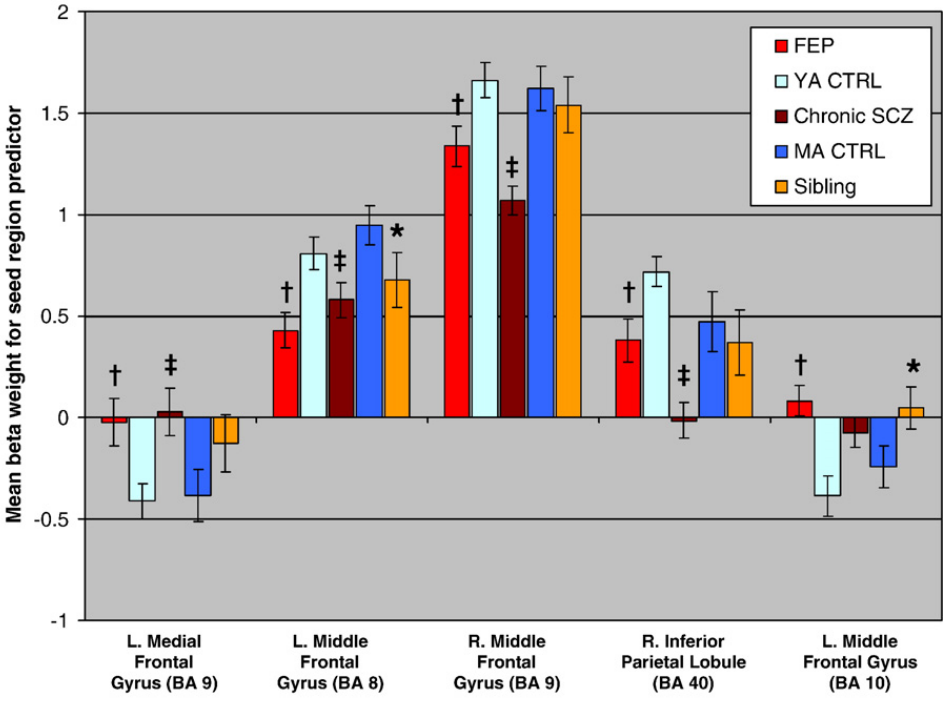

Fig. 3. Differences in functional connectivity between patients first episode (FEP) and chronic patients with schizophrenia, unaffected siblings of individuals with schizophrenia, and young and middle aged healthy comparison subjects. FEP and chronic patients demonstrated less connectivity with seed region (right BA 9) than their respective control groups, young and middle aged controls, respectively, in multiple areas of the brain. Unaffected siblings demonstrated less connectivity with left BA 8 and BA 10 than middle aged controls. Note: predictor beta weights were extracted from the five clusters derived from the patients vs. controls functional connectivity contrast with right BA 9 seed region.

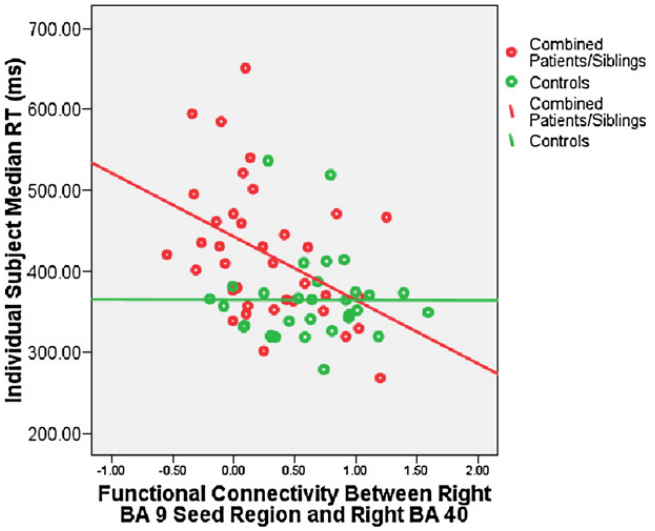

Fig. 4. Correlation between right BA 9 functional connectivity with right BA 40 and choice reaction time in controls and the combined patients/unaffected siblings group. Correlation differed between groups ($p<.05$).

