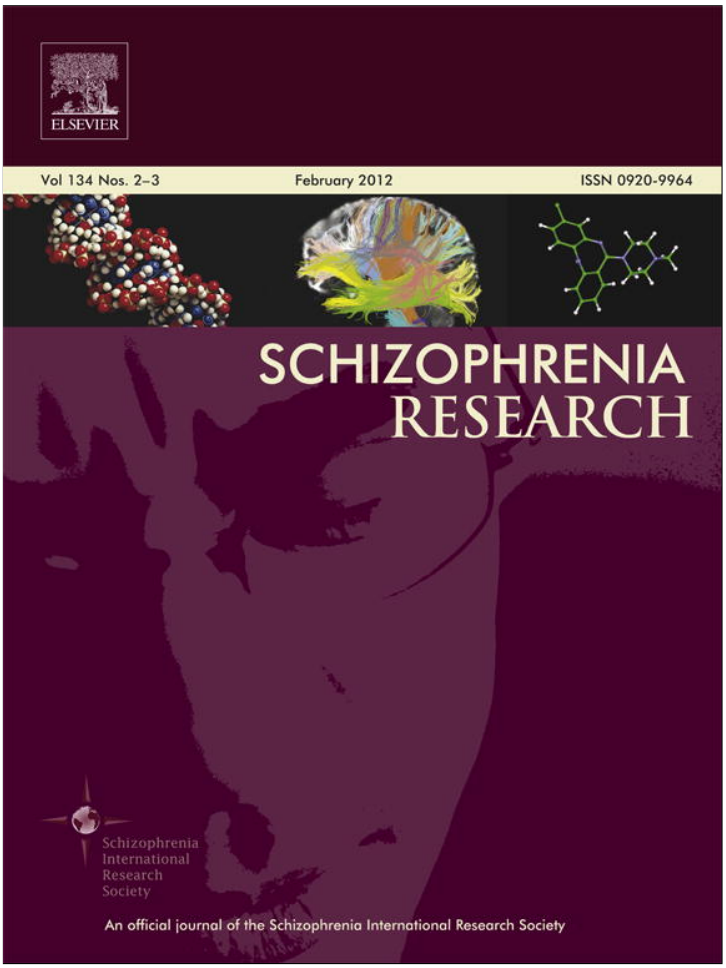

Schizophrenia Research 134 (2012) 171–179

Contents lists available at SciVerse ScienceDirect

Schizophrenia Research

journal homepage: www.elsevier.com/locate/schres

Decreased activity in right-hemisphere structures involved in social cognition in siblings discordant for schizophrenia

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ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 8 November 2011
Accepted 9 November 2011
Available online 3 December 2011

Keywords:
Schizophrenia
Siblings
Social cognition
Theory of mind
fMRI
Lateralization

ABSTRACT

Background: Social cognitive deficits contribute to functional disability in schizophrenia. Social cognitive tasks in healthy persons consistently evoke activation of medial prefrontal cortex, inferior frontal gyrus, temporoparietal gyrus, and posterior cingulate cortex/precuneus. We tested the hypothesis that patients with schizophrenia and their unaffected siblings share dysfunction of the same neural networks.
Methods: Neural activation during emotion processing (EP), theory of mind (ToM), and control tasks was measured using functional magnetic resonance imaging (fMRI) in 14 patients with schizophrenia, 14 nonpsychotic siblings of patients with schizophrenia, and 14 matched healthy subjects.
Results: Compared with healthy controls, patients with schizophrenia showed reduced activation of right hemisphere structures involved in EP and ToM including inferior frontal gyrus, middle frontal gyrus, and right temporoparietal junction. These deficits were shared, in part, by unaffected siblings. The latter group demonstrated deficits in bilateral precuneus activation during ToM, not present in patients.
Conclusions: Schizophrenia appears to be associated with a deficit in activation of right hemisphere components of a ToM network. Such deficits are shared in part by those at high genetic risk but unaffected by schizophrenia.

1. Introduction

Social functioning disturbances have long been recognized as an essential feature of schizophrenia, usually present before the onset of other symptoms, and persisting after the latter have abated (Kraepelin, 1899). The syndrome has a strong heritability, but it is highly unlikely that a single genetic alteration will account for the whole range of clinical manifestations (Weinberger, 2002). This has led to an effort to dissect the many behavioral abnormalities of the disease into discrete pathophysiological mechanisms, with the hope that each will have a simpler heritability than the syndrome as a whole (Weinberger, 2002). This might, in turn, facilitate the search for specific genes involved in the etiology of the disease. Such discrete pathophysiological features, termed endophenotypes or intermediate phenotypes, are quantifiable psychophysiological, neurocognitive, and motor variables, shown to be altered not only in schizophrenia, but also in at-risk subjects — notably, the patients' close relatives, who share with them a significant proportion of genes (Radant et al., 2010; Stone et al., 2011). These are shown to contribute to deficits of social cognition and disability seen in schizophrenia (Sergi et al., 2007). There is a paucity of studies seeking to define social cognitive intermediate phenotypes of social cognition, and to the extent of our knowledge, available studies exploring brain activity during social cognitive tasks in patients and at-risk subjects have involved emotion processing (EP) paradigms only (Habel et al., 2004; Rasetti et al., 2009). This might reflect the fact that brain networks subserving theory of mind (ToM) in normal conditions have only recently been outlined, and its components are still surrounded by controversy (Mar, 2011). Moreover, some data indicate that EP and ToM are closely interrelated phenomena, as judging other persons' intentions in facial expression does involve the evaluation of their emotional status as well as one's own response to it (Ochsner, 2008). This is most evident in tests involving recognition of facial expressions,

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doi:10.1016/j.schres.2011.11.010

which are often used in the measurement of either variable (Ochsner, 2008).

The aim of this study was to determine whether EP and ToM networks in patients with schizophrenia and their unaffected siblings differ from those in healthy controls. We hypothesized that brain activation patterns underlie performance during social cognition tasks (Benedetti et al., 2009), and on the basis of previous results on task performance (Anselmetti et al., 2009; de Achaval et al., 2010) expected brain activation during EP and ToM tasks to be similar in patients and their nonpsychotic siblings. More specifically, we predicted that abnormalities in relatives would include deficits that are less severe than those seen in patients, and perhaps with additional abnormalities as a consequence of untreated deficits (de Achaval et al., 2010).

