

Dynamic study of the hippocampal volume by structural MRI in a rat model of depression

Yifeng Luo · Zhihong Cao · Dongqing Wang ·
Liwei Wu · Yuefeng Li · Weibin Sun ·
Yan Zhu

Received: 18 December 2013 / Accepted: 13 May 2014 / Published online: 15 June 2014
© Springer-Verlag Italia 2014

Abstract The reduction of hippocampal volume remains controversial in depression because of the variability among individuals in clinical studies. Here, a reliable experimental rat model of depression, established by chronic unpredictable mild stress (CUMS), was used. Thirty rats were randomly divided into two groups (CUMS group and control group). Hippocampal volume was dynamically measured every 2 weeks in a 56-day chronic stress procedure using structural magnetic resonance imaging, and the correlation between the hippocampal volume and the learning and memory changes was investigated. Our results demonstrated that CUMS rats showed significantly smaller volumes of the bilateral hippocampus compared to that of the controls, changing dramatically with the development of CUMS procedure. The left hippocampal volume was reduced earlier and more markedly than the right one from the 2nd week to the 8th week of the CUMS procedure (on the 8th week: left: approximately 15.3 %; right: approximately 8.4 % reduction). Additionally, the hippocampal volume of CUMS rats was significantly negatively correlated with the learning and memory changes. Of note, it showed that the more obviously the hippocampal volume reduced, the more severely the learning and memory damaged. In conclusion, the hippocampal volume decreased gradually and dynamically and

was correlated with the impairment of the learning and memory in depression.

Keywords Magnetic resonance imaging · Hippocampus · Volume · Rat model · Depression

Introduction

The hippocampus is a functionally complex brain area that has been extensively studied with regard to depression. Previously, it has been revealed that the hippocampus is particularly sensitive to stress, which will induce functional and morphological alterations in hippocampus [1, 2]. In addition, as an important structure involved in the processing of cognition and emotion, the hippocampus is presumed to play a very important role in learning and memory in both animals and humans [3, 4].

To date, because some factors, such as depressive burden, treatment history, age at onset of depression and heterogeneous causes of depression, cannot be taken into account equally and perfectly between groups in clinical studies, reports from volumetric magnetic resonance imaging (MRI) studies of hippocampal volume have yielded conflicting results [5]. Furthermore, it is difficult to do long time follow-up study persistently to patients and the possibility for mechanistic investigation in depressive patients is limited by ethical reasons. Therefore, in order to improve the understanding of the neurobiological processes that induce depression and depression–cognition interactions, animal studies are really indispensable and critical. There was a reliable experimental rat model of depression that was commonly used [6–8]. The rat model which was initially described by Willner [9] and established by a regimen of chronic unpredictable mild stress (CUMS), not

Y. Luo · Z. Cao (✉) · L. Wu
Department of Radiology, The Affiliated Yixing Hospital
of Jiangsu University, 75 Tongzhenguan Road,
Wuxi 214200, Jiangsu, China
e-mail: luoyifeng1207@163.com

D. Wang · Y. Li · W. Sun · Y. Zhu
Department of Medical Imaging, The Affiliated Hospital
of Jiangsu University, Zhenjiang, China

only can mimic daily hassles and stress levels in humans, but also can imitate a core symptom and hallmark of depression—anhedonia, which is a fundamental feature of clinical depression. A former study by Xi et al. [10], however, reported that the volume of hippocampus either in the left or in the right side was not remarkably influenced by 6 weeks of CUMS procedure in a rat model of depression. In fact, we did not know the time point when the hippocampal volume began to decrease significantly, and maybe the volume was reduced in 8 or 10 weeks of CUMS procedure. Thus, a study of the rat model with longer CUMS procedure was necessary. In addition, to our knowledge, no previous study has dynamically investigated the hippocampal volume at multiple time points of the CUMS procedure in the rat model, and ours may provide a better understanding of the hippocampal volumetric change in depression.

Thus, in this study, we used the noninvasive techniques of structural MRI to dynamically compare the hippocampal volume every 2 weeks in a 56-day CUMS procedure and investigated the correlation between the hippocampal volume and the learning and memory changes in the rat model.

Materials and methods

Animals

A total of 30 adult males Sprague–Dawley rats (180–200 g) were housed with free access to food and water. All rats were kept in a conventional animal facility (12:12-h light/dark cycle with lights on at 7 a.m., 21 ± 2 °C, 55–65 % humidity) to take a sucrose preference test (SPT), forced swim test (FST), the Morris water maze (MWM) test and to measure hippocampal volume before and after CUMS procedure. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Animal Care and Use Committee of our university.

