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ARTICLE

Neural markers of negative symptom outcomes in distributed working memory brain activity of antipsychotic-naive schizophrenia patients

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Abstract

Since working memory deficits in schizophrenia have been linked to negative symptoms, we tested whether features of the one could predict the treatment outcome in the other. Specifically, we hypothesized that working memory-related functional connectivity at pre-treatment can predict improvement of negative symptoms in antipsychotic-treated patients. Fourteen antipsychotic-naive patients with first-episode schizophrenia were clinically assessed before and after 7 months of quetiapine monotherapy. At baseline, patients underwent functional magnetic resonance imaging while performing a verbal *n*-back task. Spatial independent component analysis identified task-modulated brain networks. A linear support vector machine was trained with these components to discriminate six patients who showed improvement in negative symptoms from eight non-improvers. Classification accuracy and significance was estimated by leave-one-out cross-validation and permutation tests, respectively. Two frontoparietal and one default mode network components predicted negative symptom improvement with a classification accuracy of 79% ($p = 0.003$). Discriminating features were found in the frontoparietal networks but not the default mode network. These preliminary data suggest that functional patterns at baseline can predict negative symptom treatment–response in schizophrenia. This information may be used to stratify patients into subgroups thereby facilitating personalized treatment.

Introduction

In schizophrenia, symptoms are often divided into positive symptoms, such as hallucinations, delusions and disorganized thinking, and negative symptoms, such as anhedonia, alogia and avolition. Negative symptoms are characterized by an absence of normal behaviour and share many characteristics with the cognitive deficits also present in schizophrenia patients. For example, unlike positive symptoms, negative symptoms and cognitive impairment: both remain relatively stable over time (Harvey *et al.* 2006); both respond poorly to antipsychotic medication including second generation antipsychotics (Lieberman and Stroup, 2011); both are good predictors of functional outcome (Allott *et al.* 2011); both

are associated with dysfunction in similar brain regions (Williamson, 2007).

Working memory (WM) is often impaired in schizophrenia (Lee and Park, 2005) and has been directly linked to the presence of negative symptoms (Seamans and Yang, 2004). While neuroimaging studies have failed to pinpoint a specific region of dysfunction relating to observed WM deficits (Glahn *et al.* 2005), they have consistently shown impaired functional connectivity within networks activated by WM tasks (Schlosser *et al.* 2003; Meda *et al.* 2009; Henseler *et al.* 2010). Of note is that one study was able to differentiate schizophrenia patients from healthy control subjects with 96% accuracy based on functional connectivity during an *n*-back WM task (Meyer-Lindenberg *et al.* 2001). These findings of dysconnectivity tie in with the notion that schizophrenia is primarily associated with impairment of functional integration within brain networks rather than affecting neuronal processing in a single brain area (Stephan *et al.* 2009).

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In the present study, we employed a multivariate approach, which allows the detection of subtle large-scale alterations in network dynamics that have been proposed to underlie schizophrenia. The strength of using a multivariate classification approach is exemplified by recent work showing that spatially distributed information in brain tissue in schizophrenia patients at baseline predicted the frequency of psychotic episodes at 6 yr follow-up (Mourao-Miranda *et al.* 2012). Although this multivariate approach is fundamentally data-driven, the features that are selected to make the classification are driven by theory. Both task-positive and task-negative networks are implicated in the dysfunctional working memory processes in schizophrenia (Anticevic *et al.* 2011). Considering the many similarities between negative symptoms and cognitive impairment, we hypothesized that the functional networks underlying working memory at pre-treatment baseline in antipsychotic-naive, first-episode patients would be predictive of negative symptom improvement after 7 months of atypical antipsychotic treatment with quetiapine. This study is partly motivated by the need for clinical aids in order to guide treatment choice in the clinic, and also by the need to better understand the underlying pathophysiology of symptoms that respond poorly to drug treatment.