Tasks involving the face and eyes have traditionally been used to measure abilities of EP and ToM, i.e., the capacity to infer the mental states and intentions of others (Baron-Cohen et al., 1997). In this study, we used the same stimuli in both control and experimental tasks by asking the participant to state the gender or the emotion/mental state respectively (Baron-Cohen et al., 1999a, 1999b; Russell et al., 2000; Habel et al., 2004). Given the nature of the stimuli (human expressions) and responses (choosing the correct word), we expected to observe involvement of mirror neuron systems and mentalizing or ToM systems on functional magnetic resonance imaging (fMRI) scanning (Pelphrey et al., 2004; Amodio and Frith, 2006; Van Overwalle and Baetens, 2009). In addition, stimuli used in the present study would be expected to evoke significant activation of language areas in the left hemisphere in all groups (Baron-Cohen et al., 1997; Lee et al., 2006). However, language aspects probed in our study are less concerned with phonology, syntax, and morphology (i.e., functions typically associated with the dominant hemisphere), and more with motor nonverbal expressions conveying actual meaning (associated with right-hemisphere processing; Mitchell and Crow, 2005). Since it has been argued that language is in general less lateralized in patients with schizophrenia (Crow, 1997), we predicted similar bilateral activation in schizophrenia patients and relatives, compared with healthy persons, expected to show preeminent activation of left language areas (Baron-Cohen et al., 1997).

2. Methods and materials

2.1. Participants

Two psychiatrists (SMG, EYC) and a psychologist (DDA) assessed all participants, who were seen at the Cognitive Neurology Section and the Psychiatry Department at FLENI Hospital, Buenos Aires. All participants were right-handed and provided written informed consent as approved by the local bioethics committee, and have therefore been performed in accordance with the ethical standards set by the 1964 Declaration of Helsinki.

2.1.1. Patients

Psychiatry outpatients were invited to participate in the study if they (a) had a DSM-IV-TR diagnosis of schizophrenia, any subtype, confirmed with a Composite International Diagnostic Interview (Robins et al., 1988) administered by a consultant psychiatrist (EYC), (b) were aged 18 to 50 years, and (c) had been on the same medications for at least two weeks. Patients reported having been on antipsychotic medications during the whole disease process, i.e., eight years on average (Table 1), but this could not be confirmed with chart review, nor were data available on exposure to typical vs. atypical antipsychotics during that period.Exclusion criteria were (a) misuse or addiction to illegal substances in the previous 6 months, (b) active symptoms having recently (<2 weeks) warranted antipsychotic dose adjustment or admission to the hospital, day hospital, or intensive outpatient treatment, or (c) a history of mental retardation. Current symptom severity was assessed with the Positive and Negative Syndrome Scale (Kay et al., 1987). A total of 14 patients

Table 1
Demographic and clinical data, MATRICS consensus cognitive battery scores, and response latency and accuracy.

	Patients (n = 14)	Siblings (n = 14)	Controls (n = 14)	Statistic	p
Age (years)	30.6 ± 7	30.4 ± 4.8	28.4 ± 8.3	F = 0.475	0.062
Education (years)	14 ± 2	15.1 ± 2.4	15.2 ± 1.8	F = 1.508	0.234
Parental education (years)	11.2 ± 3.6	12.8 ± 3.3	14.4 ± 3.6	F = 2.834	0.071
Women, n (%)	1 (7)	6 (43)	6 (43)	$X^2 = 5.57$	0.357
Age at onset (years)	23.5 ± 4.8				
Disease duration (years)	7.8 ± 4.5				
MMSE score	28.8 ± 1.5	28.9 ± 1.4	29.5 ± 0.9	F = 1.329	0.276
WAT score	32.4 ± 4.3	33.1 ± 5.6	34.6 ± 7.9	F = 0.454	0.638
FRT score	22.7 ± 2.6	24.6 ± 1.9	23.1 ± 4.9	F = 1.262	0.294
MCCB (percentile)					
Speed of processing	4 ± 0[a]	29 ± 26[a]	47 ± 18[a]	F = 17.98	<0.001
Attention/Vigilance	17 ± 21	27 ± 30	53 ± 22[a]	F = 7.815	0.001
Working memory	17 ± 19[b]	38 ± 33	58 ± 26	F = 8.501	0.001
Verbal learning	23 ± 22	32 ± 30	59 ± 24[a]	F = 7.406	0.002
Visual learning	28 ± 29[b]	51 ± 41	65 ± 23	F = 4.774	0.014
Reasoning/Problem solving	24 ± 22	29 ± 29	49 ± 31[a]	F = 7.071	0.002
Social cognition	21 ± 27	32 ± 32	63 ± 26[a]	F = 8.228	0.001
Symptom severity					
PANSS, positive	13.4 ± 6.5				
PANSS, negative	21.6 ± 7.6				
PANSS, total	36.6 ± 11.2				
Hamilton Depression score	6.4 ± 4.3[a]	3.0 ± 3.6	0.9 ± 1.4	F = 9.527	<0.001
Hamilton Anxiety score	8.9 ± 6.3[a]	4.7 ± 4.5	1.5 ± 2.1	F = 8.986	0.001
Medications					
Valproic acid, n (%)	1 (7.1)				
Risperidone, n (%)	5 (35.7)				
Olanzapine, n (%)	3 (21.4)				
Clozapine, n (%)	1 (7.1)				
Quetiapine, n (%)	2 (14.3)				
Paliperidone, n (%)	4 (28.6)				
CMZ equivalent (mg/day)	146.4				
SSRI, n (%)	4 (28.6)				
Promethazine (%)	1 (7.1)				
Biperidene	1 (7.1)				
Clomipramine	1 (7.1)				
Benzodiazepine, n (%)	8 (57.1)				
Response latency (ms)					
BET					
Experimental condition	241 ± 48[b]	218 ± 46	188 ± 44	F = 4.757	0.014
Control condition	189 ± 53[b]	160 ± 40	135 ± 34	F = 5.396	0.009
FToM					
Experimental condition	293 ± 54	273 ± 41	215 ± 53[a]	F = 9.331	<0.001
Control condition	182 ± 42[a]	153 ± 21	127 ± 21	F = 12.01	<0.001
EToM					
Experimental condition	280 ± 54	280 ± 34	248 ± 42	F = 2.388	0.105
Control condition	183 ± 51[b]	158 ± 34	142 ± 32	F = 3.795	0.031
Response accuracy (%)					
BET					
Experimental condition	95 ± 4[b]	97 ± 5	99 ± 2	F = 3.418	0.043
Control condition	98 ± 3	98 ± 5	99 ± 2	F = 0.254	0.777
FToM					
Experimental condition	91 ± 6 [b]	93 ± 7	97 ± 3	F = 3.309	0.047
Control condition	95 ± 3	95 ± 5	97 ± 2	F = 0.254	0.777
EToM					

