

Chronic Fluoxetine Enhances Extinction Therapy for PTSD by Evaluating Brain Glucose Metabolism in Rats: An [^{18}F]FDG PET Study

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Abstract

Recent studies suggest that selective serotonin reuptake inhibitors (SSRIs) and exposure therapies have been used to reduced footshock-induced posttraumatic stress disorder (PTSD) symptoms. However, the therapeutic effect of the combination of SSRIs treatment with exposure therapy remains a matter of debate. This study aimed to evaluate these therapeutic effect through the behavioral and the neuroimaging changes by positron emission tomography (PET) in model rats. We adopted a Pavlovian fear conditioning paradigm to establish model rats, and serial [^{18}F]fluorodeoxyglucose (FDG) PET imaging was performed to investigate the neurofunctional changes before and after the therapies (chronic fluoxetine and/or extinction). Behavioral tests indicated combined therapy group performed lower freezing (%) than the two other groups. Compared with the baseline, fear conditioning increased ^{18}F -FDG uptake in the right amygdala but decreased uptake in the left primary somatosensory cortex. After extinction retrieval, there was increased [^{18}F]FDG uptake in the left striatum but decreased uptake in the anterior cingulate cortex in the extinction group. Fluoxetine increased [^{18}F]FDG uptake in the left hippocampus but decreased uptake in the cuneiform nucleus. The combined therapy increased [^{18}F]FDG uptake in the left hippocampus, left striatum, right insular cortex but reduced uptake in the cerebellar lobule. c-Fos expression was significantly higher in the hippocampal dentate gyrus and anterior cingulate cortex in the fluoxetine and combined groups than that in the extinction group, with no significant difference between the two groups. Taken together, chronic fluoxetine enhanced the effects of extinction training in a rat model of PTSD. In vivo PET imaging may provide a promising approach for evaluation chronic fluoxetine treatment of PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric disorder caused by exposure to traumatic events such as natural disasters, combat and sexual abuse. According to a survey, the worldwide lifetime prevalence of PTSD is almost 3.9% (Koenen et al., 2017). The core symptoms of PTSD include persistently re-experiencing the traumatic events, avoiding cues associated with the trauma, and experiencing alterations in cognition or moods and hyperarousal/hypervigilance (Guideline Development Panel for the Treatment of PTSD in Adults, 2019). Due to the variability in these symptoms of PTSD, the evaluation of the effects of treatment is challenging.

For decades, two common treatments, including pharmacological and non-pharmacological therapy, have been used for PTSD. Exposure therapy is considered a representative non-pharmacological treatment for alleviating the symptoms of PTSD (Bisson et al., 2019). Exposure therapy reduces or eliminates a subject's fear response by repeatedly exposing the subject to an environment that includes the fear-inducing stimuli. Behavioural extinction training for fear in animals has neurobiological mechanisms similar to those of exposure therapy. Pharmacological therapy for PTSD includes tricyclic antidepressants, monoamine oxidase inhibitors and selective serotonin reuptake inhibitors (SSRIs), of which SSRIs are considered the first-line medication choice. There are six clinically used SSRIs, namely,

citalopram, escitalopram, sertraline, paroxetine, fluvoxamine and fluoxetine. Fluoxetine has been shown to be effective in the treatment of PTSD (Cipriani et al., 2018). In addition, animal studies of PTSD examining the above treatments have shown good therapeutic effects in recent years (Karpova et al., 2011). However, the number of studies examining combination therapies remain insufficient. The mechanisms underlying exposure or pharmacological therapies have not been fully elucidated.

As an advanced non-invasive molecular imaging technology, positron emission tomography (PET) has allowed the in vivo visualization of metabolic changes in the body and brain. In particular, 2-deoxy-2- $[^{18}\text{F}]$ fluoro-D-glucose ($[^{18}\text{F}]$ FDG) PET has been used to detect changes in glucose metabolism in the brain. Statistical Parametric Mapping (SPM) 8 software was adopted for the quantitative analysis.