Method

Participants

Fourteen antipsychotic-naive, first-episode patients (11 male and three female) were clinically assessed and scanned with magnetic resonance imaging (MRI) at baseline and clinically reassessed 6.89 (S.D. = 0.57) months after quetiapine drug treatment at clinically efficacious doses (mean dose = 518 mg; S.D. = 291 mg). Patients were recruited as part of a larger prospective study on first-episode schizophrenia. Structural and univariate functional MRI (fMRI) findings have been reported elsewhere (Ebdrup *et al.* 2010, 2011; Nejad *et al.* 2011). A total of 17 patients of whom we had fMRI data at baseline were followed up after quetiapine treatment at 7 months. Two were excluded based on poor imaging quality and one patient was excluded based on missing symptom assessment data.

Inclusion criteria were a diagnosis of schizophrenia, no prior exposure to antipsychotic medication, aged 18–45 yr, no medical or neurological co-morbidity and no history of significant head injury. MRI scans were without pathology as evaluated by a neuroradiologist. Diagnoses were based on the schedules for clinical assessment in neuropsychiatry (SCAN), version 2.1 (Wing *et al.* 1990). Duration of untreated illness (DUI) was estimated from clinical interviews, records and, where possible, interviews with relatives. DUI was defined as the time between onset of unspecified psychotic symptoms to the date of the MRI scan. Psychopathology was assessed with the positive and negative syndrome scale (PANSS; Kay *et al.* 1987) by trained raters. Interviews were video-recorded for validation purposes. An intra-class correlation of 0.92 was achieved in a two-way mixed effect model of 10 random interviews. All participants were right-handed as assessed by the Edinburgh handedness inventory (Oldfield, 1971). For all participants parental-socioeconomic status (P-SES) was assessed (Hansen, 1986). Six patients were prescribed benzodiazepine but abstained on the day of MRI scanning. One patient received SSRI medication (fluoxetine) at the time of scanning and two patients last received SSRI medication ⩾16 months prior to study inclusion. Four patients fulfilled lifetime DSM-IV criteria for substance abuse, two of whom had no history of abuse for the past year and one had none for the past month. One patient had smoked cannabis on a few occasions in the month prior to the MRI scan. Substance dependence was an exclusion criterion. All subjects had a negative urine screening for substance intake before the examinations.

The study was conducted in compliance with the Helsinki II Declaration and approved by the Ethics Committee of the Capital Region (H-KF-01-78/97). All participants gave written consent after receiving a written and oral description of the study.

Experimental working memory task

We used the verbal *n*-back task to probe working memory functional networks. This task has been found to reliably activate known working memory areas (Rottschy *et al.* 2012) and has been used extensively in schizophrenia research (Glahn *et al.* 2005). The verbal *n*-back task consisted of four conditions: rest; 0-back; 1-back; 2-back. Participants performed seven blocks of each condition in a pseudo-random order. During the rest condition, a fixation cross was continuously displayed and subjects lay still with their eyes open. During the *n*-back conditions, 15 letters were presented for 0.5 s in the centre of the screen every 2 s. Participants pressed the response key 'yes' with their right index finger to every stimulus that was presented in the previous trial for the 1-back condition or two trials previously for the 2-back condition. In the 0-back condition, participants were instructed to respond 'yes' whenever the letter 'X' appeared on the screen. On average, each block contained four targets out of 15 stimuli. Subjects responded to non-targets by pressing the response key 'no' with their right middle finger. Participants performed a practice run outside the scanner.

MRI

MRI was performed using a 3-T Siemens Trio scanner (Siemens, Germany). A high-resolution anatomical brain scan was acquired using a 3D T1-weighted sagittal magnetization prepared rapid gradient echo (MPRAGE) sequence of the whole head (TR = 1540 ms; TE = 3.92 ms;

flip angle $= 9°$; voxel size $= 1$ mm³; 192 slices). Echo planar imaging (EPI) was used to measure blood oxygen level dependent (BOLD) signal as an index of regional neural activity (TR $= 2000$ ms; TE $= 30$ ms; flip angle $= 90°$; FOV $= 240$ mm; voxel size $= 3.8$ mm, isotropic resolution). Each acquired brain volume consisted of 31 contiguous slices oriented parallel to the anterior commissure–posterior commissure plane. A total of 477 whole-cerebrum measurements were acquired during WM task performance. We also acquired a B0 field map (TR $= 400$ ms; TE1 $= 5.19$ ms; TE2 $= 7.65$ ms; flip angle $= 60°$; distance factor $= 25$%; FOV $= 240$ mm; 31 slices; slice thickness $= 3$ mm).