Table 1 (*continued*)

	Patients (n = 14)	Siblings (n = 14)	Controls (n = 14)	Statistic	p
Experimental condition	88 ± 5	88 ± 6	91 ± 5	F = 1.903	0.163
Control condition	84 ± 3	85 ± 3	85 ± 4	F = 0.549	0.582

MMSE: Mini-Mental State Exam; WAT: Word Accentuation Test; FRT: Facial Recognition Test; MCCB: MATRICS consensus cognitive battery; SSRI: Specific serotonin reuptake inhibitor; CMZ: chlorpromazine; PANSS: Positive and negative symptom scale; BET: Basic Emotions task; FToM: Theory of Mind task, faces; EToM: Theory of Mind task, eyes; shown are mean ± standard deviation or number (%) of cases. a: significantly different from healthy controls; b: significantly different from the other groups, Tukey's HSD post hoc test. See the text for details.

with schizophrenia (1 female, age 30.6 ± 7 years) were recruited for this study.

2.1.2. Siblings

Siblings were recruited from patients participating in this study (n = 8), and from families where patients did not fulfill the symptom stability criteria (n = 6). Exclusion criteria included (a) the lifetime presence of any DSM-IV-TR Axis I psychotic disorder diagnosis as detected by a psychiatric interview with consultant psychiatrist (EYC) and (b) a medication history of antipsychotics, antidepressants, or mood stabilizers. Given the reported increased prevalence of nonpsychotic psychiatric disorders in first-degree relatives of schizophrenia patients, we planned to exclude siblings with syndromes warranting psychopharmacological treatment, so as to avoid that significant depressive or anxiety symptoms interfere with the results. No potential participants were excluded per this criterion, and anxiety and depressive symptoms were not higher in siblings of schizophrenia patients as compared to control participants (Table 1). A total of 14 siblings (6 females, age 30.4 ± 4.8 years) were recruited to the study.

2.1.3. Controls

Healthy comparison individuals were recruited from the local community. Exclusion criteria included (a) the lifetime presence of any DSM-IV-TR Axis I anxiety, mood, or psychotic disorder diagnosis as detected by a psychiatric interview with a psychiatrist (EYC) and (b) a medication history of antidepressants, antipsychotics, or mood stabilizers. A total of 14 healthy subjects (6 females, age 28.4 ± 8.3 years) were recruited to the study.

2.2. Neurocognitive screening measures

2.2.1. Cognitive measures

Previous to fMRI studies, all participants were screened for general cognitive impairments with the MATRICS Consensus Cognitive Battery (MCCB; Kern et al., 2008; Nuechterlein et al., 2008) and Mini Mental State Examination (MMSE; Folstein et al., 1975). They were also screened for premorbid intelligence with the Word Accentuation Test (WAT; Del Ser et al., 1997), and for possible impairments in face perception, which could interfere with expression recognition, with the Facial Recognition Test (FRT; Benton and Van Allen, 1968).

2.2.2. Mood status measures

Subjects were also assessed for depression symptom severity with the Hamilton Depression Rating Scale (Hamilton, 1960) and for anxiety symptom severity with the Hamilton Anxiety Rating Scale (Hamilton, 1969) prior to the fMRI scanning.

2.3. fMRI stimuli

2.3.1. Basic emotion task (BET)

We used photos from the Picture of Facial Affect (Ekman and Friesen, 1976). The task requires the subject to select from two emotions, which best describes how the person in the photo is thinking or feeling. The stimulus portrays the whole face in a close-up, black and white photograph (Fig. 1, top panel).

2.3.2. Faces theory of mind task (FToM)

We used a modified version of the Cambridge Mindreading Face–Voice Battery Test (Golan et al., 2006). This entails judging which of two complex mental states such us proud, nostalgic, or enthusiastic, best describes what the person in the photo is feeling (Fig. 1, middle panel).

2.3.3. Eyes theory of mind task (EToM)

We used a modified version of the 'Reading the Mind in the Eyes' Test (Baron-Cohen et al., 1999a, 1999b). This also entails judging which of two complex mental states such us jealous, enthusiastic or threatened, best describe what the person in the photo is thinking or feeling. In this task, the stimulus portrays only the eyes in a close-up, black and white photograph (Fig. 1, bottom panel).

2.4. fMRI paradigm

We used a block design paradigm ABAB…for the three tasks (BET, FToM, EToM). Each task consisted in 14 blocks of 25 s; half of them were an experimental condition and the other half a control condition.

In the experimental condition, a series of 4 photographs of faces or eyes were presented to the subjects, and they were asked to press a button to indicate which of the two simultaneously presented words best described the mental state of the photographed person. In the control condition, subjects were presented the same stimuli as in the target condition but were asked to indicate which of the two simultaneously

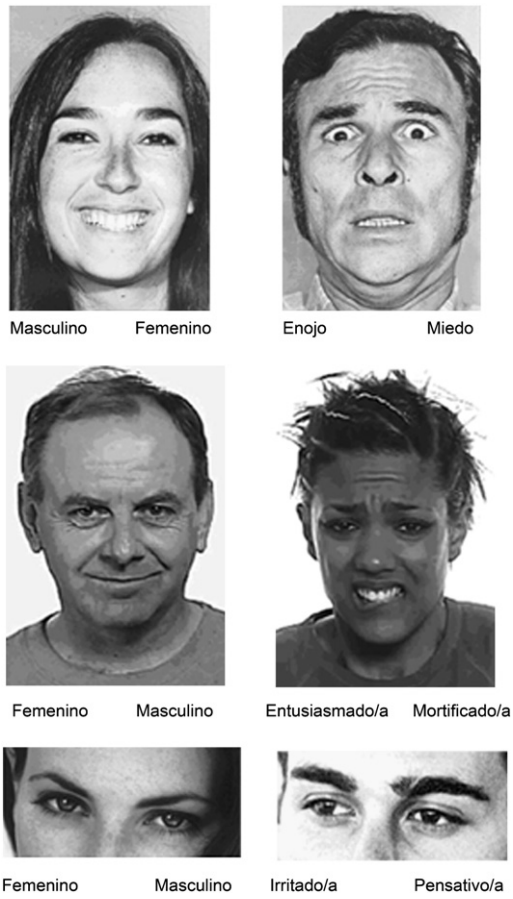

Fig. 1. Examples of stimuli used for assessment of basic emotion processing (top panel), theory of mind in faces (middle panel) and theory of mind in eyes (bottom panel). During control tasks, the same stimuli were used but the participant was asked to decide the gender of the depicted person. Please see the text for details.

presented words best described the gender of the photographed person. Thus, the key difference between the two conditions was the type of judgment the subject had to make.