Experimental procedure

After 2 weeks of acclimation, 30 rats were randomly divided into 2 groups: control group ($n = 15$) and CUMS group ($n = 15$). The rats of CUMS group were subjected to isolation housing with CUMS procedure, while the ones of control group were housed four per cage without CUMS. The SPT, FST, MWM test and MRI scans were carried out at 5 time points: before stress (0 week as the baseline) and every 2 weeks of CUMS procedure.

CUMS procedure

The CUMS procedure began 1 day after the baseline data acquired, which included 7 different stressors: 5-min swimming in water at 4 °C, 17 h of 45° cage tilt, overnight illumination, 1-min tail pinch, 23-h food and water deprivation, 21-h wet cage and 2-h immobilisation. The 7 stressors were randomly arranged day and night in 2 weeks and were repeated across 56 consecutive days.

Sucrose preference test

Sucrose preference test was used to evaluate the anhedonia. After 23 h of food and water deprivation, the rats were presented simultaneously with 2 bottles for 1 h, one containing sucrose (1 %), the other water. The positions of the 2 bottles (right/left) were varied randomly and were reversed after 30 min. The sucrose preference was calculated according to the following formula:

$$\text{sucrose preference (\%)} = \left[\frac{\text{sucrose intake (g)}}{\text{sucrose intake (g)} + \text{water intake (g)}} \right] \times 100 \%$$

Forced swim test

Forced swim test was performed to measure the immobility time that was considered an indicator of behavioral despair and depressive-like behavior [11]. In the test, the rats were placed in a cylindrical glass container (40 cm height, 30 cm diameter) with tap water (25 ± 1 °C) of 28 cm depth for 15 min; 24 h later, the rats were subjected to a 5-min swimming test. Periods of time when a rat was passively floating in the water, exerting minimal activity except respiration, was measured as immobility.

Morris water maze test

The MWM test was used to examine the spatial learning and memory. The MWM apparatus consisted of a black circular pool filled with opaque water at 23 ± 1 °C, and an escape platform was placed 1 cm under the water surface in the middle of one of the quadrants.

In the acquisition trials, the rats were trained for 90 s per trial and 4 trials per day from a different starting point in a random order with 30-min intervals. If the rat was unable to escape during the 90-s trial period, it was then physically guided to the platform and was recorded 90 s. Before the CUMS procedure, 4 consecutive days of trials were used. The trials on the previous 3 days were for adaption, and the escape latency in the 4th day was get as the baseline. Every 2 weeks of the CUMS procedure, 4 consecutive days of trials were repeated and the escape latency in the 4th day was measured.

MRI acquisition

A 3.0 T MRI scanner (SIEMENS, Germany) with a 50-mm rat brain quadrature coil for radiofrequency reception was used for data acquisition. Rats were anesthetised with isoflurane/O₂ (3 % for induction and 1.5–2 % for maintenance). During scanning, to minimize motion artifacts, the animal's head was fixed in a custom-made head holder. A moderate spin-echo pulse sequence was used for acquisition of the images with the following parameters: repetition time (TR) = 3,200 ms, echo time (TE) = 110 ms, number of average = 6, number of slices = 23, slice thickness = 0.9 mm, no gap, field of view = 8.5 cm × 4.5 cm, matrix size = 384 × 384. First, a sagittal scout image was taken to control for proper image alignment. The 23 coronal sections used for the volumetric analyses were taken perpendicular to a line connecting the superior end of the olfactory bulb with the superior end of the cerebellum (Fig. 1a).