fMRI data processing

Pre-processing was conducted with SPM5 (Wellcome Trust Centre for Neuroimaging, UK). To correct for scanner gradient nonlinearities, the structural MPRAGE image was unwarped before being normalized to the Montreal Neurological Institute (MNI) template using the VBM5.1 toolbox. The EPI images were unwarped using a voxel displacement map created from the gradient nonlinearity and acquired B0 field maps (Andersson *et al.* 2001). Subsequently, the mean EPI image was co-registered to the MPRAGE image. All EPI images were then realigned to the mean EPI image. Next, EPI images were transformed into MNI space (2 mm isotropic resolution) by applying the spatial normalization transformation parameters of the MPRAGE image. Finally, the EPI images were smoothed with a Gaussian kernel of 8 mm full-width half-maximum.

Demographic and behavioural data analyses

Statistical analyses were performed with SPSS version 18 software. Paired *t* tests tested for clinical changes in PANSS scores from baseline to follow-up. Patients were grouped according to improvement on the PANSS negative symptom scale by subtracting patients' negative symptom score at follow-up from their baseline score. Percentage changes were calculated after transforming the PANSS scores from interval to ratio scale (Obermeier *et al.* 2011). We used the traditional threshold of a 20% reduction in PANSS symptom scores (Mortimer, 2007) to define patients as negative symptom improvers. Mann–Whitney *U* tests tested for group differences between improvers and non-improvers on all PANSS scores, mean quetiapine dose, DUI, age and handedness scores. Sex ratio, benzodiazepine prescriptions and P-SES were analysed for between-group differences with χ^2 tests.

For each *n*-back condition, we calculated the mean reaction time (RT) and mean signal detection sensitivity, *d'*. RT was measured from the time of stimulus presentation until the response. The sensitivity measure, *d'*, which indicates how well participants are able to respond correctly to both targets and non-targets, was calculated as the *z*-transform of the hit rate minus the *z*-transform of the false-alarm rate (MacMillan and Creelman, 2005). Higher values of *d'* denote better WM performance.

A repeated-measures general linear model tested for group (improvers, non-improvers) and group-by-WM load interaction effects. WM load (1-back and 2-back) was a within-subject factor. RT and *d'* were dependent variables. Significance was set at a two-tailed level of 0.05.

fMRI data analysis

The fMRI data analysis proceeded in the following steps: (1) selection of features: spatial independent component analysis (ICA) was used to extract temporally coherent functional networks (i.e. components) from fMRI signals. Those components that showed the strongest associations with the task were selected for further analyses; (2) classification: a linear support vector machine (SVM) classifier was employed to determine whether the information contained in the selected functional networks distinguished patients who would later go on to improve in negative symptoms from those who would not; (3) visualization: finally, we visualized the brain areas which best differentiated the two groups by use of weight maps.

Selection of features

Spatial ICA for group analysis was run using the Group ICA of fMRI Toolbox (http://icatb.sourceforge.net, version 2.0c). Spatial ICA reduces the functional imaging data into time-courses and associated spatial maps, which account for most of the variance in the temporal data. The brain areas within the resulting spatial maps share a common pattern in their time series. Those spatial maps whose temporal patterns show the strongest correlations with onset of task conditions are presumed to signify task-related networks.

As a first step, the dimensionality of the pre-processed fMRI data from all subjects was reduced by singular value decomposition to 20 dimensions per subject and temporally concatenated. Independent components, *a priori* set to 20, were then estimated from the aggregate data using the Infomax algorithm. We estimated 20 independent components as this number has previously been shown, in a study using a similar verbal *n*-back task, to divide data into meaningful task-related networks (Gordon *et al.* 2012). Next, subject-specific spatial maps of each component were generated through back reconstruction of the group components onto subject-specific functional data, after which individual component maps were Z-transformed with voxel values expressing signal deviation from the group-averaged component (higher voxel values indicating greater expression of the group component's temporal pattern).