Each photograph was presented for 5 s and was followed by a 0.75-s interval in which the screen was white. Correct words were counterbalanced to left and right side of the screen. All the stimuli were presented via Presentation ®. Subjects were trained with an example of each task before scanning.

The three tasks were presented in a counterbalanced order, half of the subjects starting with the BET task and the other half taking the ETOM first. There was a short break between tasks but the subject did not leave the scanner or move between tasks.

2.5. fMRI data acquisition

MRI data were acquired on a 3 T GE HDx scanner with an 8 channel head coil. Change in blood-oxygenation-level-dependent (BOLD) T2* signal was measured using a gradient echo-planar imaging (EPI) sequence. Thirty contiguous slices were obtained in the AC-PC plane (TR: 2.37 s, TE: 30 ms, flip angle: 90°, FOV: 24 cm, 64×64 pixels per inch matrix, voxel size = 3.75×3.75×4). A structural MRI was acquired with the fast SPGR–IR sequence (120 slices, 1.6-mm thick slices, TR 12.956 ms, TE 6.1 ms, flip angle 15°, FOV 24 cm, 512×512 matrix). Three sessions of 155 volumes were taken per subject.

2.6. Statistical analysis

2.6.1. Analysis of behavioral data

Discrete variables in patients, siblings and controls were compared using a chi-square test, and continuous variables were compared using a one-way ANOVA followed by a Tukey HSD test. Significance was assumed at $\alpha < 0.05$, and all reported results were two-tailed. All tests were performed with the SPSS version 13.0 software (SPSS Inc.).

2.6.2. fMRI analysis

2.6.2.1. Image processing. Image processing was carried out using SPM2 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB 7 (Mathworks Inc., Sherborn, MA, USA). Slice-timing correction was applied to each volume. The imaging time series was realigned to the first volume and spatially normalized to the stereotactic space of Talairach and Tournoux (Talairach and Tournoux, 1988) using Montreal Neurological Institute reference brain (Ashburner and Friston, 1999). The normalized volumes of $2\times2\times2$ mm^3 were spatially smoothed by an isotropic Gaussian kernel of 8 mm at full width half-maximum (Friston et al., 2000).

2.6.2.2. Statistical analysis. Individual analysis was computed using the general linear model including the control (GENDER) and experimental (EMOTION) conditions. The design matrix also included correction for head movements. The effects were modeled using a canonical hemodynamic response function to create regressors of interest. A linear contrast EMOTION–GENDER was applied to the design for each subject and these individual contrast images were subjected to a random effect analysis to see effects at a group level. For this operation we used a statistical threshold (uncorrected) of $p < 0.001$ combined with an extended cluster size threshold of 10 voxels.

A two-sample *t* test was also performed using the individual contrast images (EMOTION–GENDER) in each task for: PATIENTS vs. CONTROLS, SIBLINGS vs. CONTROLS, and PATIENTS vs. SIBLINGS.

We also performed a 2×3 ANOVA of repeated measures within each group to evaluate hemisphere vs. task effects in three different pairs of region of interest (ROIs): 1) in the left inferior frontal gyrus (IFG) defined as a sphere centered at (−45, 18, 2), and the right IFG centered at (40, 24, 2); 2) in the left middle frontal gyrus (MFG) at (−48, 15, 26) and the right MFG at (48, 15, 26) and 3) in the left superior temporal gyrus (STG) at (−51, −45, 3) and the right STG at (51, −45, 3). All ROIs were spheres of 10 mm radius built using MarsBar algorithm. The selection of the MNI coordinates was based in the overlapping of active clusters for the three groups within Brodman's areas 44–45, 9 and 22 respectively. Signal percent was calculated for each subject and entered to the SPSS 13.0© for the statistical analysis. Post hoc calculations were performed using the Bonferroni correction with a significant threshold of $p < 0.05$. We explored the relationship between activation in each ROI and reaction time or performance in all groups, whereas in patients we quantified the relationship between activation in each ROI and chlorpromazine equivalent, neurocognitive performance (MCCB score) and psychotic symptoms (PANSS score), by means of a linear regression analysis.

3. Results

3.1. Demographic data

Patients, unaffected siblings, and healthy subjects were similar in age, gender, years of education, years of parental education, basic cognitive screening (as assessed by the MMSE), premorbid intelligence (as assessed by the WAT), and ability to recognize facial features (as assessed by the FRT). They were also similar from an ethnic and cultural point of view (native Argentine Caucasian) and spoke Spanish as a native language. Patients performed poorly as compared to healthy individuals in all seven domains of the MCCB (Table 1). Nonpsychotic siblings of schizophrenia patients showed decreased performance in five domains of the battery, but their performance was better than that of the patients in speed of processing, working memory, and visual learning (Table 1). Patients had more symptoms of depression and anxiety than participants in the other two groups, and their siblings did not differ from controls in this regard (Table 1).

3.2. Task performance and reaction time

Performance in all groups was between 80% and 100% accurate during the three tasks. Patients showed less accuracy of responses than healthy controls in the BET task ($p = 0.033$) and in the FToM task ($p = 0.047$). During the EToM task, groups did not differ regarding accuracy of performance. Siblings did not show significant performance differences with either healthy persons or patients (Table 1).

Compared to controls, patients with schizophrenia took longer to decide which emotion or mental state the person was displaying in both tests that involve faces (BET $p = 0.01$; FToM $p = 0.001$) and to choose the gender of the person in all tests (BET $p = 0.006$; FToM $p < 0.001$, EToM $p = 0.025$). Siblings took more time than comparable healthy subjects to discern the mental state of the person only in the FToM task ($p = 0.009$). Patients had a significant longer reaction time than siblings when they had to decide the gender during the FToM task ($p = 0.039$) (Table 1).

3.3. Subtraction analysis per groups

3.3.1. Task effects in BET (Fig. 2A, supplementary Table 1A)

Patients (red) exhibited significant active clusters in the MFG and IFG (Brodmann's areas [BA] 44/45 bilaterally, corresponding to Broca's area in the left hemisphere), and precentral gyrus and fusiform gyrus in both hemispheres, though activation was more significant on the left side. They also showed significant activation of the left STG, in the caudal part of BA 22 (Wernicke's area).

