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An fMRI investigation of procedural learning in unaffected siblings of individuals with schizophrenia

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Abstract

Vulnerability for schizophrenia is related, in part, to genetic predisposition. The identification of pathophysiological abnormalities associated with the disorder that are also present in unaffected family members of individuals with schizophrenia may assist in delineating the genetic contributions to vulnerability for schizophrenia. Previous functional Magnetic Resonance Imaging (fMRI) investigations of procedural learning in patients with schizophrenia identified reduced activity in the frontal cortex, basal ganglia, and parietal cortex during performance of the serial reaction time (SRT) task suggesting that abnormal function of these regions may relate to genetic vulnerability for schizophrenia. In order to examine this hypothesis, 12 unaffected siblings of patients and 15 controls underwent fMRI during performance of the SRT task. Unaffected siblings demonstrated normal performance on the SRT task. However, compared to controls unaffected siblings demonstrated less activity in regions of the frontal and parietal lobes and, to a lesser extent, basal ganglia, during procedural learning. Interestingly, unaffected siblings demonstrated greater activity in regions of the frontal cortex during the control condition compared to the procedural learning condition of the SRT task, an idiosyncratic pattern that was also observed in patient groups but not control subjects of two prior imaging studies. The findings support previous investigations suggesting that altered cerebral neurophysiology during performance of cognitive tasks may be related to genetic vulnerability for schizophrenia. Identification of genes related to the function of cerebral regions such as the prefrontal cortex, parietal lobe, and basal ganglia may assist in delineating the genetic contributions to schizophrenia.
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1. Introduction

A genetic basis for schizophrenia is strongly suggested by the increased familial risk for the disorder (Gottesman, 1991). However, the lack of 100% concordance in monozygotic twins and aggregation of illness risk in affected families implies that schizophrenia is a polygenetic disorder with a complicated etiology involving a dynamic interplay between multiple susceptibility genes and environmental factors (Gottesman and Shields, 1967; Shields and Gottesman, 1972; Caspi et al., 2005). Schizophrenia is characterized by abnormalities in neuropsychological function, cerebral morphology, and neurophysiology (Heinrichs and Zakzanis, 1998; Shenton et al., 2001; Kircher and Thienel, 2005) and searching for similar deficits in unaffected family

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members may provide insight into underlying pathological mechanisms that are related to genetic liability. This approach has identified abnormalities in neuropsychological function, cerebral morphology, and neurophysiology, in unaffected family members, including siblings of patients, that are similar to those observed in affected family members (Cornblatt and Keilp, 1994; Myles-Worsley and Park, 2002; Sitskoorn et al., 2004; Cannon et al., 2002; Narr et al., 2002; Steel et al., 2002).

Abnormalities in cerebral activity detected in patients during performance of a variety of cognitive tasks are also observed in their unaffected relatives; although only a handful of studies have been carried out. Callicott and colleagues identified abnormalities in cerebral activity related to a verbal working memory task in two independent groups of unaffected siblings that were remarkably similar to the alterations observed in patients (Callicott et al., 2003a, 2000, 2003b). Consistent with their findings in patients using the same working memory paradigm, unaffected siblings evinced greater activity in the dorsolateral and inferior prefrontal cortex (PFC) and parietal lobe during performance of a verbal N-back task, despite performing relatively normal compared to controls. Alterations in working memory related cerebral activity in prefrontal cortex and parietal lobe has also been reported by others (Thermenos et al., 2004; Brahmbhatt et al., 2006). Abnormal cerebral neurophysiology in unaffected first degree relatives of patients is not limited to working memory tasks. Abnormal activity in the frontal lobes and basal ganglia during performance of eye tracking and antisaccade tasks, respectively, has been documented (O'Driscoll et al., 1999; Raemaekers et al., in press) as have differences in the neural timing of activations in the PFC during performance of a stimulus-response incompatibility task (MacDonald et al., 2006). Combined, these studies suggest that unaffected relatives demonstrate abnormal activation of cortical-sub-cortical circuits during performance of a variety of tasks and that, in some cases, the alteration in brain activity is not accompanied by impaired behavioral performance.