functional connectivity between the seed region and these areas was associated with longer reaction times and worse accuracy (see online Supplementary Table 1). The correlations were recalculated within the controls and the combined patient/sibling group separately to determine if the pattern of correlations varied as a function of activity in the seed region. Linear regression with RT entered as the dependent variable and seed region functional connectivity with right BA 40 and group (combined patients/sibling and controls) entered as predictors revealed a significant interaction between group and connectivity ($t(64)=2.06$; $p<.05$) indicating that the correlation between connectivity and RT differed between controls ($r=.01$, $p<.99$) and the combined patient/sibling group ($r=-.43$, $p<.01$). This correlation was significant within the unaffected sibling group ($r=-.65$, $p<.03$) As shown in Fig. 4, greater connectivity between right BA 9 and right BA 40 correlated with faster RTs in patients and siblings, but not controls. No additional significant correlations between connectivity and CRT performance were observed in the combined patients/sibling or control groups for the remaining 4 clusters identified in the patients vs. controls functional connectivity analysis.

4. Discussion

This study simultaneously examined behavioral performance and the neural correlates of CRT in medication free FEP patients, stable chronic patients, and unaffected siblings of individuals with schizophrenia. The inclusion of FEP and chronic patients, and unaffected siblings, combined with our data analysis approach that attempted to: 1) confirm the presence of behavioral deficits and changes in brain function in both patients groups, and 2) determine if the exact same changes are present in unaffected siblings using a hypothesis driven approach, are strengths of the study. Consistent with previous findings (Pellizzer and Stephane, 2007; Krieger et al., 2001; Krieger et al., 2005); both FEP and chronic patients demonstrated longer RTs and a subtle deficit in accuracy on the CRT task. Unaffected siblings also made more errors; however, in contrast to a prior twin study, RT was not impaired (Cannon et al., 2000). The failure to identify a significant reaction time deficit in unaffected siblings likely relates to the reduced genetic concordance between siblings, compared to twins, and the smaller number of unaffected siblings included in the current study. It should be noted that although both patients and unaffected siblings made slightly more errors than controls, task performance was very high (>90%) in all groups suggesting that the imaging results were not compromised by poor performance in the patient and unaffected siblings groups.

CRT is an ideal paradigm to use for imaging potential alterations in brain function related to genetic vulnerability for schizophrenia due to the fact that CRT is heritable, associated with genetic vulnerability for schizophrenia in twin studies, and the deficit observed in patients appears state-independent; three key criteria for an endophenotype (Krieger et al., 2001, 2005; Cannon et al., 2000; Wright et al., 2001; Gottesman and Gould, 2003). Our analyses revealed that patients demonstrated greater BOLD response than controls during CRT in the right dorsolateral PFC (dlPFC) and SMA. Importantly, the exaggerated BOLD response observed in the right dlPFC was also detected in unaffected siblings. Consequently, these results suggest that altered activity in the right dlPFC, but not SMA, may relate to genetic vulnerability for schizophrenia. However, it should be noted that although both patient groups demonstrated over-activation of the right dlPFC, compared to their respective control groups, the relative degree of over-activation was greater in chronic patients. This suggests that the alteration in dlPFC activation during CRT may also relate to non-genetic factors such as medication and/or chronic illness/severity effects on brain structure and function.

It is noteworthy that the area of the PFC demonstrating over-activity in the current study overlaps considerably with regions that showed a similar hyper-active response during an N-back working memory task in patients and unaffected siblings (Callicott et al., 2000, 2003b,a) suggesting that exaggerated response of the PFC may relate to a cognitive process tapped by both CRT and some N-back working memory paradigms. It has been hypothesized that the greater BOLD response observed in the PFC during some N-back working memory tasks in patients and unaffected siblings reflects an "inefficient" maladaptive response (Weinberger et al., 2001). It is possible that the current results reflect a similar mechanism in that the exaggerated response observed in the PFC of patients and unaffected siblings also relates to decreased cortical "efficiency." Evidence that activity in the PFC increases linearly with the number of response options in healthy individuals lends further support to this hypothesis (Schumacher et al., 2003).