The aim of this study was to explore the effects of treatment with chronic fluoxetine and behavioural extinction training in a PTSD rat model. Accordingly, we hypothesized that $[^{18}\text{F}]$ FDG PET is suitable for evaluating changes in glucose metabolism in the brain during the control, fear-conditioning, and extinction-retrieval phases. Neural activity was identified by the expression of c-Fos.

Materials And Methods

Animals

Male Sprague–Dawley rats (Shanghai SLAC Laboratory Animal Co., Ltd.) weighing 250–290 g ($n = 30$) were used. The rats were single housed in plastic cages under standard laboratory conditions (12 h light/dark cycle) at a temperature of $22 \pm 2^\circ\text{C}$ and relative humidity of $55 \pm 5\%$. The rats were provided ad libitum access to food and water. All animals and the experimental protocol were approved by the Institutional Animal Care and Use Committee of Zhejiang University.

Behavioural Apparatus

In this study, we adopted a Pavlovian fear conditioning paradigm to establish a rat model of PTSD. The animals were repeatedly exposed to an environment in which a neutral tone (as the conditional stimulus, CS) was associated with an unavoidable foot shock (as the unconditional stimulus, US). The animals showed PTSD-like symptoms and fear memories.

Two standard experimental chambers (50 cm wide \times 60 cm long \times 60 cm high) provided by Anlai Software Technology Co., LTD were used for the fear conditioning (designated Context B) and extinction training (designated Context C).

The chambers were enclosed in separate soundproof boxes. The CS was a neutral tone (75 dB, 30 s) emitted by a loudspeaker located on the top of the chambers, and the US was an electrical foot shock (0.8 mA, 1 s) produced by a stainless steel fence at the bottom during the final second of the CS.

Behavioural Procedures

On day 1, all rats were placed in Context A for a 5-minute adaptation period, and then they were exposed to 10 CS presentations at 2-minute intervals. The rats underwent fear conditioning in Context B on day 2. All rats were trained with 10 sets of CS presentations that co-terminated with a US presentation after 5 minutes of adaptation. The rats that successfully established conditioned fear were randomly divided into the following three groups: the extinction training (EXT), fluoxetine (FLX), and extinction training plus fluoxetine (EXT + FLX) groups (n = 10 per group). The rats in the EXT and EXT + FLX groups were exposed to extinction training in the extinction chamber (Context C) from day 15 to day 17. The rats were presented with 20 sets of the CS at 60-s intervals. On day 18, all rats were placed in Context A for 30 minutes to test their extinction memory. Two minutes after the CS in the final behavioural session, the rats were returned to their home cage. Freezing behaviour was characterized by the disappearance of all muscle movements except for breathing. All behaviour of the rats was recorded by a camera on the top of the chambers. In the present study, we performed the behavioural procedures and PET scans during the light cycle.

Drug Treatment

Fluoxetine hydrochloride was dissolved in 0.9% normal saline. The rats in the FLX and EXT + FLX groups were gavaged with fluoxetine at a dose of 10 mg/kg/d, and the rats in the EXT group were gavaged with normal saline for 16 days (days 3–18) after fear conditioning. All rats were weighed daily to accurately calculate the dose of fluoxetine.

MicroPET Scan and Analysis

PET scans were performed on days 1, 2 and 18 (Fig. 1). All 30 rats were fasted overnight before the PET scans. [¹⁸F]FDG, which was used as the radioactive tracer for the PET imaging, was injected intraperitoneally at a dose of (0.91 ± 0.16)mCi into the rats before the behavioural training (Tian et al., 2014). The rats were anesthetized with 5% isoflurane, and then placed in a prone position on the bed of the scanner. 2% isoflurane was used to maintain anesthesia during scanning. The data acquisition was performed using a microPET R4 scanner (Siemens Medical Solutions) for 30 minutes after a 40-minute uptake period of [¹⁸F]FDG. The [¹⁸F]FDG PET image was reconstructed by the maximum a posteriori (MAP) algorithm, which generates 128*128*63 voxels of 0.85*0.85*1.21 mm size. Then, the PET images were processed and analysed by SPM8 software (Institute of Neurology, University College of London, UK) and the improved toolkit for rat brain images. This toolkit contains a rat brain FDG PET template and the rat brain atlas by Paxinos & Watson. Individual PET images were spatially normalized to a custom PET [¹⁸F]FDG template and smoothed with an isotropic Gaussian kernel (2-mm FWHM). Proportional scaling was used for global normalization. The difference in the level of [¹⁸F]FDG in the brain among the groups was compared, the difference in the activation signal in the brain was calculated when the rats were in different behavioural states ($p_{\text{(uncorrected)}} < 0.001$), and more than 50 consecutive voxel aggregation highlights were considered significant differences among the groups.