The goal of the current experiment was to identify the neural correlates of performance on the Serial Reaction Time (SRT) task (Nissen and Bullemer, 1987), a commonly used test of procedural learning, in a sample of unaffected siblings of patients with schizophrenia and an aged matched group of controls in order to determine if the functional alterations observed in patients in prior studies is related to genetic liability for schizophrenia. Procedural learning refers to the ability to acquire a motor skill or cognitive routine in the absence of declarative knowledge (Cohen and Squire, 1980), and the SRT task is frequently used to examine procedural learning in healthy, psychiatric, and neurological populations. Three previous imaging studies of SRT performance in schizophrenia produced several important findings. The first study, by Kumari et al. (2002), revealed a performance deficit in patients that was accompanied by an absence of activity in the frontal cortex, striatum, thalamus, and cerebellum relative to an age-matched control sample. Unfortunately, the results of this study are difficult to interpret because patients were receiving typical antipsychotic drugs (APDs) at the time of scanning, drugs that interfere with procedural learning (Purdon et al., 2003; Stevens et al., 2002; Kumari et al., 1997), and there were marked performance differences between patients and controls. Two subsequent studies by Zedkova et al. (2006) and Reiss et al. (2006) avoided the treatment confound by scanning subjects being treated predominantly or exclusively with atypical APDs, drugs that have a more benign D2 binding profile (Kapur and Seeman, 2001; Seeman, 2002) and do not impair procedural learning (Purdon et al., 2002, 2003; Stevens et al., 2002). Both studies confirmed that patients fail to activate the striatum, caudate in particular, during performance of the SRT. Additional abnormalities were identified in left premotor cortex in both studies and, in the case of one study, reduced activity in the left parietal cortex and increased activity in the anterior cingulate and temporal lobe, relative to controls, was also observed (Zedkova et al., 2006). Interestingly, reduced volume of the presupplementary area is inversely correlated with procedural learning on the SRT task in schizophrenia (Exner et al., 2006). The abnormalities identified in the two subsequent studies could not be explained by differential performance between controls and patients as the patient groups in both studies demonstrated the same degree of procedural learning as controls. Interestingly, in both the Zedkova et al. (2006) and Reiss et al. (2006) studies, patients demonstrated greater activation in the PFC during the control condition relative to the procedural learning condition, an idiosyncratic pattern that was not observed in the control groups of either study.

The initial findings reported by Kumari et al. (2002) suggested that SRT performance deficits in schizophrenia result from a failure to activate structures central to PL circuitry and that this may reflect a core deficit in schizophrenia, but may also be a deleterious side effect of treatment with typical APDs. On the other hand, the findings reported by Zedkova et al. (2006) and Reiss et al. (2006) suggest that patients do not demonstrate the same degree of activity in the prefrontal cortex,

striatum, and perhaps parietal cortex, despite performing the SRT task relatively normally, at least when receiving predominantly atypical APDs. Moreover, preliminary evidence suggests that patients may compensate for a failure to activate regions normally implicated in the SRT task by recruiting alternate regions. If siblings also fail to activate similar regions or recruit alternate ones compared to controls during performance of the SRT task then the alterations observed in patients may relate to genetic liability for schizophrenia. Conversely, if siblings demonstrate normal cerebral activity then the abnormalities observed in patients likely reflects disease specific alterations unrelated to genetic liability for schizophrenia or a medication induced alteration in neural activity.

2. Methods

2.1. Subjects

Twelve, right handed, unaffected siblings of individuals with schizophrenia and fifteen, right handed, age-matched controls were recruited for this study. Controls were recruited largely from employees and students of the University of Alberta. Siblings were recruited from first episode and chronic patients with schizophrenia seen at the Edmonton Early Psychosis Intervention Clinic (EEPIC) or the Schizophrenia Clinic at the University of Alberta Hospital. All subjects were provided a verbal and written description of the study prior to solicitation of written informed consent to participate. Exclusion criteria included current or prior history of any DSM-IV Axis I psychopathology, as determined using the Structured Clinical Interview for DSM-IV Axis I disorders (SCID: First et al., 1996), current use of any psychotropic medication, history of head injury or neurological disease, presence of systemic medical disease likely to affect central nervous system functions, current or previous alcohol/substance abuse or dependence, and the presence of ferromagnetic objects in the body. In addition, positive family history of schizophrenia was also an exclusion criterion for control subjects. Demographic data for the subjects is presented in Table 1.

Table 1
Sample characteristics[a]

Variable	Controls	Siblings	Test statistic
n	15	12	
Age	31.3 (11.2)	36.9 (13.3)	$t(25)=1.19$, $p<.248$
Education	17.2(2.6)	15.0 (2.3)	$t(25)=2.37$, $p<.027$
Parental SES[b]	2.6 (0.5)	3.2 (0.8)	$t(25)=2.18$, $p<.040$
Sex (men/women)	10/5	5/7	$x^2=1.68$, $p<.195$

[a] Mean and (SD).
[b] Parental Socioeconomic Status (SES). Note: Lower scores equal higher status.