Finally, multiple regression of the 1-back and 2-back WM task parameters against the time series of the group mean components identified those components which were strongest associated with the changes in the task,

i.e. task-related components representing networks modulated by WM processing. These components were then used to train a classifier in distinguishing negative symptom improvers from non-improvers.

Classification

Machine learning classifiers attempt to find a dividing plane, given the data, which best separate sets of grouped data. We used a linear SVM (Cortes and Vapnik, 1995) as our classifier using LIBSVM version 3.1 software (http://www.csie.ntu.edu.tw/~cjlin/libsvm). SVMs are well-suited for multivariate pattern discrimination between two classes of high dimensional data, i.e. for group analysis of functional neuroimages. The sequential minimal optimization algorithm, incorporated in LIBSVM, was used to define the hyperplane that best separated data labelled as negative symptom improvers and non-improvers. The C-parameter, which penalizes for incorrect predictions, was fixed at the default setting of 1.

Classification accuracy was estimated by leave-one-out cross-validation. The classifier was trained on all data labelled as a member of negative symptom improvers or negative symptom non-improvers. One data set remained unlabelled and was used to test whether the trained classifier was able to correctly classify it as belonging to the 'improver' or 'non-improver' group. This procedure was repeated until each subject's data was left out of classifier-training and used as a test data set. The accuracy rate of classification was indicative of the percentage of times the classifier correctly identified the unlabelled data sets. We further calculated sensitivity (proportion of true positives, i.e. correctly classified improvers) and specificity (proportion of true negatives, i.e. correctly classified non-improvers).

The statistical significance of the classifier was tested by rerunning the leave-one-out procedure on 1000 random permutations of the training-set class labelling. The *p*-value was thus a non-parametric indication of how often a classifier trained on a random class labelling could perform with better leave-one-out accuracy than the classifier trained on the true labelling of improvers and non-improvers.

Visualization

When applying SVM, it is possible to obtain voxel values indicating how important a given voxel is for defining the dividing hyperplane between two classes. These values, or classifier weights, can be used to identify brain areas which make a strong contribution to classification. For this, we constructed reproducible activation volumes using the procedure outlined in Rasmussen *et al.* (2011) employing the NPAIRS split-half resampling technique (Strother *et al.* 2002). This method estimates a reproducible activation map of *Z*-scores by plotting the similarity of classifier weights obtained from 200 independent split-half samples. The resulting volumes

Table 1. Demographic and Positive and Negative Syndrome Scale (PANSS) assessments at baseline and follow-up for all study participants

	Baseline		Follow-up	
	Mean	S.D.	Mean	S.D.
Age (yr)	26.78	4.99		
Edinburgh handedness score	97.61	6.25		
Negative PANSS	21.21	6.05	20.71	5.24
Positive PANSS	20.64	3.59	16.21*	3.85
General PANSS	40.64	8.54	39.57	9.49
Total PANSS	82.50	13.89	76.50	17.52

*Significant improvement over time.

were thresholded at Bonferroni's corrected *p* value of 0.05. The surviving clusters were those which were reproducibly important for the classifier to draw the dividing hyperplane between negative symptom improvers and non-improvers.

Results

Demographic and behavioural data analyses

Demographic and clinical data are presented in Table 1. Patients improved significantly on the positive symptom subscale after 7 months of quetiapine treatment ($t_{13}=4.0$; $p=0.002$). However, we did not observe any significant improvements from baseline to follow-up on the negative, general or total PANSS scores ($p>0.15$ for all).