Immunohistochemistry

After the extinction retrieval, a subset of rats ($n = 5$ per group) was sacrificed for c-Fos immunostaining in specific brain regions where increased or decreased [^{18}F]FDG accumulation was found. For the immunohistological analysis, the animals were perfused through the heart with normal saline and 4% paraformaldehyde (PFA) in PBS, and the brains were removed and postfixed in 4% PFA overnight at 4°C. The brain tissues were processed, embedded in paraffin, and cut into 4- μm thick coronal sections. Haematoxylin and eosin (H&E; Beyotime, Beijing, China) staining was performed according to a standard protocol. The slides were incubated with a polyclonal antibody against c-Fos (1:1000; Santa Cruz Biotechnology) overnight at 4°C, and c-Fos positive cells were counted using ImageJ software (NIH).

Statistical Analysis

All data are presented as the mean \pm SEM. Kolmogorov–Smirnov (KS) test and Levene test are used to check the normality and homoscedasticity of the data. The behavioural data of the control phase and conditioned fear were analysed by a one-way analysis of variance (ANOVA). A two-way repeated-measures ANOVA with Bonferroni correction for multiple comparisons was used to analyse the relevant phase of the study (fear conditioning and extinction retrieval). The data were analysed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA) and statistical significance was set at $p < 0.05$.

Results

Behavioural extinction training combined with chronic fluoxetine promoted the extinction of fear memory

The rats received 10 sets of CS-US presentations in Context B to acquire fear memories. Compared with the freezing (%) in the control phase, the freezing (%) of the rats with conditioned fear memory was significantly increased (control vs. fear: $23.54 \pm 4.83\%$ vs. $69.06 \pm 7.74\%$, $p < 0.05$) (Fig. 2a). The repeated-measures ANOVA revealed that all rats with conditioned fear showed less freezing (%) following the treatment (all $p < 0.05$). In the extinction retrieval phase, the Bonferroni adjustment for multiple comparisons showed that there were significant differences between the EXT+FLX group and the two other groups (EXT group and FLX group) (all $p < 0.05$), but there were no differences between these two groups ($p > 0.05$) (Fig. 2b). These behavioural results demonstrated that extinction training combined with fluoxetine treatment led to lower freezing (%).

Brain glucose utilization increased in the amygdala following fear conditioning

Compared with the level of glucose metabolism in the control phase, the rats exposed to conditioned fear showed increased glucose metabolism in the right amygdala and left primary visual cortex but decreased glucose metabolism in the left primary somatosensory cortex ($p < 0.001$) (Table 1, Fig. 3).

Chronic fluoxetine treatment increased glucose utilization in the left hippocampus and enhanced the effects of behavioural extinction

After the extinction retrieval, the rats in the EXT group presented an increased [^{18}F]FDG uptake in the left striatum, left cochlear nucleus and right primary visual cortex but decreased uptake in the anterior

cingulate cortex ($p < 0.001$) (Table 2, Fig. 4a). [^{18}F]FDG accumulation was significantly increased in the left hippocampus and right primary visual cortex but decreased in the bilateral primary somatosensory cortex, left primary/secondary motor cortex and cuneiform nucleus of the rats in the FLX group ($p < 0.001$) (Table 3, Fig. 4b). At the same time point, the EXT+FLX rats exhibited increased [^{18}F]FDG uptake in the left hippocampus, left striatum, right insular cortex, left posterior parietal cortex and right secondary visual cortex but reduced [^{18}F]FDG uptake in the cerebellar lobule ($p < 0.001$) (Table 4, Fig. 5)

The expression of c-Fos in the anterior cingulate cortex and hippocampal dentate gyrus was increased following the treatment with chronic fluoxetine

After the extinction retrieval, compared with the EXT group, c-Fos expression in the dentate gyrus of the hippocampus and anterior cingulate cortex was significantly increased in the FLX and EXT+FLX groups (all $p < 0.05$), whereas no significant difference was found between the FLX and EXT+FLX groups ($p > 0.05$) (Fig. 6).