Hippocampal volumetric measurements

Two experienced tracers who were “blind” to the group assignments outlined the regions of interest (ROI) of the hippocampus manually using OsiriX (<http://www.osirix-viewer.com>) on 6 coronal slices according to Wolf et al. [12]. Firstly, the DICOM images were imported to the OsiriX, and then in the viewer windows, the tool called “closed Polygons” in the ROI pull down menu was chosen to outline the hippocampus. The closed Polygons was overlaid on the original two-dimensional images and manually modified to closely match the hippocampus. Figure 1b–d includes representative MRI images showing the hippocampus. To account for variations in brain size, hippocampal volume was normalized to intra-cranial volume (ICV), which was extracted based on the PCNN (Pulse Coupled Neural Network) method. The total hippocampal volume was computed by the summing size of the target areas multiplied by slice thickness, and the average of the normalized hippocampus volume from the two tracers was

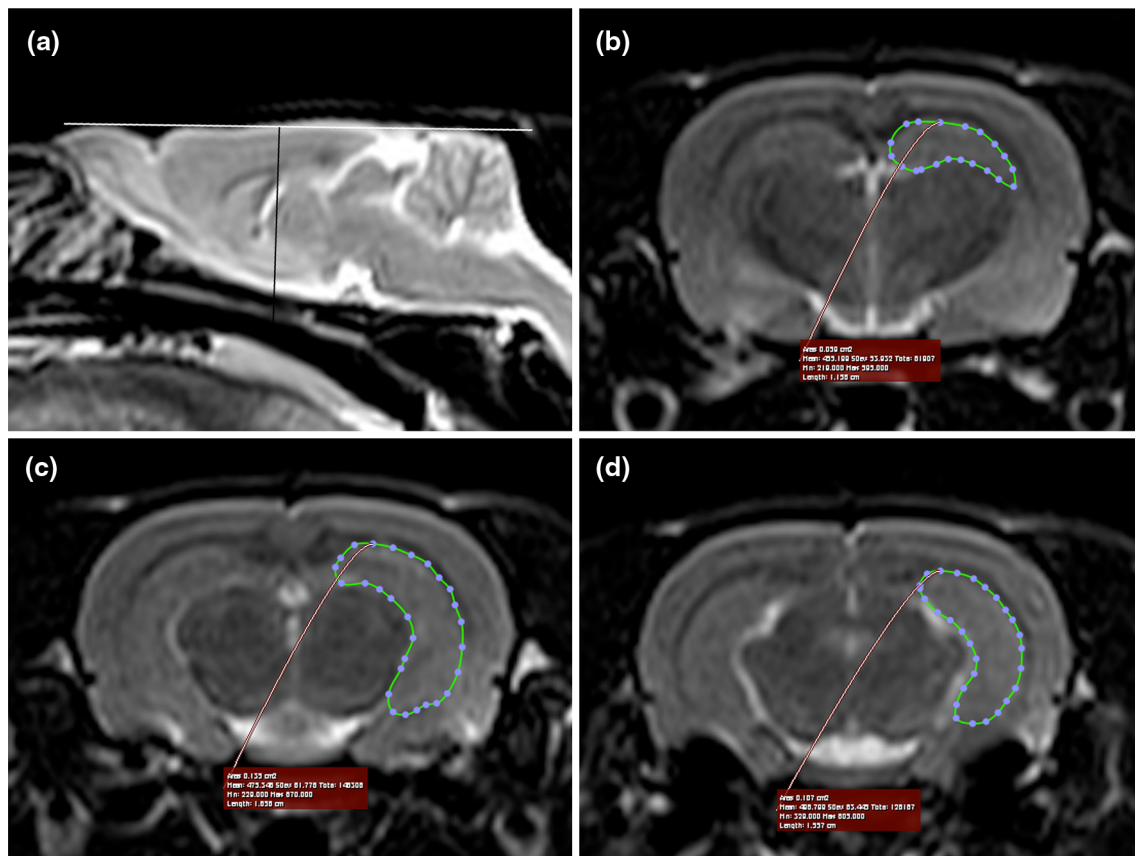


Fig. 1 MRI acquisition for hippocampal volumetric analyses. **a** Resliced sagittal image indicating the line connecting the superior end of the olfactory bulb with the superior end of the cerebellum (white line). A line perpendicular to this one (black line) was used to orient

the coronal image acquisition creating the images used for the volumetric analysis. **b–d** Three representative coronal sections from the MRI brain images

