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Save the Global: Global Signal Connectivity as a Tool for Studying Clinical Populations with Functional Magnetic Resonance Imaging

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Abstract

The global signal is commonly removed from resting-state data, as it was presumed to reflect physiological noise. However, removal of the global signal is now under debate, as this signal may reflect important neuronal components, and its removal may introduce artifacts into the data. Here, we show that the functional connectivity (FC) of the global signal is of functional relevance, as it differentiates between schizophrenia patients and healthy controls during rest. We also demonstrate that other reported findings related to various clinical populations may actually reflect alternations in global signal FC. The evidence of the clinical relevance of the global signal propose its usage as a research tool, and extend previously reported perils of global signal removal in resting-state data of clinical populations.

Key words: fMRI; functional connectivity; global signal; neuroimaging; resting state; schizophrenia

Introduction

Spontaneous brain activity has been detected in both human and nonhuman species using different methodologies (Arieli et al., 1996; Biswal et al., 1995; Fox and Raichle, 2007; Fox et al., 2007; Raichle, 2009). This phenomenon is characterized by widespread fluctuations, predominantly oscillating at frequencies lower than 0.1 Hz, which appear not only during task performance but also during rest (Cordes et al., 2000, 2001; Lowe et al., 1998). One common method for analyzing resting-state data is termed functional connectivity (FC), and refers to the inspection of temporal correlations between time courses (TCs) of spatially remote brain areas (Biswal et al., 1995; Calhoun et al., 2009; Salomon et al., 2013).

A relatively novel tool for studying FC, termed global brain connectivity (GBC), examines the FC of each voxel TC and all other voxel TCs (Cole et al., 2010; Salomon et al., 2011). Thus far, this measure has been used to characterize resting-state network architecture (Cole et al., 2010), and to study cognitive abilities (Cole et al., 2012) in healthy participants. In addition, the GBC is also used to study clinical populations. For example, specific prefrontal cortex functional alternations in schizophrenia (Cole et al., 2011), bipolar disorder (Anticevic et al., 2013), and obsessive-compulsive disorder (Anticevic et al., 2014) were studied using this method. Recently, this measure was also used to study the relationship between spontaneous FC and schizophrenia-like symptoms in healthy participants (Driesen et al., 2013).

We previously used the GBC method to study the connectivity of all cortical voxels in schizophrenia patients and healthy controls while they performed cognitive tasks and during rest (Salomon et al., 2011). We measured the voxel-wise GBC in each participant separately and compared these voxel values between schizophrenia and control participants (Fig. 1). Using this quantitative measure, we found a widespread disruption of FC in schizophrenia patients across all tasks, and most prominently in the resting-state condition.

Though the GBC measure shows promise as a clinical research tool, it is unclear what component of brain connectivity it reflects. From a descriptive view, since this method is global in nature, it may be linked to the averaged whole-brain signal, known as the global signal (for a mathematical formulation of

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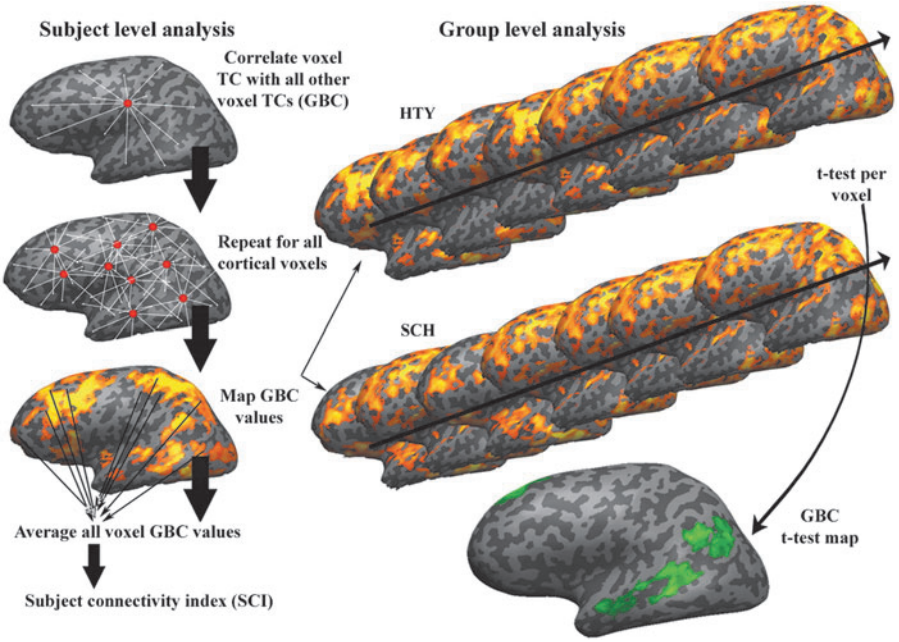