2.2. Behavioral paradigm and statistical analysis of behavioral data

The paradigm and analysis of behavioral data were identical to those used in our previous report on procedural learning in patients with schizophrenia (Zedkova et al., 2006) and is described in more detail in the online Supplementary Material. Briefly, subjects were instructed to identify the location of a target that could appear in one of four spatial locations on each trial as quickly and accurately as possible by pressing the response key that corresponded to the location of the target. Sixty trials comprised a block and the blocks were either sequenced (S) or random (R). Within S blocks the location of the target followed a 12-element sequence that repeated five times. During R blocks the location of the stimulus appeared randomly with the caveats that all 4 locations appeared with equal frequency within a block, and no location repeated consecutively. Subjects completed two scanning runs, each consisting of 3 S and 3 R blocks alternating in a blocked AB manner, with each block separated by an 18 second fixation point resting period. Subjects completed 5 consecutive blocks of 72 sequenced trials consisting of 6 repetitions of the SOC sequence immediately before entering the scanner.

2.3. fMRI data analysis

2.3.1. Image acquisition

All structural and functional MRI images were acquired during a single session on a Siemens Sonata 1.5T scanner located at the University of Alberta In Vivo Imaging Center. 25 contiguous axial (approximate range $Z=70$ to $Z=-30$), 4 mm thick functional images acquired parallel to the AC-PC line using a single-shot, T2* EPI sequence (matrix = 128 × 128; voxel size 1.72 × 1.72 × 4mm; TR = 3000 ms; TE = 50 ms) were collected. 159 volumes were acquired during each of the two runs but the first three volumes of each run were discarded. A high resolution, 144 slice, 1 × 1 × 1 mm voxel size 3D structural image was also acquired using an MPRAGE sequence.

2.3.2. Functional imaging statistical analysis

To reduce the number of voxels included in the statistical analysis and to examine activations in specific

a-priori defined regions of the brain, two anatomical masks were used (Zedkova et al., 2006). The masks reduced the number of comparisons performed within the statistical parametric maps (SPMs) by limiting subsequent statistical analyses to only those voxels contained within the cortex and sub-cortical regions of interest (ROIs). The first mask, a cortex based mask, was created to examine areas of activation within the cortex (described in detail in Goebel et al., 1998; Kriegeskorte and Goebel, 2001; Zedkova et al., 2006). Evidence acquired from a broad array of sources including lesion, neurological, and functional imaging studies, implicates the caudate, putamen, thalamus, and globus pallidum in procedural learning. As such, an ROI approach was undertaken to better identify activity in these relevant sub-cortical structures. The second structural, sub-cortical mask restricted the statistical analyses to only those functional voxels included in the thalamus, caudate, putamen, and globus pallidum.

Statistical analyses 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 S and R blocks entered as predictors in a fixed effects general linear model (GLM) analysis corrected for serial autocorrelations. SPMs comparing S to R blocks were created for each group in order to identify the pattern of activations unique to each sample. Since no cortical ROIs were specified a priori, the threshold for the cortex based statistical analysis was set to $p<.005$ with a cluster threshold of 6 functional voxels (Forman et al., 1995). A statistical threshold of $p<.01$ with no minimum cluster size threshold specified a priori was used to identify significant voxels for the sub-cortical ROI analysis. In addition, an ANCOVA analysis with reaction time advantage during the scanning session (median reaction time for R blocks minus median reaction time for S blocks) entered as a covariate was performed in order to identify regions where the BOLD signal change between conditions correlated with reaction advantage. Within group correlations are reported at the statistical thresholds described above for the cortical and subcortical ROI SPMs. Significant differences in activations between groups were examined by entering all subjects into a voxelwise random effects GLM analysis that was restricted to only the voxels that demonstrated a significant effect of condition (S>R or R>S) in either the control group or sibling group. A p-value of .05, with a cluster size threshold of 6 functional voxels for the cortex based analysis, was applied to this analysis since the between groups contrast only included voxels that exceeded threshold in the within groups analyses.

3. Results

3.1. Behavioral data

Behavioral data from one control subject was lost due to experimenter error leaving complete behavioral data for 14 controls and 12 siblings. Mean median reaction times during the pre-scanning and scanning sessions are presented in Fig. 1. Analysis of the SRT RTs for the pre-scanning session revealed a main effect of block ($F(4,21)=4.83$, $p<.007$), but no main effect of group ($F(1,24)=1.94$, $p<.177$) or block by group interaction ($F(4,21)=0.25$, $p<.908$). Subjects got progressively faster over blocks such that the mean of the median RTs for the final block was approximately 40 ms faster than it was for the first block of trials ($F(1,24)=16.39$, $p<.001$). The block 5 vs. block 1 time advantage did not differ between controls and siblings ($t(24)=0.36$, $p<.973$). Accuracy rates were high in both controls (96%) and siblings (95%) and did not differ between groups ($t(24)=0.81$, $p<.430$).

