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Brain functional and perfusional alterations in schizophrenia: an arterial spin labeling study

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A B S T R A C T

Schizophrenia is a severe mental disorder that affects the anatomy and function of the brain, with an impact on one's thoughts, feelings, and behavior. The purpose of the study was to investigate cerebral blood flow (CBF) and brain connectivity in a group of patients with schizophrenia. Pseudo-continuous arterial spin labeling (pCASL) images were acquired from 28 patients in treatment and 28 age-matched healthy controls. Mean CBF and connectivity patterns were assessed. Schizophrenia patients had decreased CBF in the bilateral frontal pole and superior frontal gyrus, right medial frontal gyrus, triangular and opercular parts of the inferior frontal gyrus, posterior division of the left supramarginal gyrus, superior and inferior divisions of the left lateral occipital cortex, and bilateral occipital pole. Moreover, through different methods to assess connectivity, our results showed abnormal connectivity patterns in regions involved in motor, sensorial, and cognitive functions. Using pCASL, a non-invasive technique, we found CBF deficits and altered functional organization of the brain in schizophrenia patients that are associated with the symptoms and characteristics of the disorder.

1. Introduction

Schizophrenia is a disabling psychiatric disorder that affects around 1% of the population worldwide. Delusions, hallucinations, disorganized thought, and cognitive deficits are the main features of schizophrenia, which has been further characterized by abnormal brain connectivity and behavioral disturbances (Watkins and Andrews, 2016). Because of the altered level of neuronal activity in schizophrenia, brain function and perfusion have been extensively investigated, and cerebral blood flow (CBF) alterations during resting state have been reported in patients compared to healthy age-matched subjects (Andreasen et al., 1997; Kanahara et al., 2013).

Since CBF is directly coupled with neuronal activity, its synchronous fluctuations in brain regions that form functional networks have allowed the investigation of resting brain networks (RBNs) and functional connectivity (FC) patterns (Melie-García et al., 2013). In this context, arterial spin labeling (ASL) is an important tool for obtaining CBF maps, using blood water as an endogenous contrast and thus consisting of a completely non-invasive technique that provides quantitative maps with better temporal and spatial resolutions than other imaging methods (Alsop et al., 2015; Wintermark et al., 2005; Xu et al., 2009).

Recent ASL studies have demonstrated CBF changes in schizophrenia at the resting state condition. Notably, decreased CBF was reported in patients when compared with healthy individuals in regions of the frontal lobe, anterior cingulate cortex, temporal lobe and occipital lobe (Kindler et al., 2015; Ota et al., 2014a; Pinkham et al., 2011; Scheef et al., 2010; Walther et al., 2011; Zhu et al., 2015). Aiming to overcome the problems due to small sample sizes, Zhu et al. compared large groups (100 patients and 94 healthy controls) and reported reduced CBF in schizophrenia in the left insula, left middle frontal gyrus, bilateral anterior cingulate cortices, and bilateral middle occipital gyri. In addition, schizophrenia patients exhibited increased CBF in bilateral inferior temporal gyri, thalami and putamen (Zhu et al., 2015).

Moreover, alterations in ASL-CBF connectivity in schizophrenia have also been reported. Kindler and colleagues investigated functional connectivity between regions of the default mode network (DMN) and found changes in schizophrenia patients (Kindler et al., 2015). Another study used anatomical brain areas with reduced CBF in schizophrenia patients as seeds, and its seed-based analysis showed decreased connectivity between the left insula and postcentral gyrus, and between the

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left thalamus and right medial superior frontal gyrus (Zhu et al., 2015). In addition, two other studies described topological alterations of CBF in schizophrenia using anatomical information (Cui et al., 2017; F. Liu et al., 2016). However, functional connectivity and its relationship with CBF at rest remain to be assessed in other RBNs.

Therefore, we investigated brain perfusion and functional connectivity at rest in schizophrenia patients, assessing information collected from the whole brain. To our knowledge, the present study is the first that used individual CBF temporal fluctuations to assess functional connectivity considering the whole brain, not only specific networks, through different approaches (seed-based, independent component analysis and graph theory), and correlated the results with regional CBF in schizophrenia.

2. Materials and methods

2.1. Subjects

Twenty-eight patients with schizophrenia and twenty-eight healthy age-matched subjects participated in the study. Patients were recruited at the Department of Neuroscience and Behavior of the Ribeirao Preto Medical School University Hospital of the University of Sao Paulo (HCFMRP-USP) by a trained psychiatrist who applied the diagnostic criteria of the DSM-V (Diagnostic and Statistical Manual of Mental Disorders – *Fifth Edition)*. To be eligible for inclusion in the study, participants had to be between 18 and 45 years of age and have a diagnosis of schizophrenia with the presence of positive or negative symptoms (i.e., score equal or greater than 4 in any item of the Positive and Negative Syndromes Scale - PANSS). All patients were in antipsychotic treatment when the MRI scans were performed. For both groups, exclusion criteria included the presence of systemic medical illness such as cardiovascular disease, severe hypertension, kidney failure, liver disease, hypothyroidism, and epilepsy; any indication of neurovascular diseases (atherosclerotic, stroke or tumor); pregnancy; breastfeeding; drug and alcohol abuse; and presence of prosthesis incompatible with the magnetic resonance environment. The demographic and clinical characteristics of the two groups are described in Table 1.