FIG. 1. Schematic of GBC analysis pipeline. Left: subject level analysis. For each subject's functional scan, each voxel's time course was correlated with the time courses of all other voxels (top). This was repeated for all cortical gray matter voxels, so that each voxel had a single correlation index that was the mean of its correlation with all other voxels (middle). This index was projected onto spatial maps; the results from all voxels were averaged to produce the SCI (bottom). Right: group-level analysis. The GBC maps for all subjects in each group were subjected to a voxel-by-voxel between-group *t*-test. The resulting GBC disparity map shows voxels in which one group had significantly higher GBC. HTY, healthy controls; SCH, schizophrenia patients; FC, functional-connectivity; GBC, global brain connectivity; SCI, subject connectivity index; TC, time course. (adapted from Salomon et al. [2011]).

this relationship to the global signal, see Theory section in Supplementary Data; Supplementary Data are available online at www.liebertpub.com/brain). Indeed, we did not remove the global signal from resting-state data in our earlier work, though this is commonly done in order to subtract the influence of physiological noise, such as variations in heart rate and respiration, in addition to instabilities in the magnetic resonance measurement (Aguirre et al., 1998; Bianciardi et al., 2009; Birn et al., 2006; Macey et al., 2004; Shmuel and Leopold, 2008; Shmueli et al., 2007; Wise et al., 2004). Global signal removal has also been found to sharpen observed correlations within resting-state networks, as well as the anti-correlations between the intrinsic and the extrinsic systems (Fox et al., 2009; Nir et al., 2006).

However, it has also been claimed that regression of the global signal does not remove breathing artifacts from functional data and may introduce artificial anti-correlations (Anderson et al., 2011; Carbonell et al., 2011; Murphy et al., 2009; Weissenbacher et al., 2009; but also see Chang and Glover, 2009; Chai et al. 2012). Furthermore, it has recently been shown that global signal removal decreases 1 year test-retest reliability of seed-based FC measures (Guo et al., 2012). Importantly, the problematic nature of global signal regression has been recently demonstrated using simulated data. When the global signal was removed, artificial FC group differences emerged in regions that were designed to have the same degree of connectivity between groups, while true connectivity differences between groups were attenuated (Saad et al., 2012). As the authors noted, this finding is specifically relevant to clinical research that compares patients and healthy controls after global signal regression. Indeed, this finding was recently supported experimentally using resting-state data of participants with autism (Gotts et al., 2013). However, given the enormous variance in neural manifestations of different mental pathologies, it is currently unclear whether these findings may be generalized to other clinical populations.

Regardless of the influences of global signal regression on resting-state data, it is also possible that the global signal itself may be a source of neural information. First, although the most common methods of calculating the global signal is averaging the TCs of all the voxels in the brain (Macey et al., 2004), it has been shown that the global signal mainly maps to gray matter voxels rather than to white matter and ventricles (Fox et al., 2009; Vincent et al., 2008). This enables another form of global approximation, which is the averaging of all cortical gray matter voxels (see, for example, Dinstein et al. [2011] and Ramot et al. [2011]). Thus, it is quite likely that the global signal calculated in resting-state data is, in fact, the averaged spontaneous activity over the cortex or over all gray matter voxels. In line with this claim, it has been found that neural activity contains a global component, apparent over the entire cortex. This has been observed using a combination of electrophysiological and functional magnetic resonance imaging (fMRI) measurements in resting monkeys (Schölvinck et al., 2010) and humans (Wong et al., 2013).

Here, we explore the relationship between the GBC method and the global signal, using resting-state data acquired from schizophrenia patients and healthy participants. First, we replicate our previously reported finding of reduced GBC in schizophrenia patients, attesting to the robustness of the GBC measure. Next, we reveal a tight relationship between the GBC measure and voxel-wise FC of the global signal, using both theoretical and experimental methods. Since the GBC method is widely used to characterize neural pathologies (Anticevic et al., 2013, 2014; Cole et al., 2011), its high resemblance to global signal FC suggests that the global signal holds value as a research tool of various clinical conditions. Finally, these findings extend previous suggestions related to the perils of global signal regression from data of clinical populations (Gotts et al., 2013; Saad et al., 2012) to schizophrenia, in which resting-state data have been extensively used (Calhoun et al., 2009).