A main effect of condition was observed during the scanning session ($F(1,24)=29.78$, $p<.001$) due to the

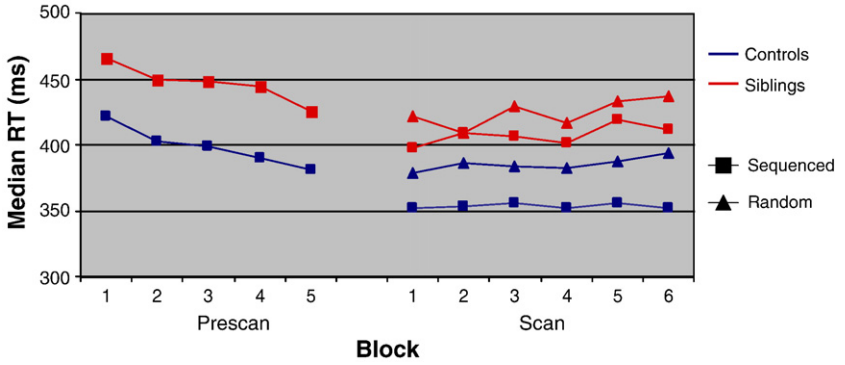

Fig. 1. Median SRT reaction times for controls and siblings.

Table 2
fMRI results in controls and unaffected siblings

Group	Brain region	Talairach X	Talairach Y	Talairach Z	t score	Size (mm^3)
Controls						
S>R Contrast: Cortex Based Analysis						
	L. Middle Frontal Gyrus (BA 6)	−30	6	52	4.22	459
	L. Superior Frontal Gyrus (BA 9)	−18	40	34	3.12	216
	L. Superior Frontal Gyrus (BA 10)	−22	58	12	3.22	351
	R. Superior Frontal Gyrus (BA 9)	14	52	19	3.99	378
	L. Angular Gyrus (BA 39)	−43	−67	32	3.77	1863
	R./L. Anterior Cingulate (BA 24/32)	−4	33	−5	3.87	1593
	L. Inferior Frontal Gyrus (BA 47)	−49	28	−8	3.66	351
	L. Inferior Frontal Gyrus (BA 10)	−48	43	−1	3.61	513
	L. Medial Frontal Gyrus (BA 10)	−10	53	7	3.46	513
	R. Middle Temporal Gyrus (BA 21)	60	−1	−9	3.78	837
		45	7	−24	3.49	324
	L. Middle Temporal Gyrus (BA 21)	−52	−9	−14	3.94	756
Sub-Cortical ROI Analysis						
	L. Caudate Body	−12	11	7	4.03	1944
	L. Anterior Thalamic Nucleus	−3	−2	7	2.94	135
	R. Caudate Body	15	17	4	2.72	27
	R. Caudate Body	11	2	4	2.83	54
	R. Putamen	30	−22	10	3.18	108
R>S Contrast: Cortex Based Analysis						
	R. Precuneus (BA 19)	25	−74	34	3.57	783
	R. Middle Temporal Gyrus (BA 37)	51	−57	0	3.36	378
	L. Fusiform Gyrus (BA 37)	−47	−45	−20	3.56	270
Siblings						
S>R Contrast: Cortex Based Analysis						
	L. Superior Frontal Gyrus (BA 6)	−15	14	49	3.97	918
	L. Middle Frontal Gyrus (BA 8)	−29	13	40	3.59	513
	L/R Anterior Cingulate (BA 24/32)	3	26	4	3.85	675
	L. Middle Temporal Gyrus (BA 21)	−52	−19	−17	3.14	216
	L. Inferior Frontal Gyrus (BA 47)	−22	17	−14	5.00	675
	L. Fusiform Gyrus (BA 37)	−41	−50	−18	3.59	189
S>R Contrast: Sub-Cortical ROI Analysis						
	L. Caudate	−18	14	7	2.55	27
	R. Caudate	18	14	6	2.82	54
R>S Contrast: Cortex Based Analysis						
	R. Precentral Gyrus (BA 6)	34	−7	58	3.80	270
	L. Middle Frontal Gyrus (BA 9)	−46	23	28	4.13	297
Siblings						
R>S Contrast: Cortex Based Analysis						
	R. Middle Frontal Gyrus (BA 10)	38	49	15	3.71	783
	L. Parahippocampal Gyrus	−17	−8	−11	3.41	270
R>S Contrast: Sub-Cortical ROI Analysis						
	L. Lateral Globus Pallidus	−27	−16	−5	2.68	27
	R. Globus Pallidus	18	−7	−7	4.62	2079