Functional connectivity analysis revealed marked differences in the degree of connectivity between right dlPFC and other brain regions across groups, and shed light on the relationship between cerebral neurophysiology and task performance. Specifically, functional connectivity analysis revealed two salient features of the altered right dlPFC activity observed in patients and unaffected siblings: 1) overall connectivity between dlPFC and multiple brain regions is reduced, and 2) the association between connectivity in a right fronto-parietal network and task performance is different for patients and unaffected siblings. Reduced connectivity

between the dlPFC and other brain areas, particularly posterior brain regions, is emerging as a consistent finding in imaging studies of schizophrenia and the present study extends the work in patients to unaffected siblings (Courtney et al., 1997; Williamson, 2007; Garrity et al., 2007; Salgado-Pineda et al., 2007; Zhou et al., 2007; Pomarol-Clotet et al., 2008; Yoon et al., 2008). Indeed, there is considerable evidence that fronto-parietal dysconnection contributes to a variety aspects of schizophrenia, including impaired cognition (Torrey, 2007). Similarly, the current results extend the differences between controls and patients in the relationships between cerebral function and task performance identified in prior studies to unaffected siblings (Tan et al., 2006). What remains to be determined is why the relationship between fronto-parietal connectivity and task performance differs between patients/unaffected siblings and controls in the manner in which it does (i.e. less connectivity compared to controls, but connectivity positively correlated with performance in combined patients/siblings group). One possible explanation is that the relationship between performance and connectivity may be non-linear such that connectivity may predict performance, but only up to a certain point. In this case, it may be that impaired connectivity limits performance in patients and unaffected siblings. Further investigation of the relationship between functional connectivity and CRT in control subjects would be helpful in elucidating the neural basis of CRT. Regardless, there is converging evidence that cerebral connectivity is reduced in schizophrenia and that the relationship between cerebral function/connectivity and task performance may be altered. The current results implicate a genetic basis for at least some of these alterations.

There are several limitations of the study. First, the patient sub-groups and unaffected siblings sample sizes were somewhat small and they were not precisely matched on all demographic variables (i.e. education) to the control groups. This may have compromised the sensitivity and validity of the analyses; although the imaging results remained unchanged when education was added as a covariate. The reduction in sample sizes after stratification may also explain why some of the alterations in functional connectivity where not consistently observed in both patient groups and unaffected siblings. These risks are mitigated to some extent by the hypothesis driven approach we took to examining behavioral changes and BOLD response differences between unaffected siblings and controls; however, these results were not corrected for multiple comparisons and replication of the findings is essential. A second limitation relates to the use of BOLD functional connectivity analyses to infer neuronal connectivity. fMRI functional connectivity analysis is limited by its inability to infer causality and its dependence on hemodynamic signals, which are correlated with local field potentials but do not always reflect neuronal activity directly (Logothetis, 2008). However, recent results do indicate a relationship between functional connectivity and underlying neuronal circuitry in computational models and comparison to firing rates and gamma band power modulation in the sensory cortex (Horwitz et al., 2005; Nir et al., 2008). Finally, our confidence that the findings relate to response selection and not alternative cognitive, motor, or perceptual demands of the task is limited by the fact that CRT blocks were compared to a very low level baseline condition (i.e. passive fixation viewing). However, it is noteworthy that the dlPFC region demonstrating greater activity in patients and unaffected siblings is virtually identical to the area identified in response selection studies in healthy controls (Schumacher et al., 2003; Dux et al., 2006). At the very least, the current findings suggest that follow-up imaging studies of response selection in schizophrenia patients and their unaffected relatives are warranted.

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Contributors

Author NDW conceptualized the purpose of the study, performed the literature search and statistical analyses, and wrote the first and revised drafts of the manuscript. Author BW provided assistance with data analysis, subject recruitment, and manuscript preparation. BR contributed to the data analysis, particularly functional imaging connectivity analyses, and provided comments on earlier drafts of this manuscript. Authors SEP and PT secured funding for the study and contributed to writing drafts of this manuscript. PS implemented the imaging portion of the study protocol. All authors contributed to and approved the final draft of the manuscript.

Conflict of interest

Neil Woodward, Barb Waldie, Baxter Rogers, Phil Tibbo, and Peter Seres have no conflicts of interest, financial or otherwise, to disclose. Scot Purdon has received grant/research support, served on an advisory board and/or as a speaker for Eli Lilly & Co, Janseen-Ortho, Astra-Zeneca, Pfizer, Novartis, and CV Technologies. No commercial support was received for this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2008.11.028.

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