Materials and Methods

Subjects and data acquisition

In this study, we used previously published data made available to us, from individuals scanned at the Olin Neuropsychiatry Research Center at the Institute of Living/Hartford Hospital, Connecticut (Calhoun et al., 2008). This dataset contained the resting scans of 20 control participants and 19 chronic schizophrenia outpatients. Exclusion criteria included manifestations of visual or auditory impairment, mental retardation (full scale IQ < 70), traumatic brain injury with loss of consciousness greater than 15 minutes, and presence or history of any neurological illness. Participants were also excluded if they met criteria for alcohol or drug dependence within the past 6 months or produced a positive score in a urine toxicology screen on the day of scanning. All participants gave written informed consent at Hartford Hospital and were compensated for their participation. The experiment was approved by the IRB's at Hartford Hospital/Institute of Living and Yale University. Schizophrenia patients were diagnosed according to DSM-IV TR criteria on the basis of a structured clinical interview and review of the medical file (DSM-IV-TR, 2000).

Three controls and four patients were excluded from the analyses due to severe head movements (> 1 mm). One control and three patients had a severe head movement at the end of the scan, which was cropped. The resulting control group consisted of 17 participants (6 women, mean age 27.6 ± 9), and the schizophrenia group consisted of 15 participants (3 women, mean age 41 ± 12.1). Assessment of the severity of schizophrenia symptoms according to the positive and negative syndrome scale (PANSS) was available for 11 of the schizophrenia participants. Control participants had no DSM-IV TR Axis I disorders, and were not administered psychotropic medication. All of the schizophrenic patients received treatment with atypical antipsychotic medication (Kiehl et al., 2005).

The participants were scanned in a Siemens Allegra 3T head scanner, equipped with 40 mT/m gradients and a standard quadrature head coil. The following parameters were used in the acquisition process: TR/TE/flip angle = 1500 msec/27 msec/70°; with FOV $24 \times 24\,cm^2$; matrix size 64×64. Each resting scan was 5 min and 6 sec in duration. For further details, see Calhoun et al. (2008).

Preprocessing of the imaging data

fMRI data were processed using Brain Voyager QX 2.1 software package (Brain Innovation, Maastricht, The Netherlands) and in-house Matlab code. The first two images of each functional scan were discarded. Preprocessing of functional scans included 3D motion correction, slice scan time correction, and temporal high-pass filtering with a cutoff frequency of two cycles per scan (0.007 Hz). Functional images were aligned with high-resolution anatomical volumes using trilinear interpolation, and the anatomical and functional images were transformed to the Talairach coordinate system (Talairach and Tournoux, 1988). The high-resolution anatomical image of each subject was used for segmentation of gray matter, white matter, and cerebrospinal fluid (CSF). The non-neuronal contributions to the BOLD signal were removed by linear regression of motion parameters from head movements, ventricle and white-matter TCs for each participant (Fox et al., 2009), and by low-pass filtering with a cutoff frequency of 0.1 Hz (Cordes et al., 2001). The data were then spatially smoothed using a Gaussian filter of 8 mm full width at half maximum value correction, and transformed to units of percent signal change.

GBC measure

For each participant separately, the mean correlation between the TC of each cortical voxel and the TCs of all other cortical voxels was computed (for a mathematical formulation of this method, see Supplementary Data). This procedure yielded a GBC value for each cortical voxel. To assess the frequency of negative correlations, their proportion in each participant's functional data was calculated and compared between groups using a two-tailed *t*-test. The average proportions of negative correlations were below 1.5% in both experimental groups and did not differ between groups [$t_{(30)} = -0.41$, *p*-value n.s.]. Due to the minority of negative correlations in the data, and considering recent claims that these correlations have neural bases (Chai et al., 2012; Chang and Glover, 2009; Fox et al., 2009; Keller et al., 2013; Uddin et al., 2009), we did not exclude negative correlation from our analyses. To obtain a gross measure of connectivity per participant, the GBC indices of all cortical voxels were averaged to produce a single global correlation value per participant, here referred to as the subject connectivity index (SCI). These SCI values were transformed to *z*-values using Fisher's *r*-to-*z* transformation, and a one-tailed *t*-test was used to determine whether the SCIs of the schizophrenic patients would be significantly lower than those of the controls, as was previously reported using the same methodology (Salomon et al., 2011).