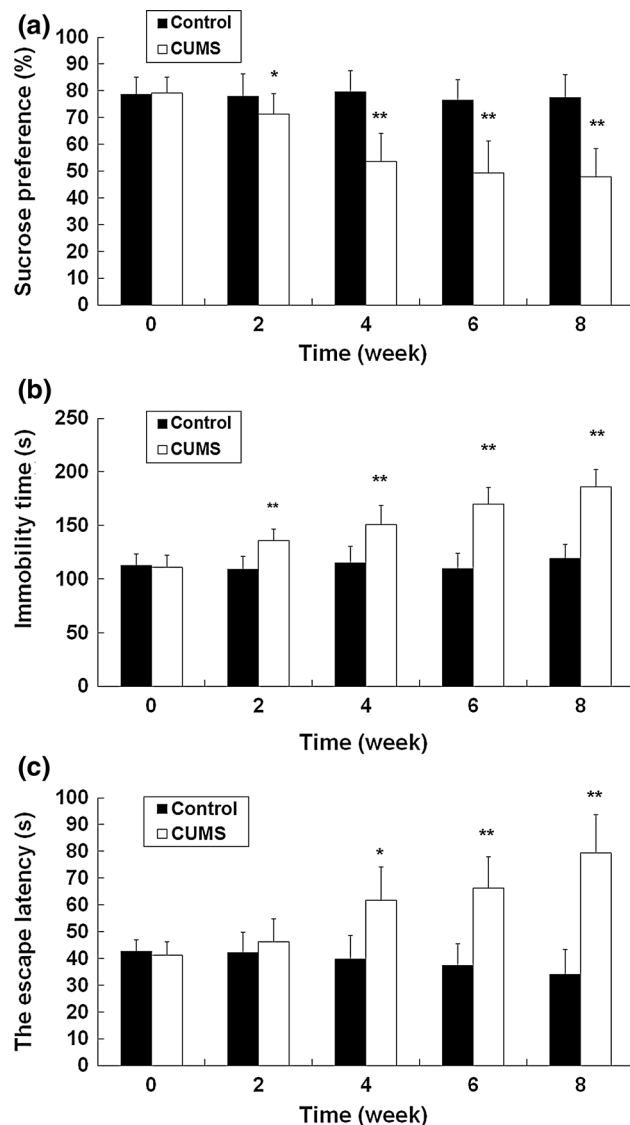


Fig. 2 Effects of CUMS on the sucrose preference, the forced swim time and the escape latency. **a** CUMS rats showed a decrease in the sucrose preference compared with control rats. **b** CUMS rats showed an increase in the immobility time compared with control rats. **c** CUMS rats displayed an increase of the escape latency. All the data are displayed as mean \pm SEM. * $P < 0.05$ and ** $P < 0.01$

used to be analyzed between the groups. The percentage change of hippocampal volume was calculated from the following equation [2]: % change = (poststress volume – baseline volume)/baseline volume \times 100. Inter-rater reliability was calculated using intra-class correlation coefficients (ICC), and test–retest reliability for a single rater (intra-rater reliability) was calculated using the same statistic with a 4-week interval between the measurements.

Statistical analysis

Data were analyzed using SPSS version 17 (SPSS Inc., Chicago, Illinois, USA). Values were presented as

mean \pm SD. The SPT, FST, MWM test and bilateral hippocampal volumes were analyzed by one-sample t test. Pearson correlations between the escape latency in the MWM test and the bilateral hippocampal volumes were performed. Data were considered statistically significant if $P < 0.05$.

Result

Effects of CUMS in SPT, FST and MWM

At the beginning of CUMS procedure, there was no remarkable difference between the 2 groups of the sucrose preference in SPT ($t = 0.18$, $P > 0.05$), the immobility time in FST ($t = 0.45$, $P > 0.05$) and the escape latency in MWM ($t = 0.84$, $P > 0.05$). After 2 weeks of CUMS procedure, CUMS group showed a significant decrease in sucrose preference ($t = 2.21$, $P < 0.05$) and a remarkable increase in immobility time ($t = 6.28$, $P < 0.01$). After 4 weeks, CUMS group indicated a marked increase in the escape latency ($t = 5.51$, $P < 0.01$). With the development of CUMS procedure, the significance of the sucrose preference, the immobility time and the escape latency between the 2 groups was more prominent, which meant the worse of the anhedonia, the despair and the damage of the spatial learning and memory of the model rats (Fig. 2).

The hippocampal volumetric changes

Inter-rater (intra-rater) intra-class correlations were 0.91 (0.95) for the left hippocampus and 0.92 (0.96) for the right one, which meant that our measurement was highly reliable.

From the 2nd week, the left hippocampal volume of CUMS group began to decrease, and on the 4th week, it was significantly reduced ($t = 2.36$, $P < 0.05$), while the right one began to reduce. On the 6th week, the right hippocampal volume was decreased significantly ($t = 2.07$, $P < 0.05$), while the left was reduced more (Table 1). Overall, CUMS group showed a remarkable loss in hippocampal volume from prestress to poststress (Fig. 3; left: approximately 15.3 %; right: approximately 8.4 % reduction on the 8th week).