Areas activated by nonpsychotic siblings (yellow) included the left superior frontal gyrus (SFG), somatosensory area (SMA), left inferior occipital gyrus (IOG), and both cerebellar hemispheres. The task also induced activity in voxels in the left IFG and the anterior part of the STG, as well as the left and right MFG, in all cases significant at voxel level only, and somewhat higher in the left side.

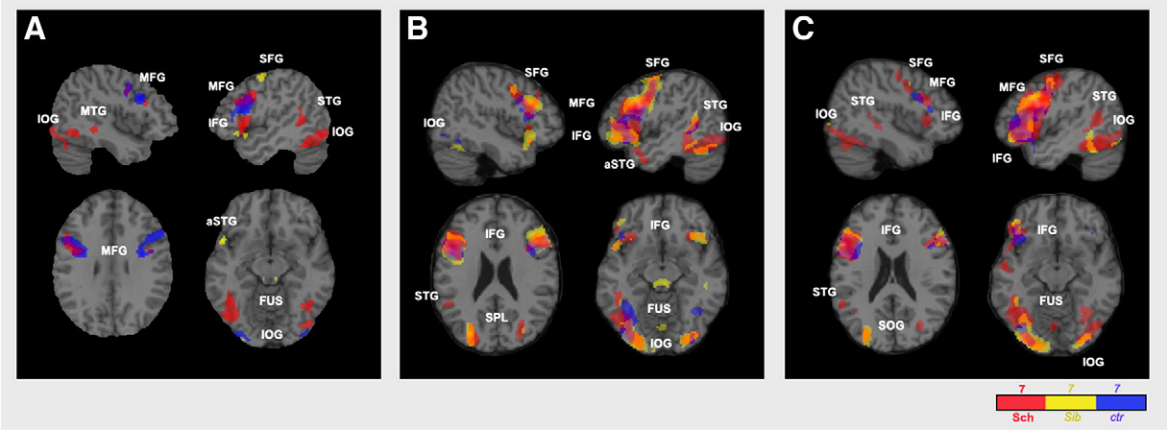

Fig. 2. Group analysis for schizophrenia patients (red), siblings of patients (yellow), and healthy subjects (blue) for the Emotion vs. Gender contrast in the Basic Emotion (A), Theory of Mind (Faces, B) and Theory of Mind (Eyes, C) tasks. MFG: middle frontal gyrus; IFG: inferior frontal gyrus; SFG: superior frontal gyrus; MTG: middle temporal gyrus; aSTG: anterior superior temporal gyrus; IOG: inferior occipital gyrus; FUS: fusiform gyrus; SPL: superior parietal lobule. Please see the text for details.

Healthy individuals (blue) activated the MFG, precentral gyrus and occipital regions in both hemispheres, as well as the IFG and the dorsal portion of left Brodmann's areas 44/45. Activation in this group was greater on the right side.

3.3.2. Task effects in FToM (Fig. 2B, supplementary Table 1B)

Patients with schizophrenia (red) showed bilateral active clusters in the caudal and ventral part of the middle, medial, and inferior frontal gyri in BA 45, with greater activation on the left side. Activity was also present in the parietal lobule and precuneus in both hemispheres, and the fusiform and middle occipital gyri. Left STG (Wernicke's area) also showed activation by this task in patients.

Unaffected siblings (yellow) activated superior, middle, and inferior frontal gyri bilaterally, including BA 44. Activity was also found in the left superior parietal lobule (SPL), left middle temporal gyrus (MTG), STG, and in the midbrain (red nucleus). Wernicke's area was also activated in this group. Similarly to the patients, unaffected siblings were found to have bigger activation clusters and higher z scores in the left side overall.

Healthy participants (blue) activated IFG (BA 44) bilaterally, right MFG, left STG near Wernicke's area and left fusiform gyrus extended to the middle occipital gyrus (MOG). Overall, brain activity in healthy individuals was similar in both hemispheres, showing similar z-scores with the exception of a greater activity in the left IFG.

3.3.3. Task effects in EToM (Fig. 2C and supplementary Table 1C)

In patients with schizophrenia (red), EToM induced an enhanced BOLD signal in the MFG, IFG, and insula bilaterally, although the clusters on the left side were considerably larger. Activity in the cingulate gyrus extending into the MFG was also observed predominantly on the left hemisphere, with some voxels extended over the right hemisphere. Activation in the MTG and STG were present on the left side, near BA 22, whereas on the right side activation was less intense and located more posteriorly. Superior parietal lobule (SPL) and precunei were activated bilaterally during this task, but the activation cluster on the left side was larger.

Siblings of patients (yellow) showed a large cluster of activation including the SFG, MFG and IFG; activation on the right side involved a relatively small cluster in the MFG. Additional areas of activation included MOG and fusiform region, as well as voxels in the left SPL. The task also induced activation in a posterior region of the left temporal lobe near Wernicke's area.

Healthy subjects (blue) activated the left IFG (BA 45/47), right MFG (BA 9/46), IOG, and a small group of voxels in the MTG.

3.4. Comparison analysis between groups

3.4.1. BET (Fig. 3A)

During emotion processing, healthy subjects activated right prefrontal structures to a greater extent than patients with schizophrenia (a) and their nonpsychotic siblings (b). Compared with the latter, control individuals also activated portions of the left cingulum and precentral gyrus (b). Activation of the left postcentral gyrus (a) and left STG (c) was greater in patients than in healthy controls or unaffected siblings, respectively.

3.4.2. FToM (Fig. 3B)

Individuals with schizophrenia presented greater overall brain activity in response to this task than comparable healthy subjects (a) and their nonpsychotic siblings (c). Differences with healthy individuals were most evident in the posterior sector of the left STG and MTG (Wernicke's areas), left parietal lobe, left medial frontal gyrus and right insula (a). Compared to their siblings, patients with schizophrenia showed more activation in the left parietal lobe, left anterior cingulate gyrus, and right cingulate cortex, but less activation in the thalamus (c). Compared with healthy persons, nonpsychotic siblings showed activation in the left precuneus and lentiform thalamic nucleus, and in the right MFG (b).

3.4.3. EToM (Fig. 3C)

Overall BOLD signal enhancement brought about by the performance of EToM task was greater in individuals with schizophrenia than in comparable control persons (a) and in their unaffected siblings (c). Compared to healthy controls, patients with schizophrenia activated more intensely areas in the left medial prefrontal cortex, SFG, and SPL (a). Compared with their nonpsychotic siblings, patients showed greater activation in left SFG, MFG, and IFG, left precuneus, bilateral cingulum, and bilateral insula (c). Healthy persons showed more activation than nonpsychotic siblings in the right precuneus and left posterior cingulate cortex (b).