Group-level analysis

In order to assess whether the reduction in connectivity in the schizophrenia group was ubiquitous across the entire cortex, a voxel-wise analysis was conducted. First, group maps were created by averaging the GBC values of each voxel across all participants of each experimental group. The resulting averaged voxel connectivities were then projected onto a representative individual's cortical surface.

Next, voxel-wise GBC values were compared between experimental groups. Specifically, a voxel-by-voxel one-tailed *t*-test was performed on the *z*-transformed GBC values in order to determine whether the schizophrenia group had reduced correlations compared with the control group, as was previously reported using the same methodology (Salomon et al., 2011). The resulting *t*-values were projected onto a representative individual's cortical surface. A correction for multiple comparisons was performed by thresholding these values [$t_{(30)} = 1.7$, $p < 0.05$] and calculating the probability of a false positive from the frequency count of cluster sizes within the entire cortical surface, using a Monte–Carlo simulation, as implemented in Brain Voyager (Brain Innovation).

Global signal analyses

The global TC was calculated for each participant separately by averaging across all TCs of gray matter cortical voxels. For each participant, this mean signal was linearly regressed from each unsmoothed cortical TC, along with

the previously described nuisance variables. The GBC analysis was then conducted again, using the spatially smoothed residual TCs, in the same manner mentioned earlier. Group and disparity maps were also created as described earlier.

In a separate analysis conducted within each participant, the global TC was correlated with each cortical voxel TC, prior to global signal regression, yielding a vector of correlation coefficients. This vector was then correlated with a vector holding the GBC values for each voxel of the same participant. Correlation coefficients between global FC and the GBC values were then converted to z-values using Fisher's r-to-z transformation, and were compared between groups using a two-tailed t-test.

Removal of high-variance voxels

The variance of each cortical voxel TC was calculated for each participant. Then, the global signal of each participant was recalculated as described earlier, while excluding all voxels with variance values which exceeded two standard deviations of the mean variance of all TCs of that participant. The global connectivity analyses described earlier were then repeated, excluding voxels of high variance.

Group differences in global signal characteristics

Differences in global signal characteristics between the groups were examined in two ways. First, the global signal of each participant was calculated as described earlier. The variance of the global signal was then computed and compared between participants of the two groups using the Ansari–Bradley test. Second, the variance of each cortical voxel TC was computed, and the mean of all voxel TC variances was calculated. This mean variance was then compared between groups using the Ansari–Bradley test.

Correlation to schizophrenia symptoms

PANSS scores were correlated with SCI values in a subsample of schizophrenia participants for whom PANSS scores were available, under the hypothesis that lower SCI values would predict more severe symptoms. Resulting p-values were corrected for multiple comparisons by using the Bonferroni correction.

Principal component analysis

The contribution of the global signal to the functional data of each participant was evaluated by applying principal component analysis (PCA) decomposition on the TCs of cortical voxels (using the princomp function in Matlab's statistics toolbox). Group differences in the variance explained by the first principal component of each participant's data, which estimates the global signal (Carbonell et al., 2011), were tested using an F-test.

For analyses of the effects of head motion and age on global signal FC, see Supplementary Data.

Results

Differences in GBC between schizophrenia and control groups

In order to test our previous finding of reduced GBC in resting schizophrenia patients (Salomon et al., 2011), a larger dataset of 15 patients and 17 controls was used. The correlation of each cortical voxel TC with all other cortical voxel TCs (GBC) was computed for each participant, and group maps were created (see Materials and Methods section). As can be clearly seen in the top two panels of Figure 2, while the control group demonstrated strong and wide spread correlations, the patient group showed a marked reduction of connectivity. As seen in the bottom panel of Figure 2, a direct comparison between voxel-wise GBC values revealed significant and widespread differences between the groups, which encompassed areas of the superior frontal sulcus, cingulate cortex, central sulcus, post-central sulcus, pre-central sulcus, insula, inferior parietal sulcus, superior temporal sulcus, parieto-occipital sulcus, occipito-temporal sulcus, lateral occipital sulcus, and the parahipocampal gyrus.