Abbreviations: L: left; R: right; BA: Brodmann's Area; ROI: Region.

fact that subjects responded approximately 24 ms faster during S blocks compared to R blocks. The main effects of block ($F(5,20)=1.92$, $p<.137$) and group ($F(1,24)=2.10$, $p<.161$) were not significant nor were any of the interaction terms (all F-statistics <2.80, $p<.108$). Repeated measures analysis of the accuracy rates indicated that subjects performed equally well during S (97.1%) and R (96.7%) blocks ($F(1,24)=1.60$, $p<.219$); however there was trend for controls to be slightly more accurate overall than siblings (98.0% vs. 95.8%; $F(1,24)=3.90$, $p<.061$). There was no interaction between condition and group ($F(1,24)=0.46$, $p<.504$) with respect to accuracy rates.

Correlations between the RT advantage observed during scanning and demographic variables age, gender, education, and SES were performed to ensure that demographic differences between the two groups did not account for the behavioral results. None of the demographic variables was correlated with procedural learning in the combined total sample, control group, or sibling group.

3.2. Imaging results

The imaging results were based on the complete sample of 15 controls and 12 siblings. The pattern of activations observed in controls and siblings is presented in Table 2 and Fig. 2. In controls, significant activations during S, relative to R blocks, were observed in several cortical areas, primarily in the left hemisphere, including the rostral ventral anterior cingulate corresponding to Brodmann's Area (BA) 24/32, regions of the superior, middle, and inferior frontal gyri, and inferior parietal lobe, particularly regions typically involved in motor function (BA 6), spatial attention (BA 39), and subregions of the PFC (BA 9 and 10). In addition, greater activity was also observed bilaterally in the middle temporal gyrus. Sub-cortical activations were observed

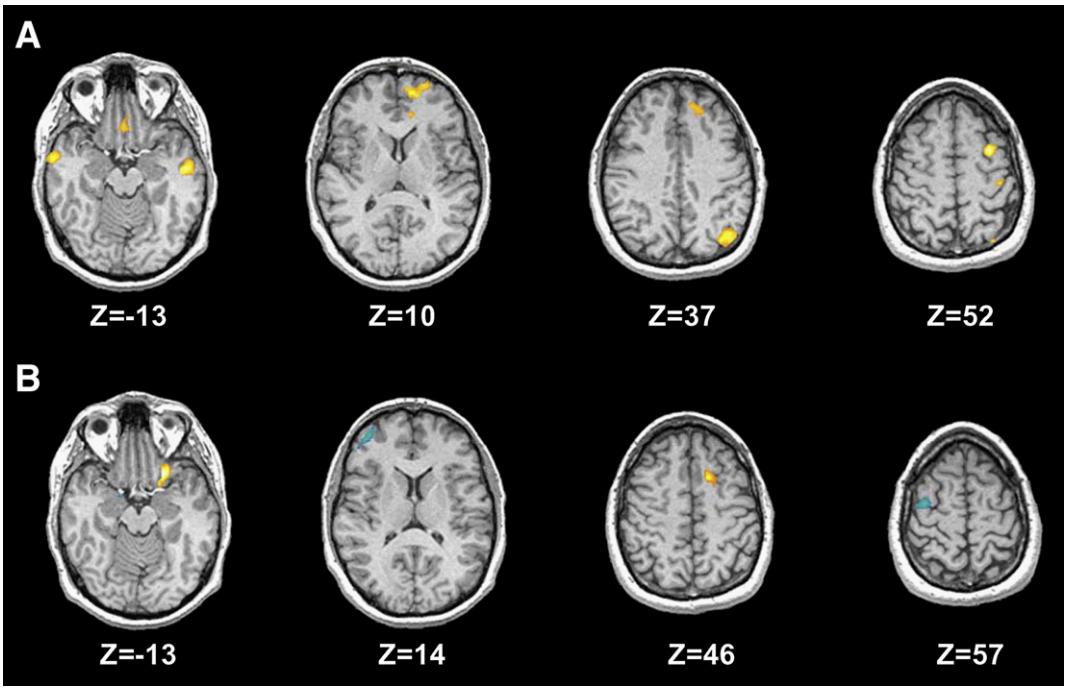

Fig. 2. Regions active during procedural learning in controls (panel A) and unaffected siblings (panel B). Region more active in controls when S blocks were contrasted with R blocks (warm colors) included left superior and middle frontal gyri corresponding to Brodmann's Areas (BA) 6, 9, and 10, left angular gyrus (BA 39), and bilateral middle temporal gyrus (BA 21). Regions more active during S blocks in siblings included left premotor cortex (BA 6) and left inferior gyrus (BA 47). Siblings also demonstrated greater activity during R blocks (cool colors) in the right precentral gyrus (BA 6) and right middle frontal gyrus (BA 10). Note, left/right orientation reversed on axial slices.

primarily in the left caudate and anterior thalamic nucleus, and, to a lesser extent, right caudate and putamen. The reverse contrast (R > S) revealed only three regions in the control group; right precuneus, right middle temporal gyrus, and left fusiform gyrus, that were more active during R blocks compared to S blocks.

