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Research report

Increased hippocampal fissure width is a sensitive indicator of rat hippocampal atrophy

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ABSTRACT

Objectives: Volume loss within the hippocampus is known as the most replicated finding of structural brain imaging studies of neuropsychiatric diseases. Although voxel-based auto or semi-auto volumetric measurements are widely used in the determination of the human hippocampus, the detection of hippocampal atrophy in rats is still a dilemma as it relies on a relatively primitive and complex approach. In this study, we aimed to develop a convenient way to measure the atrophy of the hippocampus in rats.

Methods: Twenty-four male Wistar rats were exposed to chronic unpredictable mild stress (CUMS) and a wheel running test (WRT) to simulate the conditions of hippocampal volume atrophy and improvement. The hippocampal volume and hippocampal fissure (HiF) width were dynamically measured using 7 T structural magnetic resonance imaging (MRI) with the grayscale method at week 0, 2, 4, and 8. The changes in the hippocampal volume and HiF width in rats were compared. In addition, hematoxylin-eosin (HE) staining of the HiF was used to verify the MRI findings.

Results: The hippocampal volume and the HiF width presented opposite trends based on the MRI findings and the histology data. The atrophy of the hippocampal subfields was closely related to the corresponding increase in the HiF width.

Conclusion: Determination of the HiF width may serve as a sensitive and convenient indicator of rat hippocampal atrophy.

1. Introduction

The hippocampus is associated with several cognitive functions, such as visuospatial memory, visuospatial orientation, crossmodal sensory integration, information consolidation, and attention (Epstein and Kanwisher, 1998; Iaria et al., 2003; Laroche et al., 2000). To date, volume loss within the hippocampus is known as the most replicated finding of structural brain imaging studies of neuropsychiatric diseases (Han et al., 2016). Quantitative measurements of hippocampal volume using high-resolution MRI provide histologic and clinical information, which have been confirmed by previous MRI studies (Malmgren and Thom, 2012; O'Doherty et al., 2015; Pardoe et al., 2009; Scorzin et al., 2008). The voxel-based auto or semi-auto volumetric methods have been widely used in these studies. Unfortunately, the detection of hippocampal atrophy in rats still relies on a relatively primitive and complicated approach, despite advances in the design of MRI magnets with high field-strength and progress in image analysis software (Rao et al., 2011). One obvious deficiency is the confusion surrounding the anatomical hippocampal boundary in MRI. Unlike the human hippocampus, the hippocampal structure of rats is smaller, and it is difficult to identify the anatomical landmarks. Another typical deficiency is the complex calculation method, which is similar to the calculus principle. Moreover, the method of layer by layer summation used in MRI might lead to some inevitable mathematical mistakes (Luo et al., 2014).

Abbreviations: PHFs, perihippocampal fissures; HiF, hippocampal fissure; AD, Alzheimer disease; CSF, cerebrospinal fluid; NPH, normal pressure hydrocephalus; CUMS, chronic unpredictable mild stress; WRT, wheel running test; MRI, magnetic resonance image; FST, forced swim test; FDR, false discovery rate; ANOVA, one-way analysis of variance

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Several pathologic conditions [e.g., Alzheimer disease (AD), depression and epilepsy] manifest as atrophic changes in the structures of the hippocampus and consequently, as dilatation of the fissures of the mesial temporal lobe, which could collectively be described as the perihippocampal fissures (PHFs) because they surround the hippocampus (Li et al., 2006). The PHFs include the lateral part of the transverse fissure, the choroidal fissure, and the hippocampal fissure (HiF) (Sasaki et al., 1993). Several studies have reported that atrophy of the hippocampus and the resultant enlargement of the PHFs were useful radiologic markers for the diagnosis of AD using CT or MR examinations. For example, de Leon et al. (1993) reported that dilatation of the perihippocampal cerebrospinal fluid (CSF) and the associated hippocampal atrophy could serve as predictors of the development of AD in nondemented individuals. In addition, Golomb et al. (1994) reported that the hippocampal volume was preserved in nondemented patients with normal pressure hydrocephalus (NPH), and dilated PHFs and atrophic hippocampi were observed in patients with NPH and concurrent dementia. These results suggested that hippocampal atrophy and the associated dilatation volume of the PHFs could be evaluated on routine MR images. Currently, dilation of PHFs appears to be a sensitive and specific marker for hippocampal atrophy (Holodny et al., 1998), but the method of volume calculation shares the same shortcomings as the hippocampal volume measurement. Therefore, it is urgent to develop a more convenient and accurate method to measure hippocampal atrophy. The aim of this study was to describe whether increased HiF width could be used as a potential surrogate marker for hippocampal atrophy in rats.