SCI values were calculated by averaging the GBC values within each participant. These averaged values were found to differentiate control from schizophrenia participants [$t_{(30)} = 1.76$, $p < 0.05$, see left bars of Fig. 3].

Connectivity differences after regression of the global signal

If there is a strong connection between the GBC and global signal FC, the linear regression of the global signal from the data should diminish the difference in GBC between the groups. To test this hypothesis, we projected the global signal of each participant from the participant's functional data. We then recalculated the GBC for each participant, and created group GBC maps. The GBC values within the control group map ranged between -0.02 and 0.04 (compared with a range of 0–0.6 before global regression), and the values for the patients' map ranged between -0.03 and 0.03 (compared with a range of 0–0.37 before global regression). The negligible correlation values indicate that the GBC is tightly linked to the global signal. In addition, a voxel-wise comparison of GBC values between the groups showed no area of significant difference.

SCI values were also calculated after global regression, but contrary to the finding before global removal, no significant difference in SCIs was found between the groups [$\overline{\mathrm{SCI}}_{\mathrm{HTY}} = 0.004$ $\mathrm{SE}_{\mathrm{HTY}} = 0.002$, $\overline{\mathrm{SCI}}_{\mathrm{SCH}} = 0.004$ $\mathrm{SE}_{\mathrm{SCH}} = 0.002$, $t_{(30)} = -0.09$, n.s.]. Figure 3 displays the mean SCI values of each group before and after the removal of the global signal. It is clearly evident that the difference which was found before global removal was no longer present after the global signal was regressed from the data.

Correlation between GBC values and global FC values

To assess the magnitude of relationship between the GBC and global signal FC, we conducted a global FC analysis for each participant. The resulting voxel-wise FC values were correlated with the voxel-wise GBC values within each participant (see Materials and Methods section). In both groups, the correlation coefficient between these two measurements was very high, with the average of 0.96 and 0.88 in the control and schizophrenia groups, respectively. A significant group difference was also found [$t_{(30)} = 3.35$, $p < 0.005$, Fig. 4, left panel].

Removal of high-variance voxels

Mathematically, the GBC and the global signal FC are equal, to the level of TC z-score (see Theory section in Supplementary Data). Since z-score normalization manipulates