For the negative symptoms, six patients with positive difference scores were grouped as negative symptom improvers (range 18.75–100% change). Eight patients displaying negative ($n=6$; range 6.7–100% change) or null ($n=2$) difference scores were grouped as negative symptom non-improvers (Table 2). Two of the patients that we grouped as 'improvers' fell just below our predefined threshold at a symptom reduction of 19%, whereas the rest displayed >25% symptom reduction. Nevertheless, the large gulf between 'improvers' and 'non-improvers' (>19%) indicates that there was a qualitative clinical difference between our defined groups. Handedness, P-SES, mean quetiapine dose, benzodiazepine prescriptions and sex ratio were not significantly different between improvers and non-improvers ($p>0.2$ for all). Negative symptom improvers were significantly ($U=7.0$; $Z=-2.19$; $p=0.023$) younger than non-improvers, but did not differ in DUI ($p=0.34$). The baseline PANSS negative symptom subscale scores differed significantly between the improvers and non-improvers ($U=8.5$; $Z=-2.01$; $p=0.043$), otherwise no significant differences were found on the PANSS total or other subscales ($p>0.28$ for all). Finally, improvers and non-improvers did not show any significant between-group differences or interactions in *d'* ($p>0.70$ for all) or

Table 2. Demographic and positive and negative syndrome scale (PANSS) assessments for negative symptom improvers and non-improvers

	Negative symptom improvers ($n=6$)		Negative symptom non-improvers ($n=8$)	
	Mean	S.D.	Mean	S.D.
Baseline age (yr)	23.93	3.76	28.92*	4.89
Edinburgh handedness score	98.33	4.08	96.99	7.96
Baseline negative PANSS	24.83	7.55	18.50*	2.78
Baseline positive PANSS	20.33	5.28	20.88	1.96
Baseline general PANSS	39.83	5.91	41.25	10.47
Baseline total PANSS	85.00	15.39	80.63	13.42
Positive PANSS difference[a]	4.50	6.12	4.37	2.26
General PANSS difference[a]	2.83	6.94	−0.25	10.33
Total PANSS difference[a]	12.83	12.04	0.87	15.24
Mean quetiapine dose (mg)	436	290	578	295
Duration of untreated illness (wk)	169	234	317	324

* Significant difference between groups.
[a] Values reflect means of difference scores (baseline minus follow-up symptom scale scores) with positive values denoting improvement.

RT ($p>0.17$ for all) on the *n*-back task. All participants performed the *n*-back task to an acceptable standard with a mean of 90 correct trials out of a possible 105 trials (S.D. $=8.4$) in the 2-back condition, ensuring that all patients were engaged in the task.

ICA and classification results

The regression of the group mean components' time series on the 1-back and 2-back WM task parameters identified a right-lateralized frontoparietal WM network component ($R^2=0.367$), a default mode network (DMN) component ($R^2=0.275$) and a bilateral frontoparietal WM network component ($R^2=0.187$; Fig. 1).

These three network components were used to train the linear SVM classifier. The classification accuracy was 79% (sensitivity $=75\%$; specificity $=83.3\%$), significant at $p=0.003$, in spite of the relatively small sample size.

Post hoc *classification results*

A number of confounding variables differed between the 'improver' and 'non-improver' groups. Therefore, classification was rerun with the data grouped according to these 'nuisance' variables in order to investigate whether classification improved with this relabelling and so whether these group-differences were driving our results.

Since the improvers and non-improvers significantly differed on baseline negative symptom scores (as assessed by the PANSS negative symptom scale), we trained a linear SVM classifier on high ($n=6$) *vs.* low ($n=8$) scorers, grouped according to whether they scored above or below the baseline negative symptom mean of 21. The classification accuracy declined to 43%

(sensitivity $=62.5\%$; specificity $=16.7\%$), suggesting that baseline difference in negative symptom scores did not influence our earlier result.

Also, Lysaker *et al*. (1997) previously reported that cognitive impairment could predict the stability of negative symptoms at 6-month follow-up. To test whether in our study the WM-related network components predicted the temporal stability of negative symptoms rather than the direction of symptom change, we grouped patients according to the same criteria as the Lysaker *et al*. study (stable scorers <4 point change $<$ variable scorers). Six patients were labelled as stable negative symptom scorers and eight as variable negative symptom scorers. Rerunning the leave-one-out cross-validation resulted in a classification accuracy of 57.14% (sensitivity $=16.7\%$; specificity $=87.5\%$), suggesting that differences in the magnitude of negative symptom change between negative symptom improvers and non-improvers were not driving our classification results.