Siblings also demonstrated greater activity when S blocks were contrasted with R blocks in several cortical regions including the rostral ventral anterior cingulate (BA 24/32), multiple regions of the PFC including premotor cortex (BA 6), middle frontal gyrus, and inferior frontal gyrus. The sibling group also demonstrated activity in the left middle temporal gyrus corresponding to BA 21 and fusiform gyrus corresponding to BA 21 and 37, respectively. Consistent with controls, the cortical activations were almost exclusively in the left hemisphere. With respect to the sub-cortical ROI analysis, the sibling group demonstrated greater activity bilaterally in the caudate. Several cortical and sub-cortical regions demonstrated greater activity during R blocks, relative to S blocks, in the siblings. These included several foci bilaterally within the PFC, right caudate, left parahippocampal gyrus, and left globus pallidus.

Direct comparison between groups revealed several cortical and sub-cortical regions that were more active in the controls than siblings when S blocks were contrasted with R blocks. Specifically, controls activated regions of the superior and middle frontal gyri corresponding to BA 9 and 10 bilaterally, left angular gyrus (BA 39), and left parahippocampal gyrus to a greater extent than siblings. Greater activity in controls in bilateral middle frontal gyrus corresponding to BA 9/10 and left parahippocampal gyrus was due to the fact that siblings demonstrated less activity in these regions during S blocks compared to R blocks. With respect to sub-cortical regions, controls activated the left caudate, and right anterior thalamic nucleus, putamen, and medial globus pallidus to a greater extent than siblings. The greater activity observed in controls in the right globus pallidus reflected the fact that siblings demonstrated greater activity in this region during R blocks compared to S blocks. In contrast, siblings activated the left fusiform gyrus corresponding to BA 37 more than controls. Percent signal change was extracted from each cluster identified in the between groups comparison was subjected to an ANCOVA with each subjects procedural

learning score (i.e. RT advantage) during the scanning session entered as a covariate to verify that the between groups differences identified in the above regions were not due to any potential differences in SRT performance. One control subject was excluded from this analysis due to loss of behavioral data. All clusters identified in the between groups analysis remained significant after covarying for SRT performance with the exception of the left parahippocampal cluster, which was significant at the trend level ($p<.075$). Group differences in procedural learning related activity are presented in Table 3 and Fig. 3.

Within the control group, no regions in either the cortex based analysis or sub-cortical ROI were correlated with reaction time advantage at corrected statistical thresholds. Within the sibling group, BOLD signal change in a cluster located in left middle temporal gyrus corresponding to BA 19 was inversely correlated with reaction time advantage ($r=-85$). This correlation was significantly different than the correlation observed in the same region within the control group (control group $r=.07$; $Z=2.87$, $p<.005$). No regions in the sub-cortical ROI were correlated with reaction time advantage at corrected statistical thresholds. Additional correlations between BOLD signal change and reaction time advantage at the uncorrected statistical threshold ($p<.05$) are presented in Table 4 of the online Supplemental Material.

Table 3
Group differences in activations during procedural learning (S vs. R blocks)

Contrast	Brain region	Talairach			*t* score	Size (mm^3)
		X	*Y*	*Z*		
Controls>Siblings						
Cortex based Analysis	L. Middle Frontal Gyrus (BA 9)	−46	24	29	3.22	162
	R. Middle Frontal Gyrus (BA 10)	35	51	15	2.36	270
	R. Superior Frontal Gyrus (BA 9)	14	53	20	2.71	243
	L. Angular Gyrus (BA 39)	−45	−66	32	2.79	999
	L. Parahippocampal Gyrus	−16	−8	−12	2.53	189
Sub-Cortical	R. Anterior Thalamic Nucleus	18	−7	15	3.29	54
ROI Analysis	L. Caudate Head	−15	23	6	2.25	54
	R. Putamen	27	−22	10	2.07	27
	R. Medial Globus Pallidus	18	−6	−7	4.03	1593
Siblings>Controls						
Cortex Based Analysis	L. Fusiform Gyrus (BA 37)	−44	−48	−19	3.05	243

Abbreviations: L: left; R: right; BA: Brodmann's Area; ROI: Region of Interest.