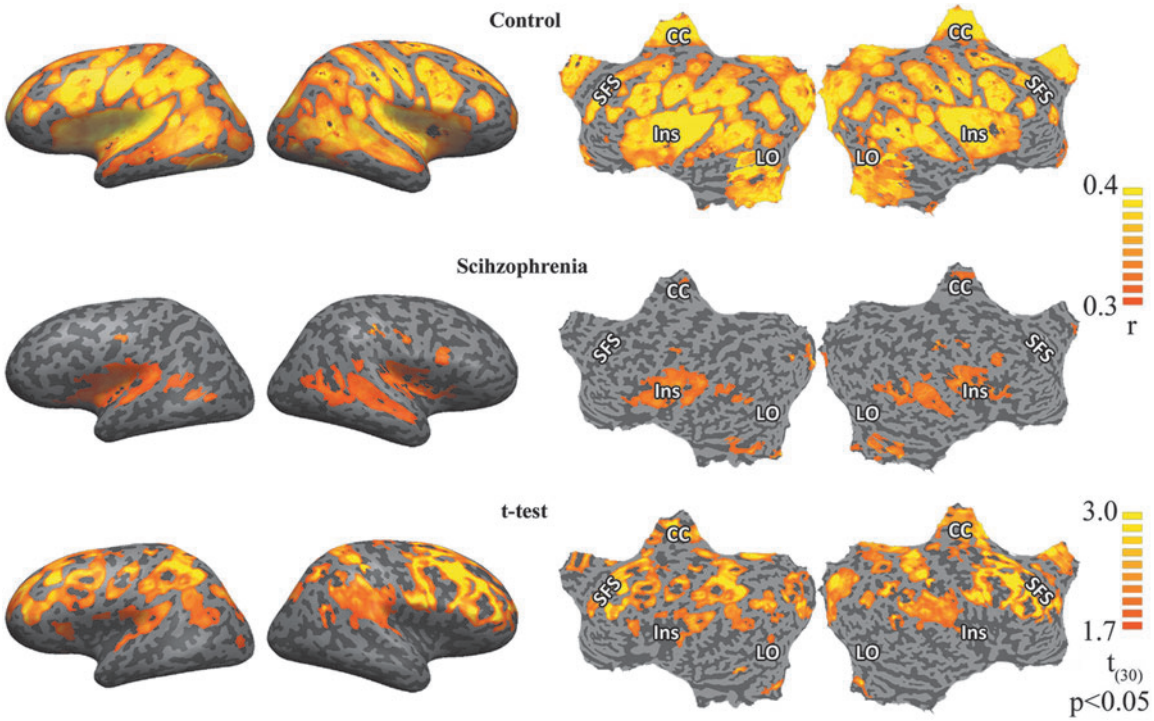

FIG. 2. GBC group maps and *t*-test map presented on inflated (left) and flattened (right) surfaces. Top: control group map (averaged correlations across voxels of all control subjects). Middle: schizophrenia group map (averaged correlations across voxels of all schizophrenia patients). Bottom: *t*-test map showing all voxels that have significantly higher GBC in the control group in comparison to the schizophrenia group (cluster-based corrected for multiple comparisons). CC, cingulate cortex; Ins, insular cortex; LO, lateral occipital cortex; SFS, superior frontal sulcus.

both the average and variance of a signal, both GBC and global FC should be similar, provided all voxel TCs of a participant have a narrow range of averages and variabilities. Since each TC was percent signal change normalized before the GBC and global FC analyses, all voxel TCs had an average of zero. We, therefore, postulated that schizophrenia patients demonstrated lower correlations between their GBC and global FC values due to a higher degree of noisy voxels, in which the temporal variance is very high. To test this hypothesis, we repeated the global FC analysis after removing all voxels with variance values that exceeded two standard deviations of the mean variance of all TCs within each participant. The average portion of removed voxels in both experimental groups was 4%. Though the percent of removed voxels was very small, after these voxels were removed, the correlation between the GBC and global FC increased in both groups to the level of almost 1 [$\overline{CORR}_{HTY} = 0.98$, $\overline{CORR}_{SCH} = 0.95$, $t_{(30)} = 2.88$, $p < 0.01$] (Fig. 4, right panel). It should be noted that after removing the influence of noisy voxels, the correlation between the global FC values and the GBC values increased in all participants.

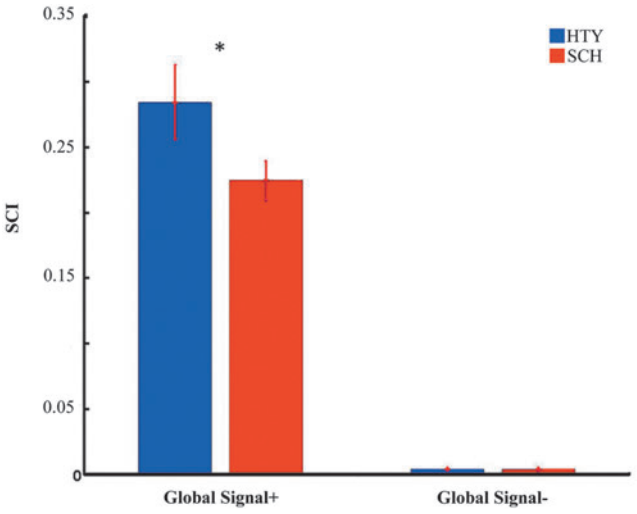

FIG. 3. Group SCI. Left bars: original data, without global signal regression (Global Signal+). Right bars: data after global signal regression (Global Signal−). Notice the negligible values of the SCI after the global signal is removed. Error bars indicate standard error of the mean. *$p < 0.05$; HTY, healthy participants (blue); SCH, schizophrenia patients (red).

Group differences in global signal characteristics

In order to examine whether other traits of the global signal could differentiate between the experimental groups, global variance was compared in two different ways (see Materials and Methods section). Neither the variance of the global signal ($W = 132$, $W^* = -0.95$, n.s.) nor the average variance of all cortical voxel TCs in each participant ($W = 124$, $W^* = -1.55$, n.s.) differentiated between the groups.