The correlation between the escape latency and the hippocampal volume

Considering that the hippocampal neuropathologic and functional alteration was induced by CUMS, the correlation between the bilateral hippocampal volumes and the escape latency in MWM was performed. A significant negative correlation between the left hippocampal volume

Table 1 Effects of CUMS on the bilateral hippocampal volume (mm^3 , $\bar{x} \pm s$)

Group	LHV (W)				RHV (W)				
	0	2	4	8	0	2	4	8	
Control	39.67 \pm 1.84	39.42 \pm 2.08	39.05 \pm 2.05	39.56 \pm 2.23	40.06 \pm 2.38	38.92 \pm 1.95	39.36 \pm 2.02	39.21 \pm 2.29	39.44 \pm 2.10
CUMS	39.49 \pm 2.01	39.01 \pm 2.12	37.13 \pm 2.40*	35.88 \pm 2.35**	33.43 \pm 2.43**	39.10 \pm 2.08	39.07 \pm 2.37	38.45 \pm 2.47	37.85 \pm 2.11*

Data presented as mean \pm SEM

LHV left hippocampal volume, RHV right hippocampal volume, W week

* $P < 0.05$; ** $P < 0.01$

and the escape latency in MWM, as well as the right one and the escape latency, was observed (left: $r = -0.927$, $P < 0.01$; right: $r = -0.989$, $P < 0.05$).

Discussion

In this study, we indicated that CUMS rats exhibited significantly smaller volumes of the bilateral hippocampus compared to the control ones, changing dramatically and decreasing gradually with the development of 56 consecutive days of CUMS procedure. Furthermore, CUMS procedure had a negative influence on the sucrose preference in SPT, the immobility time in FST and the escape latency in MWM, and the escape latency was associated with the hippocampal volume of the model rats.

The majority of volumetric MRI studies have reported smaller hippocampal volume in major depressive disorder (MDD) patients compared with healthy controls. However, several studies did not show a significant difference [13]. Because of the variability among individuals (e.g., hereditary, lifestyle and medication history), conclusive evidence of lower hippocampal volume in MDD also has been elusive. Studies have reported bilateral [14] and unilateral right [15] and left [16] deficits relative to comparison subjects. Therefore, in this study, a reliable experimental model of depression was used and it was found that the left and right hippocampal volumes of the rats changed dramatically and decreased gradually by the CUMS, which was similar with those groups who observed that stress would cause the loss of hippocampal volume of the rat or primate [2, 17]. Compared with the study by Xi et al. [10] that suggested no remarkable change of hippocampal volume in a rat model of depression, the reasons may include three points. Firstly, the measurement to detect the hippocampal volume was different with each other. The method that was developed in-house in Xi et al. was automated, while in our study, ROI of the hippocampus was manually traced. The automated method may be less prone to human and experimental errors, but in smaller ROIs or complex anatomical structures, the repeatability and accuracy may be inadequate and sometimes it will make mistakes. In the present study, we got two observers who were unaware of the group assignments to trace the outline of the hippocampus. Furthermore, the ICC was used to assess the reliability of our measurement which was found to be high with an intra-rater (inter-rater) reliability of >0.91 . Secondly, the stressors our study chose were seven different kinds in order to make balance of the effect in every 2 weeks of the CUMS procedure, while their study selected 9 ones, and the effect of every stressor may be diverse to the rat model. Thirdly, the separation in our study, namely the isolation housing which was reported that it can make

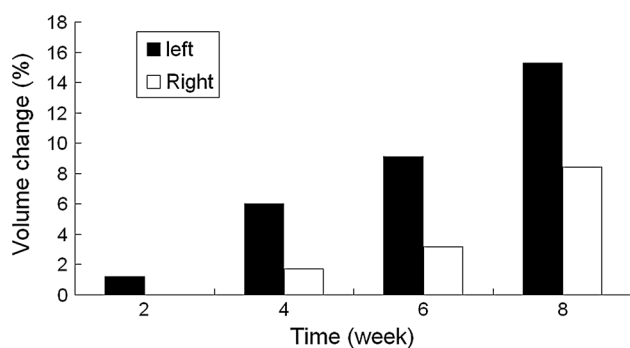


Fig. 3 Hippocampal volumetric reduction with the development of CUMS procedure

the rat to be in depression by itself [18], may make the depressive effect of the CUMS model more prominent than in the study with four rats per cage, which may lead to the atrophy of the hippocampal volume more obviously.