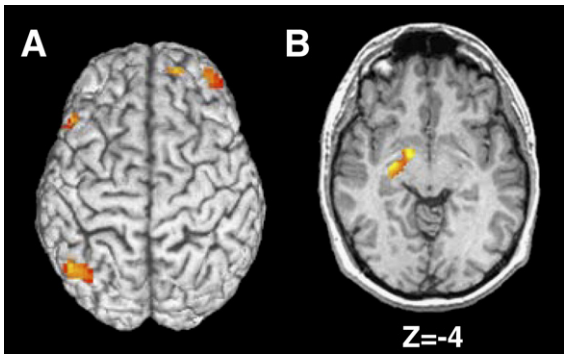

Fig. 3. Differences between controls and unaffected siblings in procedural learning related activity on the SRT task. Controls demonstrated greater activity during procedural learning in (A) prefrontal regions corresponding to right superior and middle frontal gyri (BA 9 and 10), left middle frontal gyrus (BA 9), left angular gyrus (BA 39), and (B) right medial globus pallidus. Note, left/right orientation reversed on axial slice.

4. Discussion

The present study examined behavioral performance and cerebral activity related to procedural learning, as quantified using the SRT task, in a sample of unaffected siblings of individuals with schizophrenia and an aged matched control sample with no family history of schizophrenia. Both siblings and controls demonstrated a significant reaction time advantage to blocks where the location of the target followed a repeating pattern relative to blocks in which the location of the target appeared pseudorandomly. Moreover, there was no difference between siblings and controls with respect to the magnitude of this advantage. However, given that controls demonstrated a slightly greater reaction time advantage during scanning it remains possible that a behavioral difference might have been detected had a larger sample of unaffected siblings been recruited. With respect to the functional imaging results, unaffected siblings demonstrated less activity during procedural learning in several regions of the PFC, left angular gyrus, and basal ganglia. The fact that asymptomatic siblings demonstrated abnormalities in cerebral function that are similar in several respects to the abnormalities observed in patients supports the contention that cognitive impairment/deficits in cerebral activity during performance of cognitive tasks and symptoms of schizophrenia are unrelated. It also provides further

evidence that abnormalities in cerebral function during performance of cognitive tasks is related, at least in part, to genetic susceptibility for schizophrenia.

The current results are similar in some respects to the results from our previous application of the same methods to a sample of patients with chronic schizophrenia (Zedkova et al., 2006). Patients also demonstrated intact procedural learning on the SRT task, and evinced less activity than controls in multiple regions of the PFC, left angular gyrus, and bilateral caudate. In the current study, siblings demonstrated considerably less activity in many of the same regions including multiple areas of the PFC, and left angular gyrus. However, in contrast to patients, the difference between siblings and controls in the degree of activity in the caudate was spatially circumscribed; limited to a very small region of the left caudate. This observation suggests that the abnormal cortical responses detected in both patients and siblings may relate to genetic vulnerability for schizophrenia, but that the abnormal striatal response observed primarily in patients may relate to disease specific components of the illness or the effects of treatment with APDs. Future research examining correlations between regional cortical volumes and procedural learning in unaffected relatives of patients may be informative given that studies in patients have identified correlations between procedural learning and volume of the pre-supplementary motor area (Exner et al., 2006).

However, siblings did demonstrate some abnormalities in basal ganglia function. Specifically, siblings demonstrated an idiosyncratic pattern of greater activity in the globus pallidus during R blocks, relative to S blocks, and relatively less activity than controls in this region. Moreover, BOLD signal change in this same region was positively correlated with reaction time advantage in siblings suggesting that normalization of activity in this region may be associated with greater procedural learning. Interestingly, the exact opposite pattern (i.e. greater activity in globus pallidus when S blocks were contrasted with R block) was observed in patients in our prior study. The globus pallidus is the main output structure of the basal ganglia and it can down regulate cortical activity via inhibitory inputs to excitatory thalamo-cortical projections (Alexander et al., 1990). As such, in siblings decreased activity in the globus pallidus during S blocks may be a compensatory mechanism to promote cortical activity. In patients enhanced activity in the globus pallidus may serve to down-regulate the PFC, perhaps in favor of promoting activity in another neural system to facilitate learning, a hypothesis supported but the fact that patients demonstrated greater activity in the temporal lobe during procedural learning in our previous study. Combined, these findings may suggest a gradient of impairment in fronto-striatal circuits underlying procedural learning in siblings and patients. Specifically, in patients the fronto-striatal system may be impaired to the point that another circuit, possibly involving the temporal lobe, takes over to facilitate learning. In siblings, the degree of dysfunction may be modest enough to allow compensation within the system to take place. This hypothesis is parsimonious in that it explains the gradient of activity observed in the globus pallidus and frontal cortex across siblings and patients; increasing globus pallidus activity going from siblings to patients corresponded with decreasing frontal lobe activity during procedural learning.