3.5. Region of interest (ROI) analyses within groups

Within-group ANOVA revealed a significant effect of task on BOLD signal for three ROIs in both cerebral hemispheres (Fig. 4). Significant differences were observed between the BET task and the two probed ToM tasks (which did not differ significantly regarding activation in the ROIs). In all groups, greater activation was seen in left than right hemisphere ROIs (Fig. 4).

Among patients the BOLD signal was lower during BET than ToM tasks in both hemispheres for IFG (coinciding with Broca's area and

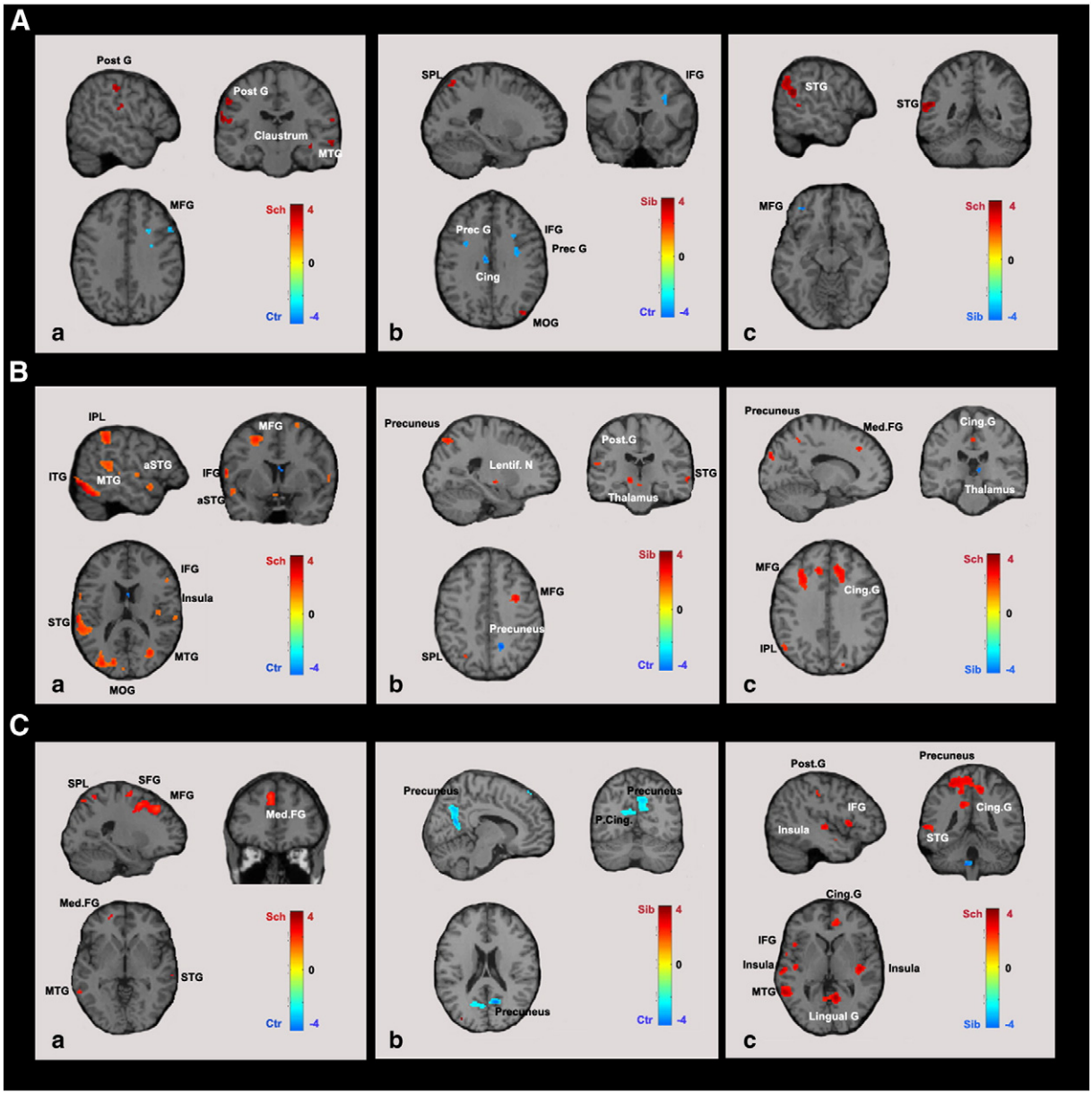

Fig. 3. Two sample t-tests between: (a) Patients (red) vs. Controls (blue), (b) Siblings (red) vs Controls (blue), and (c) Patients (red) vs. Siblings (blue), in the Basic Emotion (A), Theory of Mind (Faces, B), and Theory of Mind (Eyes, C) tasks. Abbreviations are the same as in Fig. 2. Please see the text for details.

contralateral area, Fig. 4A) and MFG (Fig. 4B). Differences were significant between tests in the left STG only (Wernicke's area) in this group (Fig. 4C).

In healthy subjects, activation was greater during the FToM than BET in the left MFG only (Fig. 4B). For the remaining combination of ROIs and hemispheres the signal percent changes for normal subjects were uniform across all three paradigms.

Siblings of patients presented intermediate results between the two other groups. FToM brought about higher activation than BET in left but not right IFG and STG. EToM resulted in greater BOLD signal than BET in the left MFG only (Fig. 4B).

3.6. Relationship between ROI activation and behavioral performance, antipsychotic dose, neurocognitive functioning, and psychotic symptomatology

Longer reaction times in BET were associated with lower activation in left ($r^2 = 0.587$, $p = 0.001$) and right ($r^2 = 0.393$, $p = 0.016$) IFG, left ($r^2 = 0.302$, $p = 0.42$) and right ($r^2 = 0.331$, $p = 0.031$) MFG, and left ($r^2 = 0.354$, $p = 0.025$) and right ($r^2 = 0.363$, $p = 0.23$) STG in nonpsychotic siblings of patients, but not in patients or healthy controls, nor in any group in FToM and EToM tests. Accuracy of responses was not related to brain activation in any group (not shown).

In patients with schizophrenia, chlorpromazine equivalent dose showed an inverse relationship with brain activation in left STG during BET ($r^2 = 0.464$, $p = 0.007$) and EToM ($r^2 = 0.528$, $p = 0.003$). No significant relationships were found between brain activation and neurocognitive performance (as measured by MCCB total score) or psychotic symptom severity (as estimated by PANSS) in this group (not shown).

