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Within-subject test-retest reliability of the atlas-based cortical volume measurement in the rat brain: A voxel-based morphometry study

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ABSTRACT

Background: Various neurological and psychological disorders are related to cortical volume changes in specific brain regions, which can be measured *in vivo* using structural magnetic resonance imaging (sMRI). There is an increasing interest in MRI studies using rat models, especially in longitudinal studies of brain disorders and pharmacologic interventions. However, morphometric changes observed in sMRI are only meaningful if the measurements are reliable. To date, a systematic evaluation of the test-retest reliability of the morphometric measures in the rat brain is still lacking.

New method: We rigorously evaluated the test-retest reliability of morphometric measures derived from the voxel-based morphometry (VBM) analysis. 37 Sprague-Dawley rats were scanned twice at an interval of six hours and the gray matter volume was estimated using the VBM-DARTEL method. The intraclass coefficient, percent volume change and Pearson correlation coefficient were used to evaluate the reliability in 96 subregions of the rat brain.

Results: Most subregions showed excellent test-retest reliabilities within an interval of 6 h while a few regions demonstrated lower reliability, especially in the retrosplenial granular cortex. The results were consistent between different methods of reliability assessment.

Comparison with existing method: To the best of our knowledge, this is the first study to quantify the test-retest reliability of the VBM measurements of the rat brain.

Conclusion: Atlas-based cortical volume of the rat brain can be reliably estimated using the VBM-DARTEL method in most subregions. However, findings in subregions with lower reliability must be interpreted with caution.

1. Introduction

Rat models of brain diseases have been widely used in translational neuroscience studies due to low cost and stable behavior. Compared to the human study, rat models are particularly suitable for the investigation of longitudinal changes of brain structure and function associated with a specific disease or pharmacological intervention as the brain characteristics can be measured *in vivo* over the lifespan. Brain volume is an important indicator for macroscopic structural changes, which could be captured non-invasively using MRI not only in humans but also in animal models (Quallo et al., 2009; Badea et al., 2010; Ellegood et al., 2010, 2013; Powell et al., 2016; Steventon et al., 2016). Many studies used the deformation based morphometry (DBM) method to measure the whole brain volume alternations, which it is dependent on the accuracy of imaging registration (van Eede et al., 2013). Another category of method is voxel-based morphometry (Ashburner and Friston, 2000), which can objectively estimate the voxel-wise whole brain cortical volumes without any prior assumption (Chetelat et al.,

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2005; Baron et al., 2001). Recent development in this line of research includes the improved registration pipeline called diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) (Ashburner, 2007). The VBM-DARTEL analysis improves inter-subject alignment by registering individual structural images to an asymmetric template generated within each group, and has demonstrated great promise in the studies of human psychiatric and neurological disorders (Mak et al., 2011; Asami et al., 2012; Matsuda et al., 2012; Liu et al., 2014). The VBM-DARTEL analysis can be easily transposed to rat models and only requires some specific preprocessing for the rat MRI data. Recent studies have demonstrated the usefulness of this method in animal studies. For example, Sumiyoshi et al. reported that regional gray matter volume increased following 7 days of voluntary wheel running exercise in rats (Sumiyoshi et al., 2014); Suzuki et al. (2013, 2015) found structural abnormalities in the hippocampus in rat models of cardiopulmonary resuscitation and heart failure. Furthermore, it is also possible to relate macroscopic cortical volume/density measures with microscopic changes in the animal brain. A recent study demonstrated that the VBM signal is correlated with the dendritic spine density in the mice brain (Keifer et al., 2015), suggesting the physiological significance of VBM measurements.

A vital question that has yet to be addressed is whether VBM measurements are reliable for quantitative studies of the rat brain. High within-subject test-retest reliability is a prerequisite of all imaging biomarkers. Identifying brain regions where cortical volume can be reliably measured will inform the interpretability of findings, particularly in longitudinal and cross-sectional MRI studies. Due to its critical importance, the test-retest reliability of structural and functional MRI measures was carefully examined in the human brain (Zuo and Xing, 2014; Andellini et al., 2015; Laumann et al., 2015; Mueller et al., 2015; Seiger et al., 2015). However, to date, the test-retest reliability of MRI measures in the rat brain has not been systematically evaluated, limiting the usage of MRI for quantitative analyses of brain anatomy in rat models. To address this challenge, here, we studied the rat brain using a test-retest design and examined the reliability of cortical volume derived from the VBM-DARTEL analysis. The intraclass coefficient (ICC), percent volume change (PVC) and Pearson correlation coefficient (PCC) were calculated to comprehensively evaluate the test-retest reliability of the cortical volume of each subregion in the rat brain. We also investigated how the test-retest reliability of cortical volume measurements might be affected by choices of data processing parameters such as smoothing (Shen and Sterr, 2013).