The idiosyncratic finding of relatively greater activity during R compared to S blocks in the sibling group, especially in BA 10 of the right middle frontal gyrus, is also strikingly similar to the findings in our previous investigation of procedural learning in patients and, to a lesser extent, the results reported by Reiss et al. (2006). In our prior investigation, patients also demonstrated significantly greater activity during R blocks compared to S blocks in BA 10 of the right middle frontal gyrus. However, patients also demonstrated relatively greater activity during R blocks in additional regions of the right PFC including superior, middle, and inferior frontal gyri corresponding to Brodmann's areas 9, 10, and 47, respectively. Similarly, Reiss et al. (2006) reported increased activations during R blocks, relative to S blocks, in the left medial, right anterior cingulate, and bilateral pre-central cortices. The fact that the regions demonstrating greater activity during R blocks relative to S blocks appears more widespread in patients compared to unaffected siblings suggests that this aspect of altered cerebral activity may reflect both a genetic vulnerability for the disorder and disease specific processes. However, it is possible that the greater activity observed during random blocks relative to sequenced blocks reflects greater general processing demands not specific to procedural learning. As such, caution is warranted in interpreting these results in siblings. Further research examining the control condition used here to fixation epochs may be helpful in determining the nature of the results.

There are several caveats to the current study that may limit generalization of the results. The first relates to the lack of independent verification that declarative memory processes were not activated by the SRT task. As such, we cannot exclude a contribution from declarative processes with complete confidence, but this seems unlikely. Explicit sequence learning typically results in a greater reaction time advantage than observed

in this study and other independent investigations that were able to rule out an explicit contribution to sequence learning (Willingham et al., 2002; Unsworth and Engle, 2005; Rauch et al., 1997; Reiss et al., 2006; Kumari et al., 2002). Moreover, it is unlikely that idiosyncratic activation of declarative processes in a few subjects would mitigate the current results because prior imaging studies of the SRT have reported similar regional activations, PFC and striatum in particular, under implicit and explicit learning conditions (Willingham et al., 2002). A second caveat is that frequency of smoking was not collected for either the control sample or unaffected siblings group. Smoking is much more common among schizophrenia patients compared to other psychiatric disorders and the general population (Morris et al., 2006; Goff et al., 2005). This may also be true for unaffected relatives of patients, although this remains to be determined. Activity at nicotinic receptors has been linked to attention processes and associated activity in the parietal lobe in fMRI studies (Thiel et al., 2005). A third caveat relates to the manner in which the SRT task was administered. Specifically, consistent with several other studies, subjects completed a pre-scan session during which several sequenced blocks where administered prior to scanning. Significant improvement occurred over the pre-scan session suggesting that subjects demonstrated some procedural learning before entering the scanner; although the addition of a random block of trials at the end of the pre-scan session would have been necessary to determine if the improvement reflected the instantiation of procedural learning or a non-specific practice effect. As such, it is possible that the results obtained during scanning reflected cerebral regions involved in the retention of procedural knowledge as opposed to, or in addition to, those regions involved in the acquisition of procedural knowledge. Regardless, this does not invalidate the current results indicating a difference in activations between controls and unaffected siblings, nor does it mitigate comparison to our previous findings in patients since the experimental procedure was identical in the two studies, as were the behavioral findings.

Finally, it remains to be confirmed that the SRT-induced regional physiological activations are, at least partially, heritable. The heritability of SRT task performance and the heritability of cerebral activation patterns for the SRT task, or any cognitive task for that matter, have not yet been demonstrated. This will be necessary before atypical cerebral physiology can be accepted having value to the delineation of susceptibility genes for psychiatric disorders (Callicott and Weinberger, 2003). Moreover, it is possible that non-genetic factors, such as urbanicity for example, that share familial transmission may underlie the results (Van Os et al., 2003). The present results suggest that physiological anomalies can be identified in patients and generalized to unaffected family members, but the true value of the anomaly will rest upon replication of the present results and a clear demonstration that functional brain activity is heritable.

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Contributors

Each of the authors listed contributed equally to this study.

Conflict of Interests

The authors have no conflicts of interest to report.

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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.2007.04.026.

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