2. Materials and methods

2.1. Animals

Twenty-four male Wistar rats (170–200 g) were purchased from the SLAC Animal Center (Shanghai, China). The animals were housed independently in standard Plexiglas cages under a normal 12 h/12 h light/dark cycle at a temperature of 22 ± 2 °C with a humidity of 55 ± 5%. The animals were allowed to adapt to the housing conditions for 1 week prior to the experiments. The animals were given free access to standard chow and water. All the study procedures were approved by the Ethical Committee of Jiangsu University.

2.2. Chronic unpredictable mild stress (CUMS)

CUMS was used to simulate the hippocampal atrophy in a stress state according to a previous study with slight modifications (Yi et al., 2014). Briefly, rats in the CUMS groups were exposed to various stressors, including food and water deprivation for 24 h, cold swimming at 6 °C for 5 min, a tail pinch at the position 1 cm from the end of tail for 1 min, physical restraint for 2 h, exposure to cat odor (removal of the cage containing the experimental rats from the procedure room and placing the experimental rats into cages in which cats had been held) for 1 h, and overnight illumination. Each stressor was randomly implemented every day and repeated throughout the 4 weeks of the experiments. The animals in the control group were housed in a separate room with no contact with the stressed rats.

2.3. Wheel running test (WRT)

Four weeks after the CUMS, the remaining rats were subjected to the WRT. The WRT was used to simulate the anti-stress effect of somatic movement. The wheel running test was based on the method developed by Sierakowiak et al. (2014). During the test, the rats were placed in a running wheel with a diameter of 35 cm for 1 h, at 9:00 am and 3:00 pm. The running wheel was driven by a motor and forced the rats to run around.

2.4. Depression-like behavioral tests (open field exploration)

The movement of the rats was tracked using Mouse Lab Tracker software during the habituation phase of the object displacement task, which resembles an open field exploration task. Open field lighting was provided by artificial illumination under a normal 12 h/12 h light (4 efficient lightbulbs 200 lx)/dark (infrared lamp) schedule. The light in the gray area was equal to that in the outer white areas. The time spent and the path travelled in the center region, defined as a circular region with the center being the center of the arena and with its radius being 1/3 the radius of the arena (15 cm), was calculated for further analysis.

2.5. MRI acquisition

MRI scan was performed at week 0, 2, 4 and 8. The rats were anesthetized using urethane (1.25 g/kg), followed by MRI data collection using a 7 T Bruker PharmaScan system (Bruker Biospin, Ettlingen, Germany) with a 38-mm-diameter bird-cage coil. T2-weighted images (T2WIs) highly sensitive to water were suitable for detecting the HiF. Subsequently, structure T2WIs were obtained using a 2D-RARE sequence with the following parameters: TR = 4600 ms, TE = 30 ms, RARE factor = 4, SBW = 100 kHz, flip angle = 90°, FOV = 32 × 32 mm^2, matrix size = 256 × 256, pixel size = 125 × 125 μm^2, number of slices = 34, slice thickness = 0.3 mm, slice gap = 0 mm, and number of repetitions = 10. Total imaging time was approximately 45 min.

2.6. Total hippocampal and subfields volumetric measurements

The total hippocampus was divided into four subfields including CA1, CA3, DG and the subiculum. Some anatomical landmarks (e.g., the lateral ventricle, the hippocampus and the ring pool) can be used as the boundary of the hippocampus and its subfields. The volume of the entire hippocampus and its subfields was calculated using Mimics 15.0 software (http://www.materialise.com). Accounting for variations in brain sizes, the hippocampal volume was normalized to the intra-cranial volume (ICV) that was extracted based on the Pulse Coupled Neural Network according to a previous description (Chou et al., 2011).

2.7. Detection of HiF width based on grayscale difference method

The hippocampal MRI images were imported into the ImageJ software. The image type was converted to 8 bits. The straight detection line perpendicular to the HiF was drawn on the MRI layer that showed the whole picture of the HiF, and the corresponding gray trough type curve was obtained. If the detection line crossed the DG, HiF and the CA1, the mean gray values (MGVs) of the DG and the CA1 were used as the starting point and the end point, respectively. The distance between the two points serving as the width of the HiF in the trough type curve was marked as $d_{DG\text{-}CA1}$. Similarly, if the detection line crossed the CA3, HiF and the CA1, the distance was marked as $d_{CA3\text{-}CA1}$. The final value (d_{HF}) was defined as the average of the $d_{dg\text{-}CA1}$ and the $d_{CA3\text{-}CA1}$ obtained from at least three measurements. The standardized method of the HiF width was the same as the calculation method of hippocampal volume (Fig. 1). The tests were performed at least in triplicate.

2.8. HE staining

After magnetic resonance scanning, six rats were sacrificed every 2 weeks to obtain their hippocampi, which were then fixed in 10% formaldehyde and prepared for pathological sections and HE staining. The hippocampal samples were cut into 20 μm coronal sections and observed under a microscope with an analog color camera (Zeiss Axiovert 135 and AxioVision 4.4, Sony CCD Camera). The HE staining results were then qualitatively compared with the MRI images.