2. Material and methods

2.1. Animals

Thirty-seven 10-week-old Sprague-Dawley rats (male, 240–260 g; Charles River, Beijing, China) were used in the experiment. All rats were housed in a temperature-controlled home cage (25 ℃ ± 2) with a 12 h light-night alternation (6 p.m. – 6 a.m. light off); the rats could freely access food and water at all times. All animal procedures were in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and approved by the ethical committee and the Institutional Review Board of Capital Medical University.

2.2. Experimental design

Test-retest reliability can be divided into two categories based on the interval between the repeated measures: long-term reliability implies repeated measurements within a few weeks or longer; short-term reliability duplicates measurements within a few hours. Long-term reliability of VBM measurements can be affected by various factors, including measurement noise, data processing methods, biological changes in the brain, stability of MRI equipment over time, and consistency of data collection procedure, etc. In the present study, we evaluated the short-term reliability of the VBM-DARTEL results by scanning the subjects twice at a short interval of 6 h, thus the influence of variance due to MRI equipment, data collection procedure, and possible biological changes was minimized.

2.3. MRI image acquisition

Prior to the MRI scan, each rat was placed into an inhalation chamber and administered preoperative anesthesia containing 5% isoflurane (mixed with oxygen and air). The anesthetized rat was then positioned in the MRI scanner bed, with the head immobilized by a tooth bar. A continuous flow of 1.5% isoflurane was used to retain anesthesia. After the preparations, the rat brain was scanned in a 7.0 T Bruker MRI scanner (16-cm horizontal diameter, equipped with transmit volume coil and a surface coil, Germany). High resolution structural images were acquired using RARE T2-weighted sequences: repetition time (TR) = 9000 ms, effect echo time (TEeff) = 48 ms, RARE factor = 8, average number = 8, number of coronal slices = 54, slice thickness = 0.5 mm, slice gap = 0, field of view (FOV) = 32 × 32 mm^2, matrix size = 256 × 256 voxels, voxel size = 125 × 125 μm^2. During the scan, the rats' respiration rate was continuously monitored, and the isoflurane might be slightly adjusted to maintain a safe respiration rate (40–50 breaths per minute). The structural MRI scanning procedure of each rat lasted about forty minutes. The signal to noise ratio (SNR) is an important quality control index for structural MRI studies and there exist many ways for the calculation of SNR, such as those reported by Solis-Najera et al. (2015), Pohmann et al. (2016). In the present study, we selected a simple but effective approach for the calculation of SNR, which is defined as the ratio of the mean image intensity in the brain divided by the standard deviation of the intensity at background (Sumiyoshi et al., 2014). The SNR was evaluated for the test and the retest imaging data, respectively.

2.4. Image processing

The VBM-DARTEL analysis was performed using statistical parameter mapping software (SPM 8, Wellcome Department of Cognitive Neurology, London, UK), and the detailed procedures were similar to the previous study (Sumiyoshi et al., 2014). First, the original T2-weighted images (0.125 mm resolution) were resized with a scale factor of 10 (Biedermann et al., 2012) to roughly match the size of the rat brain template. The transformed images thus have a spatial resolution of 1.25 mm, however, there is no information loss by resizing the images because the data were not downsampled. This is a common procedure of VBM analysis for rat/mouse brain to facilitate coregistration (Biedermann et al., 2012; Suzuki et al., 2013, 2015; Sumiyoshi et al., 2014). The images were then realigned to adjust for head motion, and coregistered to the standard rat brain template (Valdes-Hernandez and Sumiyoshi, 2011). Every image was then segmented into gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) by the unified segmentation method (Ashburner and Friston, 2005). In this step, the bias correction images were generated and used for the segmentation process. The probabilistic GM, WM, and CSF maps of the rat template brain were selected as the tissue priors. In order to accurately register the individual GM, WM, and CSF maps to the corresponding template, the non-linear DARTEL algorithm was used for normalization, which included the following steps: (i) the group-specific (test/retest group) GM, WM, and CSF templates were respectively created by the DARTEL algorithm after 6 iterative computations; (ii) the individual GM, WM, and CSF images were spatially normalized to the group-specific templates, which used the Jacobian determinant to account for the expansion or contraction of brain regions; (iii) the group-specific templates were rigid-body aligned to the standard rat brain template, and the obtained affine transformation matrix was used to coregister

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