Finally, we tested whether the significant age difference between the groups was driving the classification by grouping the patients according to whether they scored above the mean age of 27 yr ($n=7$) or below ($n=7$). The classification accuracy dropped to 50% (sensitivity $=42.9\%$; specificity $=57.1\%$), suggesting that the age difference did not influence the classification accuracy of the negative symptom improvers *vs.* non-improvers.

Mapping classifier weights

The reproducible activation map shows the brain regions which were most reproducibly distinct between the improvers and non-improvers (Fig. 2). These regions were
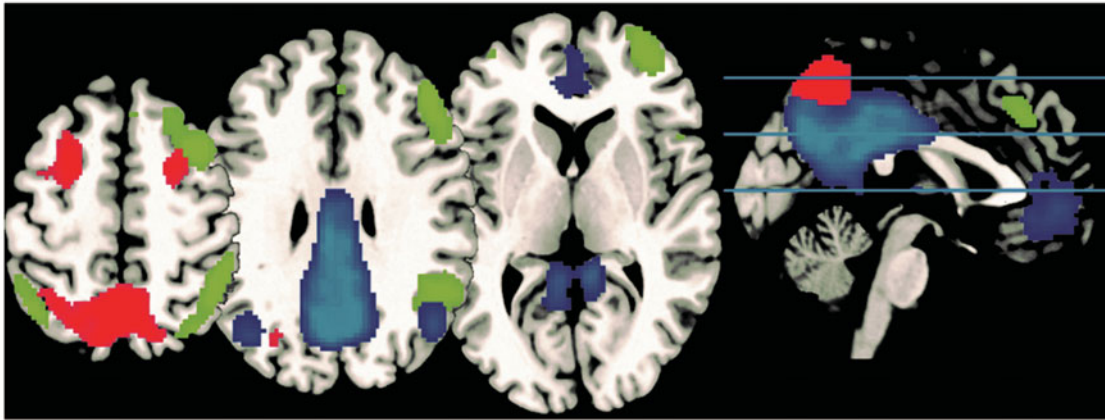
Fig. 1. Independent components of interest maps. Axial slices showing the three components used for classification (thresholded at z-score of 1.7). Red colour map indicates the bilateral frontoparietal component; green colour map indicates the right-lateralized frontoparietal component; blue colour map indicates the default mode network component.
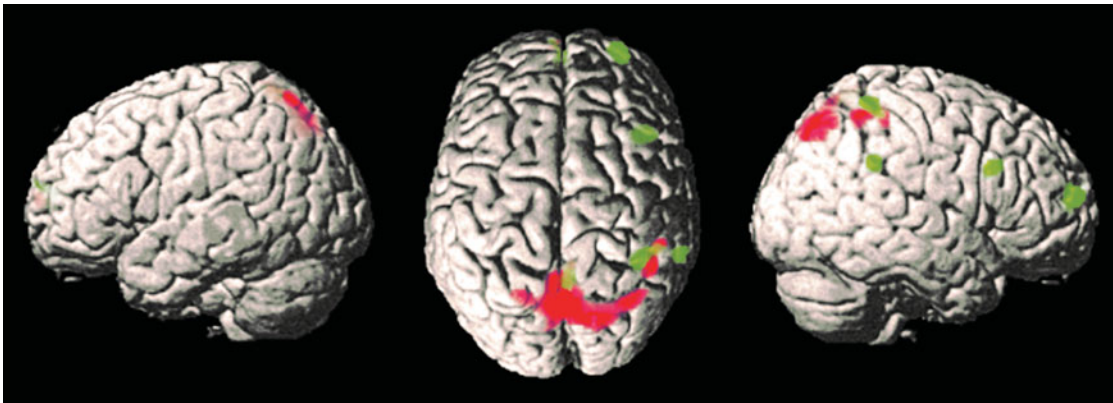

Fig. 2. Reproducible activation maps. 3D brain showing areas which are most informative to the classifier (thresholded at two-tailed Bonferroni's corrected $p = 0.05$; minimum cluster size of 20 voxels). Colour maps correspond to the respective component – red and green for the frontoparietal working memory components.

mainly located within the WM network, including precuneus, right superior and inferior parietal and right dorsolateral, ventrolateral and medial prefrontal cortices. Generally, these regions expressed low values in the subject component maps, values of which are indicative of the extent the voxel's time-course covaries with the time-course of the network represented in the mean group component. Low values therefore indicate low contribution to the variance of the network's time-course and thereby suggest that these clusters were not major nodes of the frontoparietal networks.