Correlation with schizophrenia symptoms

We next examined whether variability in SCI values of schizophrenia patients may be explained by schizophrenia

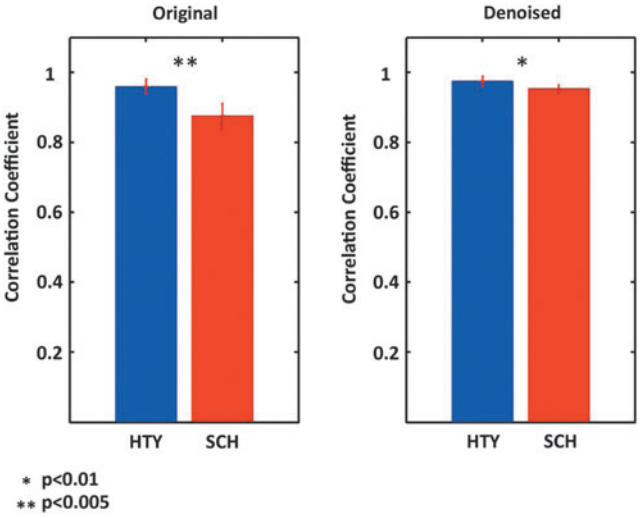

FIG. 4. Between-group comparison of correlation between global FC values and the GBC values. Left: original data. Right: data after removal of noisy voxels (see Materials and Methods section). Notice that after denoising of the data, the global FC–GBC correlations nearly reach 1 in both experimental groups; HTY, healthy participants (blue); SCH, schizophrenia patients (red).

symptoms in a sub-sample of patients for whom PNASS scores were available. No significant correlation was found between SCI values and participants' symptom scores.

Principal component analysis

It has previously been shown that the global signal can be estimated using the first principal component of the functional data (Carbonell et al., 2011). The differential contribution of the global signal to functional data of participants from the two experimental groups could, thus, be established by testing group difference in the variance explained by the first principal component. This comparison revealed a significantly higher contribution of the global signal to the data of control participants compared with schizophrenia participants [$F_{(16,14)} = 3.47$, $p < 0.05$].

For additional analyses regarding head motion and age effect on the global FC, as well as a summary table of all between-group effects, see Supplementary Data.

Discussion

Our results revealed several interesting findings: First, we replicated our previous finding, showing reduced GBC in schizophrenia patients compared with controls. Second, we showed that GBC is tightly linked to the FC of the global signal. We will now discuss these findings and their relationship to the use of the global signal in fMRI studies of clinical populations.

GBC differentiates schizophrenia patients from healthy controls

Here, we replicated our previously published finding that the correlation between each voxel TC and all other voxel TCs (GBC) differentiates healthy participants from schizophrenia patients (Salomon et al., 2011). This is now shown on a larger dataset consisting of new participants, scanned in a different magnet, and is in line with existing evidence of reduced connectivity in schizophrenia patients throughout various functional systems (Honey et al., 2005; Lawrie et al., 2002; Liang et al., 2006).

In addition to specific brain regions that differentiated between groups, significant group differences were also detected using the gross measure of SCI, which averages all cortical voxel values (including those that did not differ between groups). Though, as expected, group differences in SCI were smaller than those found in the most significant regions of the between-group *t*-map (Fig. 2), the fact that a significant between-group SCI effect was still obtained attests to the robust nature of GBC deficits in schizophrenia patients.

GBC reflects global signal FC

We mathematically formulated the close relationship between the GBC measure and the FC of the global signal (see Theory section in Supplementary Data). This was also supported experimentally by the fact that after global regression, group differences in GBC were abolished. Moreover, we showed that in both experimental groups, a voxel-wise calculation of global signal FC was almost perfectly correlated with the GBC of the same voxels within participants.

We note that before the procedure of removing high-variance voxels, the average correlation between the GBC and the global FC was 0.96 in the control group but only 0.88 in the patient group. After removing noisy voxels, the correlations in the control and patient groups were 0.98 and 0.95, respectively. This difference was still statistically significant, and may have been caused by a higher degree of noise that was not fully removed in the schizophrenia group and impacted the global FC measure. Nevertheless, it is clear that the correlations in both groups were very high both before and after this cleaning procedure, demonstrating the strong relationship between the global FC and the GBC measure.

Global signal FC can be implemented in several manners, and each differs in the way it derives the global signal. The most straightforward method simply calculates the global signal as an average across all voxel TCs. Though this method is very intuitive, it may be prone to the influence of voxels contaminated with high-variance noise. When considering this kind of noise, usage of the GBC method may be advantageous, as this method normalizes voxel variances, so that the influence of high-variance noise on the global signal is reduced (see Theory section in Supplementary Data). However, the GBC method may also overweigh noisy voxels of low temporal variance compared with the standard global FC method. A third method, global signal estimation using the first principal component of the functional data, will, by definition, underweight extreme (noisy) observations in the data, creating a clean global signal. However, this method is less intuitive and more computationally demanding. It is important to note that, regardless of the chosen method for global FC, all these approaches reflect the same underlying phenomenon, which is inseparable from the global signal itself.