4. Discussion

The main findings of this study were 1) during tasks of EP and ToM healthy individuals activate bilateral areas usually considered to subserve mirror, mentalizing, and language functions, 2) patients with schizophrenia and their nonpsychotic siblings fail to activate associated

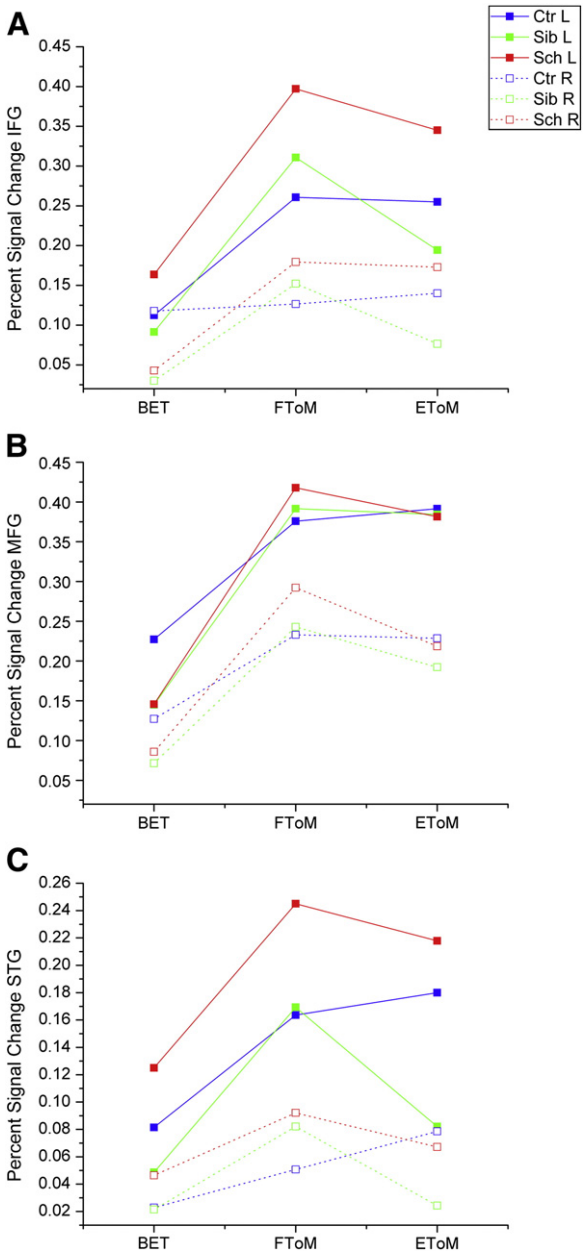

Fig. 4. Enhanced BOLD signal in IFG (in the vicinity of Broca's area and the contralateral right area, panel A), STG (coincident with Wernicke's area and the contralateral right area, panel B) and MFG (panel C). Ctr: healthy controls; Sib: unaffected siblings of schizophrenia patients; Sch: patients with schizophrenia; L: left hemisphere; R: right hemisphere. BET: Basic Emotion Test; FToM: Theory of Mind Task, Faces; EToM: Theory of Mind Task, Eyes. Please see the text for details.

right hemisphere areas to the same extent as healthy individuals, 3) there is a significant increase in brain activation overall, especially in left hemisphere structures, with increasing complexity of the social cognitive task at hand (as indicated by longer latencies of response and decreased performance) both in schizophrenia patients and their unaffected siblings, not apparent in healthy persons, 4) with increasing difficulty in social cognitive discrimination, patients and their siblings appear to recruit areas of the brain not directly involved in mirroring, mentalizing, and language processing in most previous studies, such as bilateral insula, and specific thalamic and mesencephalic areas, and 5) unaffected siblings fail to activate the precuneus bilaterally in EToM task (with reduced right sided activation in the FToM task), as compared to healthy individuals. Other areas active in the different paradigms, in all three groups, involve brain regions previously described to be involved in higher visual processing and face recognition, including occipital areas, fusiform gyrus, precuneus/posterior cingulate cortex, precentral gyrus, and cerebellum.

As predicted, healthy subjects activated areas traditionally associated with the "ToM network", mirror neurons, and language processing (Mar, 2011). However, one of our initial hypotheses (that patients would show less asymmetry than healthy persons in brain activation during tasks of EP and ToM) was not confirmed by the results of this study. In contrast, patients with schizophrenia (and to a lesser extent their unaffected siblings) showed a failure to activate right brain structures during EP and ToM tasks, as compared to healthy participants. One possible interpretation is that patients and their siblings owe their social cognitive deficits, at least in part, to a failure to recruit such structures, which underlie ToM abilities and the processing of emotional aspects of language (Mitchell and Crow, 2005; Saxe, 2006; Mar, 2011).

Left-sided structures activated by participants in this study during the EToM task are practically identical to those described in a preliminary study by Russell and colleagues with the same stimuli and control situation, and in a similar paradigm (Russell et al., 2000). In contrast with their study, however, not only did patients show a more robust activation of left hemisphere zones, but also the pattern of activation was asymmetrical, with less right hemisphere activation. A number of reasons could account for this difference, including a longer testing session influencing participant's performance, attention, and habituation to the MRI setting. Also, whereas the experimental and control condition were identical, the language was different and we used more stimuli during testing. Sociodemographic and cultural variables could also have accounted, in part, for the observed differences.

The present results should also be interpreted in light of the findings of Benedetti et al. (2009) on functional and structural abnormalities in schizophrenia patients challenged with ToM and empathy tasks. The authors explored ToM and empathy in a paradigm using cartoons depicting one (ToM) or two (Empathy) human characters respectively, and used cartoons with physical causation (also with one or two human characters) as control tasks (Vollm et al., 2006). They found increased brain activation on right temporal structures in patients with schizophrenia, who also had a decreased gray matter volume as a structural correlate in the same areas. The finding of decreased right-hemisphere gray matter in areas involved in EP and ToM, further adds to indications of right sided abnormalities in schizophrenia patients, as found in the present study. On the other hand, the finding of increased activation in the same structures is proposed by the authors to originate in a phenomenon of "physiological inefficiency," whereby less brain tissue allocated to a function results in less efficient processing and, ultimately, increased activation in the affected area (Benedetti et al., 2009). This phenomenon is not observed in the present study, which differs from that of Benedetti et al. (2009) in two important respects. First, our study involved actual human faces instead of cartoons, thereby potentially involving mirror neuron areas involved with motor phenomena. Second, participants had to choose an appropriate word with emotional connotation, thus likely eliciting activity in language-related (i.e., predominantly dominant hemisphere) areas. However, Benedetti et al.'s original observation of thinner gray matter in temporal areas shown, in our study, to have lower activation in patients and their nonpsychotic siblings, deserves further investigation as a potential explanation for the present findings.