Post hoc *comparison of features*

In order to estimate how important each component was for differentiating patients who later improved on negative symptoms from those who did not, we one-by-one removed a component from the classifier and repeated leave-one-out cross-validation (Table 3). Removing the DMN component did not change the accuracy of the classifier in discriminating between negative symptom

improvers and non-improvers. Training the classifier on each component separately revealed that the right-lateralized WM network component performed best.

Discussion

WM-related functional connectivity patterns at pre-treatment baseline predicted the improvement in negative symptoms with an accuracy of 79% in anti-psychotic-naive schizophrenia patients who were subsequently treated with quetiapine. The frontoparietal WM networks rather than the DMN contained most of the predictive information regarding the later improvement in negative symptoms. Moreover, the classifier trained on the more right-lateralized frontoparietal network component performed best of the classifiers trained on individual components. Accordingly, the reproducibility activation maps highlighted the right prefrontal and parietal clusters of the WM components as those brain regions where the activity pattern contributed most to the

Table 3. Comparison of features

	Negative symptom improvers *vs.* non-improvers		
	Accuracy (%)	Sensitivity[b] (%)	Specificity[b] (%)
Three components of interest[a]	78.57	75	83.33
Working memory network components	78.57	62.5	100
Default mode network component	50.0	62.5	33.33
Right-lateralized working memory network component	64.29	62.5	66.67
Bilateral working memory network component	57.14	50	66.67

[a] The two working memory network components and the default mode network component.

[b] True positives were those instances where improvers were classified accurately; true negatives were those instances where non-improvers were classified accurately.

discrimination between patients who showed subsequent improvement in negative symptoms from those who did not. The right-lateralized frontoparietal component also fitted best with the WM task conditions, suggesting that the most cognitively-engaged network best predicted the course of negative symptoms. Critically, our findings cannot be attributed to an overt dysfunction of WM since negative symptom improvers and non-improvers did not differ in WM performance.

Although the parietal and prefrontal regions generally constitute key nodes in the WM network (Champod and Petrides, 2007), most of the identified clusters in the reproducibility activation maps did not express strong connectivity within the frontoparietal networks in either the negative symptom improving or non-improving group. In agreement with this observation, a recent study applying graph theory to analyse resting-state functional brain connectivity in schizophrenia revealed that it was particularly the weak connections within network organization that were altered in schizophrenia patients (Bassett *et al*. 2012). Since alterations in these weak connections were found to correlate with cognition, as well as negative symptom measures, the authors concluded that dysconnectivity of peripheral rather than central network nodes might underpin cognitive deficits (Bassett *et al*. 2012). Our data suggest that baseline connectivity in weak connections of the WM network may also determine which course negative symptoms subsequently take during antipsychotic treatment.

Regions belonging to the DMN were largely absent in the reproducibility activation maps. This indicates that the classifier did not find voxels pertaining to the DMN component important in order to differentiate improvers from non-improvers. The lack of distinguishing features in the DMN is further supported by the *post hoc* comparison of features where removing the DMN component did not change the accuracy of the classifier. Some

previous findings (Bluhm *et al*. 2007; Lui *et al*. 2009), but not all (Garrity *et al*. 2007), have suggested that DMN connectivity is correlated with negative symptom severity. Although our findings do not necessarily conflict with an association between DMN connectivity and negative symptoms, they do suggest that the DMN contains little predictive value for negative symptom treatment response.