Importantly, these whole-brain approaches for global signal FC have so far proved to be valuable research tools not only in schizophrenia research (Salomon et al., 2011) but also in studies of other clinical populations. For example, GBC and global FC alternations were revealed in resting-state data of participants diagnosed with obsessive-compulsive

disorder (Anticevic et al., 2014) and participants with severe depressive disorder (Perrin et al., 2012), respectively. Studies focused on the prefrontal cortex found connectivity in this area to be aberrant using the GBC measure in schizophrenia (Cole et al., 2011; Yang et al., 2014) and bipolar disorder (Anticevic et al., 2013). Others found a reduction in global FC in a group of participants with autistic spectrum disorder in comparison to controls, and, therefore, did not regress the global signal from the data (Gotts et al., 2012). However, since the GBC measure has not been associated with global FC so far, this method has sometimes been used after applying global signal regression on data. While we encourage the use of the different global signal FC approaches to study clinical populations, we also propose that other means for removing artefacts be used before applying these methods.

Perils of global signal regression from clinical data

Global signal regression has been debated regarding claims that it distorts resting-state connectivity patterns while not cleaning physiological noise (Chai et al., 2012; Chang and Glover, 2009; Fox et al., 2009; Murphy et al., 2009; Weissenbacher et al., 2009). In addition, as detailed in the introduction, the resting-state global signal reflects the averaged spontaneous activity of gray matter voxels, and it is correlated with neural activity (Schölvinck et al., 2010). Here, we further demonstrated that there is a significant difference in the GBC (equivalent to global signal FC) between control and schizophrenia participants. Critically, our results show that removal of the global signal would have eliminated the differences between the experimental groups. Considering these findings together, we suggest that the global component should not be automatically removed, but studied as a phenomenon of interest, provided that relevant noise correction methods are applied.

Our study extends the conclusion of former studies, showing that the regression of the global signal may significantly distort results when studying clinical populations. This claim was first made based on simulated data (Saad et al., 2012), and later demonstrated on data from participants with autism (Gotts et al., 2013). Our results extend these previous reports by demonstrating that they are highly relevant to schizophrenia research as well. Specifically, as we directly demonstrated using a PCA analysis, since the global signal contributes more (has higher FC) to data of control participants, upon its removal, more variance will be removed from the control data compared with schizophrenia data, causing an imbalanced preprocessing effect. These results, based on schizophrenia patients who show very different behavioral and neural characteristics than those of autistic individuals, support the generalization of previous conclusions regarding global signal regressions to other clinical populations.

It is also important to address the assumption that the global signal mainly reflects noise, rather than true neural activity. For example, the global signal may be related to head movements, as these are likely to have a coherent effect over many brain regions. In order to ensure that our findings are not related to head motion, we used several control analyses. First, we verified that participants of the two experimental groups showed the same level of head motion in all motion axes. Second, we ensured that the global signal of each participant was not correlated with signals of any of the motion axes. In addition, we demonstrated that neither the SCI values nor the correlation between the GBC and global FC values differed between control participants who showed a relatively high amount of head movements in comparison to those who showed low levels of head movements (see Supplementary Data).

Another possible noise component that may have contributed to our reported effects is physiological noise (Birn et al., 2006; Shmueli et al., 2007; Wise et al., 2004). To account for this possible artifact, we used the TCs of white matter and CSF as nuisance regressors in our preprocessing procedure, before analyzing the data. However, since there is currently no certain way to identify the proportion of physiological noise in comparison to ''true'' neural activity in the BOLD signal (Iacovella and Hasson, 2011), we cannot rule out the influence of some noise residuals on our data. This same problem, however, exists regardless of the noise correction method used in resting-state data, even after global signal regression. Nonetheless, a global signal that is calculated after the employment of such methods should not be considered mere noise, as it more likely reflects an underlying global component of neural activity (Schölvinck et al., 2010).

Limitations

A potential limitation of this study arises from the possible effects of medication on the global signal. While such limitations apply to almost all studies on psychiatric cohorts, the effects of antipsychotic medications on the global signal are not well known, and further studies are needed in order to investigate such possible connections.

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Author Disclosure Statement

The authors report that no competing financial interests exist.

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