The study was approved by the local ethics committee, and all volunteers gave their written consent to participate after being informed about the experimental procedures.

2.2. Data acquisition

Magnetic resonance imaging (MRI) was performed in a 3 T system (*Philips Achieva*, The Netherlands) using a 32-channel head coil for reception and a body coil for transmission. Anatomical reference images were acquired using a 3D gradient-echo T1-weighted sequence (TR/TE = 7/3.2 ms, FA = 8°, matrix = 240 × 240, FOV = 240 × 240 mm^2, 160 1-mm slices). Pseudocontinuous ASL (pCASL) images were obtained using a 2D single-shot EPI sequence with the following parameters: TR/TE = 4000/14 ms, FA = 90°, matrix = 160 × 160, FOV = 240 × 240 mm^2, 20 5-mm slices (no gap), labeling duration = 1650 ms, post-labeling delay = 1525 ms, 50 control/label pairs, total scan duration = 6 min and 48 s. All subjects were instructed to stay awake, think of nothing in particular and move as little as possible during the scan. For CBF quantification, a proton density (M0) image was acquired separately with the same geometry and parameters of the pCASL sequence, but without labeling pulses.

Table 1
Demographic and clinical characteristics of schizophrenia patients and healthy controls.

Variables (Mean ± SD)	Schizophrenia patients	Healthy controls	p-value
Gender (female/male)	4/24	8/20	0.192[a]
Age (years)	32.8 ± 7.8	31.1 ± 5.8	0.178[b]
Duration of illness (years)	14.3 ± 8.3	–	–
PANSS			
Positive score	14.4 ± 6.4	–	–
Negative score	23.1 ± 8.2	–	–
Total score	69.4 ± 17.2	–	–

[a] The *p*-value was obtained by chi-square test.
[b] The *p*-value was obtained by two sample *t*-test (Welch's test).

Moreover, we have taken some care to minimize undesirable differences between groups. First, a medical physicist accompanied all exams of both groups (controls and patients) to guarantee the correct process of instructing and positioning the participants, and to check the quality of the images that were being acquired. Second, all participants were instructed to abstain from consuming coffee and alcohol for at least 12 h before the MRI section since both substances have a vasomotor effect. Third, all participants took at least a 30-min rest before entering the MR scanner to eliminate effects of previous activities. Finally, all exams were performed on the same machine, at the same period of the day to avoid variability due to diurnal CBF fluctuations, and using the same pCASL sequence with parameters previously optimized based on Alsop et al. (2015).

2.3. Data processing

All data preprocessing was performed using Statistical Parametric Mapping (SPM12; http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). For ASL images we used batch-based scripts available in ASLtbx (Wang et al., 2008) according to the following steps: motion correction, coregistration to anatomical image, temporal filtering, spatial smoothing with an isotropic Gaussian kernel (FWHM = 4 mm for CBF quantification, and FWHM = 6 mm for CBF-FC analysis), CBF quantification, and normalization to MNI standard space (resolution: 2 × 2 × 2 mm^3, matrix size: 79 × 95 × 79). The amended algorithm was used for ASL image de-noising. Residual motion and global signals were regressed out from the control/label ASL images before CBF calculations (Wang, 2012). For quantification purposes, outlier CBF images were removed using the SCORE algorithm (Dolui et al., 2017). A correction for partial volume effect (PVE) was also performed in the ASL data as previously proposed in the literature (Asllani et al., 2008).

2.4. CBF quantification

Perfusion images were obtained by sinc subtraction of control and label images. For CBF quantification, we used a General Kinect Model (Buxton et al., 1998) with the following parameters: longitudinal relaxation time (T1) of blood, 1650 ms; labeling efficiency, 0.85; blood/tissue water partition coefficient, 0.98 g/mL for gray matter (GM) and 0.84 g/mL for white matter (WM); T1 of tissue, 1020 for GM and 770 for WM, previously calculated from images acquired using a Look-Locker sequence (Karlsson and Nordell, 2000). Average CBF and normalized CBF (= voxel CBF/global gray matter CBF) maps were obtained to assess regional differences between groups. Anatomical regions of interest (ROIs) were selected from the Harvard-Oxford atlas, excluding cerebellum and vermis areas not covered by our image acquisition.

2.5. CBF resting-state networks and functional connectivity

Perfusion images were obtained by pairwise subtraction of control and label images. In the FC toolbox (version 17a) (Whitfield-Gabrieli and Nieto-Castanon, 2012), CBF images were detrended and filtered with a low-pass filter ($f < 0.07$ Hz) (Boissoneault et al., 2016; Fernández-Seara et al., 2015). In addition, WM and cerebrospinal fluid signals were removed by principal component analysis (PCA) using the Compcor algorithm (Behzadi et al., 2007). To obtain the RBNs from CBF maps, we used group-level independent component analysis (ICA) with