Additionally, this study showed that the reduction of the left hippocampal volume began markedly earlier than the right one, and until the 8th week of CUMS procedure, the reduction ratio of the left hippocampal volume was higher than the right (left: approximately 15.3 %; right: approximately 8.4 %), which was partly consistent with the study that reported a remarkable reduction of 15 % in the left hippocampal volume, and 12 % in the right one in MDD [19]. However, when longer CUMS procedure is taken, it remains uncertain of the long-term effects on the bilateral hippocampal volumes.

Brain asymmetry and lateralization have been observed at the structural, functional and biochemical level, and the right–left asymmetry in hippocampus was known to be present in humans and rodents [20–22]. It was reported that the expression of protein molecules between the left and right hippocampus was different in rat using proteomic analysis [23], and the markedly increased high-affinity choline uptake system activity was found in the left hippocampus compared to the right one of adult male rat [22]. Additionally, it was showed that chronic stress could reduce noradrenaline stores in the rat hippocampus with a right–left molecular asymmetry [24]. Therefore, the lateralization in our study that the left hippocampal volume was reduced earlier and more markedly than the right one may be due to the biochemical asymmetry and the CUMS procedure aggravated the asymmetry, and maybe the left hippocampus was more sensitive to the alterations and finally the loss of the left hippocampal volume was significant than the right; however, the underlying mechanisms of the lateralization of hippocampus remain to be further investigated to make them clear.

The possible mechanisms of the hippocampal volume's atrophy induced by CUMS may involve the dysfunction of the hypothalamic–pituitary–adrenal (HPA) axis and an

elevation of the glucocorticoid level which can be provoked by stressors in patients and CUMS rats [7, 25, 26], and it was established that excessive glucocorticoid had adverse morphologic effects, particularly on the hippocampus, including impairing neurogenesis, initiating dendritic atrophy, synaptic loss, even cell apoptosis, which finally would cause the reduction of hippocampal volume [27].

Excessive glucocorticoid also can disrupt the learning and memory [25], and the present study showed that the escape latency of the model rats was longer than that of the controls, which meant a deficit of spatial learning and memory in the model rats, and the finding was consistent with the previous studies [28, 29]. Furthermore, our study demonstrated that the escape latency of CUMS rats was negatively associated with the hippocampal volume. From the acquired data, it indicated that the more obviously the hippocampal volume reduced, the more markedly the escape latency increased, which suggested that the learning and memory was damaged more severely.

In previous studies, it was known that hippocampus was critical to the learning and memory [30, 31]. Sousa et al. [32] reported that the fine structural alterations in the various fields of the hippocampal formation in rat with a chronic unpredictable stress paradigm were accompanied with impairments in spatial learning and memory, and the study of Yin et al. [33] showed that the deficits in spatial learning and memory was associated with hippocampal volume loss in aged apolipoprotein E4 mice. Combined with the present study, it was suggested that the reduction in the hippocampal volume would lead to the impairment of learning and memory.

Future studies will be needed to further examine the changes of hippocampal histopathology and the glucocorticoid in serum correlation of hippocampal volume reduction of the rat model, analyzed dynamically for a longer time, and to investigate additional regions of the brain that are involved in depression using more sensitive measurement of MRI.

Conclusion

Our study demonstrated that the atrophy of the bilateral hippocampal volume was observed in the rat model of depression by dynamic measurements. In addition, the left hippocampal volume was reduced earlier and more remarkably than the right one, with the learning and memory impaired in CUMS rats.

Acknowledgments This study was supported by projects of Jiangsu Province Health Department (No. H200951); projects of Wuxi Municipal Technology Bureau (No. CSEWIN1111); projects of Wuxi Health Bureau (No. ML201320). We would like to thank Prof. Wang

Jichen at Nanjing Medical University and Ma Tieliang M.D at Yixing People hospital for their valuable help.