Quetiapine is a second generation antipsychotic with a complex receptor profile. Its main therapeutic effect on cognition and negative symptoms, if any, is thought to stem from its dopamine 2 (D_2) and serotonin 2A (5-HT_{2A}) receptor antagonism, which leads to an overall increase in dopaminergic activity in the prefrontal cortex (da Silva Alves *et al*. 2008). In healthy subjects, pharmacological modulation of dopaminergic neurotransmission has produced differential effects on cognitive performance and connectivity. The dopamine agonist bromocriptine increased frontostriatal functional connectivity and improved WM performance in low-WM span subjects but decreased connectivity and worsened performance in high-span subjects (Wallace *et al*. 2011). The differential effect of bromocriptine supports the presence of an inverted U-shape relationship between prefrontal dopaminergic activity and working memory (Cools and D'Esposito, 2011). This inverted U-shaped relationship may also explain the considerable inter-individual variation of quetiapine's effect on negative symptom expression in the present study. It seems that quetiapine, seemingly by way of dopamine modulation, has either beneficial or detrimental effects on negative symptoms that can be predicted by a WM-related measure; in this case, frontoparietal connectivity.

Supporting our line of reasoning, previous studies have suggested that WM-related profiles at baseline can predetermine treatment response. For instance, the Val[108/158]Met catechol-*O*-methyltransferase genotype,

which has been associated with WM performance and brain activity as well as risk for schizophrenia (Tan *et al.* 2007), predicted treatment outcome in schizophrenia patients with less WM improvement (Weickert *et al.* 2004) and poorer negative symptom outcomes (Bertolino *et al.* 2004, 2007) in Val homozygotes than Met allele carriers after antipsychotic treatment. van Veelen *et al.* (2011) found that pre-treatment WM prefrontal hyperactivity, as measured with fMRI, predicted worsened general treatment response after 10 wk. Furthermore, increases in WM (Honey *et al.* 1999; Meisenzahl *et al.* 2006) and emotional (Fahim *et al.* 2005; Stip *et al.* 2005) task-related prefrontal activity after antipsychotic treatment have been found to parallel improvements in negative symptoms.

We did not find a direct relationship between the expression of negative symptoms and WM functional connectivity at baseline. Classifying patients according to baseline negative symptom scores demonstrated much poorer accuracy (43%) than classifying based on treatment response. Also, the stability of negative symptoms could not be predicted based on WM-related network activity at baseline. Groups defined by magnitude of change in negative symptom scores were poorly classified with 57% accuracy. It therefore seems that pre-treatment functional connectivity of WM-related networks can predict the treatment response of negative symptoms but neither their temporal stability nor baseline severity. Furthermore, age differences did not seem to be driving the classification results between the negative symptom improvers and non-improvers. However, these findings do not rule out that the aforementioned factors and perhaps others such as history of substance abuse, current non-antipsychotic medication use and DUI, can interact with WM brain activity to predict the course of negative symptom change. Any possible interaction effects do not undermine our findings but would be interesting avenues to pursue in future studies.

An important limitation of this study is the relatively small sample size. The present findings, therefore, need to be replicated in a larger group of patients. A larger sample size would have allowed us to adjust classifier parameters in order to improve classification accuracy. Nonetheless, we attained reasonable accuracy using a relatively conservative kernel (linear) and an SVM classifier with default parameters. We attribute the significant results in such a small sample at least in part to the strict inclusion criteria of our study, which ensured that we studied a well-defined and relatively homogenous group of patients.

Finally, it is inherently difficult to dissociate real drug treatment effects on negative symptoms from spontaneous fluctuations in negative symptoms attributed to the natural course of the disease. These factors can only be disentangled by including an untreated control group of schizophrenia patients, which for obvious ethical reasons is not feasible.

In conclusion, we have demonstrated the applicability of emerging methods in imaging psychiatry for the prediction of treatment outcomes in schizophrenia. Identifying neural activity patterns at baseline that predict treatment resistance will not only contribute to a better understanding of the neural mechanisms involved in symptom expression and in determining the course of the disease, but ultimately may be used to tailor early and individualized treatment. Such ends might be especially important for the treatment of negative symptoms and cognitive deficits in light of their strong association with functional outcome and the limited effect current antipsychotic compounds have on these symptoms. Early intervention tailored to treat cognitive deficits and negative symptoms might improve functional outcomes for all high-risk individuals, with or without transition to psychosis (Lin *et al.* 2011). There are currently no aids to help clinicians tailor treatment to patients or those in at-risk mental state. The current study will hopefully be one of many studies to come, which aim to find and refine predictors of treatment response.

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