Schizophrenia has long been considered a "left hemisphere disorder" in some theories, with left hemisphere being considered particularly vulnerable to insults giving rise to the hypothesis that schizophrenia is due to a failure of normal, left-dominant lateralization (Crow, 1997). Different studies have observed diminished anatomical and functional asymmetry, with excess non-right-hand preference as an easily measurable proxy of abnormal brain asymmetry (Peters et al., 2006). Several discordant reports have appeared, contradicting these results, including the largest study to date on handedness and brain asymmetry in

schizophrenia, which failed to observe an association between schizophrenia and non-right-handedness, gray matter asymmetry, and neurocognitive abnormalities (Deep-Soboslay et al., 2010). The present results are in agreement with the burgeoning evidence on the role of right hemispheric abnormalities in schizophrenia. Available studies involve not only structural alterations, but also functional disturbances in response to a variety of cognitive demands, including ToM tasks (Andreasen et al., 2008; Rowland et al., 2009).

The present results suggest that failure to activate a series of frontal, temporal and parietal structures in the right hemisphere when challenged with a social cognitive task, might represent an intermediate phenotype of schizophrenia, because patients display greater deficits, while unaffected siblings have similar, albeit less intense fMRI abnormalities. The specific failure of unaffected siblings to activate the precuneus bilaterally in the EToM task in spite of similar performance and latency of response compared to healthy subjects, could represent a neural correlate of the specific deficit described previously by our group in a similar cohort, involving performance in the Reading the Mind in the Eyes task (Boly et al., 2008; de Achaval et al., 2010).

With the exception of precuneus activation in siblings in one of the tasks, not present in the other groups, we could not detect clearly distinct brain activation patterns for the paradigms usually considered to test EP and ToM, because we mainly observed quantitative differences in regional activation between tasks. The fact that, in the present study, all paradigms involving emotion or mental status recognition in faces elicited brain activity in similar areas, which overlap with regions usually considered to subserve ToM functioning, supports the opinion that the distinction between EP and ToM is to a significant extent arbitrary (Ochsner, 2008). Thus, all tests employed in the present study might be exploring different aspects, or different degrees of difficulty, of the same social cognitive construct.

Several limitations of this study should be noted. Patients were receiving antipsychotic medications, their neurocognitive performance was lower than that of healthy controls in all tested MCCB domains, and they displayed more symptoms of depression and anxiety, known to decrease attention and performance in a variety of cognitive paradigms. This resulted in greater response latencies and lower accuracy in some of the tests. Thus, greater brain activation might simply reflect more difficulty carrying out the proposed tasks. However, this does not explain lower activation of right structures in the different paradigms. Moreover, we observed that higher doses of antipsychotic medications resulted in lower activation of left hemisphere structures, where patients exhibited maximal activation compared with the other groups. This suggests that antipsychotic agents might have attenuated differences between the groups. Also, we did not observe in patients a significant effect of psychotic symptoms or neurocognitive abilities on brain activation, therefore making it unlikely that these factors accounted for the observed differences with nonpsychotic siblings and healthy control subjects. Last, among the former, longer response latencies were associated with lower brain activation, which argues against a relationship between greater task difficulty and more intense brain activity in this group.

We propose that difficulties patients exhibit in social cognition ultimately reflect a failure of right-hemisphere language functions, probably preceded by alterations in mirror and mentalizing systems, which have a significant anatomical overlap with each other (anterior prosencephalon including IFG, STG and temporoparietal junction; Saxe, 2006; Van Overwalle and Baetens, 2009; Mar, 2011). This failure is shared in part by their siblings, especially during basic emotion processing. Whereas difficulties seem to reflect semantic abnormalities, we propose that the latter are preceded by more widespread deficits in brain activation of areas concerned with mirroring of motor action (i.e., facial expressions and gaze direction) which then inform areas concerned with ToM. In this view, the choice of a semantically appropriate term to describe another person's emotion, mental state, or intention would normally depend upon relevant and accurate interpretation of facial/gaze motor features (thanks to an intact mirror system) and then the ability to interpret such information on the basis of inner experience and memory (using an intact ToM neural system). Present results suggest that abnormalities exhibited by patients with schizophrenia – and, to a lesser extent, their nonpsychotic siblings – seem to be more prominent in the right hemisphere. We believe this schema lends support to theories that conceptualize schizophrenia as a failure in development of right hemisphere functions (Crow, 1997). Originally, this theory posited that aberrant right-hemisphere language functioning was responsible for positive symptoms related to speech and logical thinking, such as auditory hallucinations, thought insertion and broadcasting, i.e. "core" or Schneiderian symptoms of schizophrenia. Our results are compatible with the view that abnormal right hemisphere ToM and language function can be the basis for social cognitive deficits as well. Future research should determine if abnormal patterns of brain activation during social cognitive tasks as described herein are related to performance-based measures of functional skills in patients and their nonpsychotic siblings.

Role of funding source

Funding sources listed in the Acknowledgment section had no role in the study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

Contributors

dDA, MFV, ECC, CBN and SMG designed the research protocol. dDA, MFV, JD, MNC, MCM, and SMG collected the data. dDA, MFV and SMG analyzed the data and wrote the first version of this manuscript. All authors contributed to and approved the final version of this manuscript.

Conflict of interest

CBN is a consultant to Xhale and Takeda, is a stockholder of CeNeRx BioPharma, NovaDel Pharma, Inc., PharmaNeuroBoost, Revaax Pharma, and Xhale, he is the owner of patents for method and devices for transdermal delivery of lithium (US 6,375,990B1) and method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay (US 7,148,027B2), he is in the board of directors of AFSP, NovaDel Pharma, Inc., and is in the scientific advisory board of American Foundation for Suicide Prevention (AFSP), CeNeRx BioPharma, National Alliance for Research on Schizophrenia and Depression (NARSAD), NovaDel Pharma, Inc., PharmaNeuroBoost, Anxiety Disorders Association of America (ADAA). All other authors report no conflict of interest.

Acknowledgments

Funding was provided by the Agencia de Promoción (Ministry of Science and Technology, Argentina, grant PICT-2007-1643, to SMG) and the University of Buenos Aires (UBACYT code 20020100101025, to SMG, MFV and DEV). DdA and MNC are doctoral fellows from CONICET. SDG. MCM is a doctoral fellow from Agencia. We are grateful for the technical assistance of Ms. Karina Buglioni.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.schres.2011.11.010.

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