References

- Duman RS (2009) Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. *Dialogues Clin Neurosci* 11:239–255
- Lee T, Jarome T, Li SJ et al (2009) Chronic stress selectively reduces hippocampal volume in rats: a longitudinal magnetic resonance imaging study. *NeuroReport* 20:1554–1558
- Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. *Neuron* 44:109–120
- Olton DS, Paras BC (2001) Spatial memory and hippocampal function. *Neuropsychologia* 17:669–682
- Koolschijn P, van Haren NE, Lensvelt Mulders GJ et al (2009) Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp* 30:3719–3735
- Segev A, Rubin AS, Abush H et al (2014) Cannabinoid receptor activation prevents the effects of chronic mild stress on emotional learning and LTP in a rat model of depression. *Neuropsychopharmacology* 39:919–933
- Xing Y, He J, Hou J et al (2013) Gender differences in CMS and the effects of antidepressant venlafaxine in rats. *Neurochem Int* 63:570–575
- Willner P (1997) Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology* 134:319–329
- Willner P, Towell A, Sampson D et al (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology* 93:358–364
- Xi G, Hui J, Zhang Z et al (2011) Learning and memory alterations are associated with hippocampal N-acetylaspartate in a rat model of depression as measured by 1H-MRS. *PLoS ONE* 6:e28686
- Porsolt RD, Le PM, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* 266:730–732
- Wolf OT, Dyakin V, Vadasz C et al (2002) Volumetric measurement of the hippocampus, the anterior cingulate cortex, and the retrosplenial granular cortex of the rat using structural MRI. *Brain Res Protoc* 10:41–46
- Posener JA, Wang L, Price JL et al (2003) High-dimensional mapping of the hippocampus in depression. *Am J Psychiatr* 160:83–89
- Sheline YI, Wang PW, Gado MH et al (1996) Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci USA* 93:3908–3913
- Steffens DC, Byrum CE, McQuoid DR et al (2000) Hippocampal volume in geriatric depression. *Biol Psychiatry* 48:301–309
- Bremner JD, Narayan M, Anderson ER et al (2000) Hippocampal volume reduction in major depression. *Am J Psychiatr* 157:115–118
- Uno H, Tarara R, Else JG et al (1989) Hippocampal damage associated with prolonged and fatal stress in primates. *J Neurosci* 9:1705–1711
- Park J, Lee S, Oh C (2013) Treadmill exercise exerts ameliorating effect on isolation-induced depression via neuronal activation. *J Exerc Rehabil* 9:234–242
- Sapolsky RM (2000) Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Arch Gen Psychiatry* 57:925–935
- Křištofiková Z, Kozmiková I, Hovorková P et al (2008) Lateralization of hippocampal nitric oxide mediator system in people with Alzheimer disease, multi-infarct dementia and schizophrenia. *Neurochem Int* 53:118–125
- Pruessner JC, Li LM, Serles W et al (2000) Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. *Cereb Cortex* 10:433–442
- Křištofiková Z, Št' Astný F, Bubeníková V et al (2004) Age- and sex-dependent laterality of rat hippocampal cholinergic system in relation to animal models of neurodevelopmental and neurodegenerative disorders. *Neurochem Res* 29:671–680
- Samara A, Vougas K, Papadopoulou A et al (2011) Proteomics reveal rat hippocampal lateral asymmetry. *Hippocampus* 21:108–119
- Spasojevic N, Jovanovic P, Dronjak S (2013) Molecular basis of chronic stress-induced hippocampal lateral asymmetry in rats and impact on learning and memory. *Acta Physiol Hung* 100:388–394
- McEwen BS, Sapolsky RM (1995) Stress and cognitive function. *Curr Opin Neurobiol* 5:205–216
- de Kloet ER, Joels M, Holsboer F (2005) Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 6:463–475
- Sousa N, Lukoyanov NV, Madeira MD (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 97:253–266
- Song L, Che W, Min-Wei W et al (2006) Impairment of the spatial learning and memory induced by learned helplessness and chronic mild stress. *Pharmacol Biochem Behav* 83:186–193
- Sun M, Alkon DL (2004) Induced depressive behavior impairs learning and memory in rats. *Neuroscience* 129:129–139
- Jarrard LE (1993) On the role of the hippocampus in learning and memory in the rat. *Behav Neural Biol* 60:9–26
- Squire LR (1992) Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychol Rev* 99:195–231
- Sousa N, Lukoyanov NV, Madeira MD et al (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 97:253–266
- Yin J, Turner GH, Lin H et al (2011) Deficits in spatial learning and memory is associated with hippocampal volume loss in aged apolipoprotein E4 mice. *J Alzheimers Dis* 27:89–98