Discussion

We mainly found that chronic fluoxetine treatment enhanced the subsequent effects of fear extinction in a PTSD rat model with in vivo PET imaging. The glucose metabolism in the hippocampus was significantly increased after the chronic fluoxetine treatment, suggesting that this region may be a particularly interesting target. To the best of our knowledge, this study is the first to evaluate glucose metabolic changes after chronic fluoxetine treatment for PTSD in a non-invasive way.

PTSD-like behaviours could be induced by a Pavlovian fear conditioning paradigm, while glucose metabolism was increased in the right amygdala and left primary visual cortex but decreased in the left primary somatosensory cortex. However, the chronic fluoxetine treatment and behavioural extinction training could alleviate the PTSD-like symptoms. Our study showed that freezing (%) was lower than before in all three groups. Fluoxetine could enhance the subsequent effects of fear extinction (Pedraza et al., 2019). In the EXT + FLX group, glucose metabolism was increased in the left hippocampus, left striatum, right insular cortex, left posterior parietal cortex and right secondary visual cortex but decreased in the cerebellar lobule after the extinction retrieval. The rats in the EXT group exhibited increased [^{18}F]FDG uptake in the left striatum, left cochlear nucleus and right primary visual cortex but decreased uptake in the anterior cingulate cortex. Glucose metabolism was increased in the left hippocampus and right primary visual cortex but decreased in the bilateral primary somatosensory cortex, left primary/secondary motor cortex and cuneiform nucleus in the FLX group.

The right amygdala was activated after fear conditioning in our study. Fear conditioning has been extensively used as a model of PTSD (Johansen, Cain, Ostroff, & LeDoux, 2011; Parsons & Ressler, 2013). The amygdala is considered the core brain region responsible for fear memory acquisition and storage (Maren & Quirk, 2004). Functional magnetic resonance imaging (fMRI) has shown greater activation of the amygdala in humans exposed to fear-relevant visual stimuli (Garrett et al., 2012). In clinical PET

imaging studies, the amygdala of PTSD patients reacted more strongly than that of the control group when exposed to odours related to prior fear memories (Vermetten, Schmahl, Southwick, & Bremner, 2007). Similar results were also observed in an animal study (Brydges et al., 2013). Our results are consistent with the previous literature.

More importantly, we found that glucose metabolism was increased in the hippocampus in the FLX group and EXT + FLX group but not in the EXT group. The hippocampal volume of PTSD patients is smaller than that of controls based on MRI analyses (Bromis, Calem, Reinders, Williams, & Kempton, 2018). In addition, the reduction in the hippocampal volume is associated with the persistence of symptoms (Chao, Yaffe, Samuelson, & Neylan, 2014) and a poor treatment response in patients with PTSD (Rubin et al., 2016). Multiple animal studies have revealed that chronic fluoxetine treatment leads to changes in the hippocampus (Castren & Hen, 2013; Patricio et al., 2015), including the regeneration and metabolism of neurons, synaptic plasticity, and the complexity of dendritic spines (Kobayashi et al., 2010; Maya Vetencourt et al., 2008). These changes may be the result of some growth factors upregulated by fluoxetine, such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF2) and serotonin (5-HT). As a major emotion regulator, BDNF plays an important role in the pathology of various mental diseases, such as depression (Rogoz, Kaminska, Panczyszyn-Trzewik, & Sowa-Kucma, 2017). Increased BDNF expression levels have been observed in the hippocampus in rodents following chronic fluoxetine treatment (Mendez-David et al., 2015). Acute, unilateral BDNF infusion into the rat dentate gyrus induced the long-term potentiation of medial perforant path-evoked synaptic transmission and, concomitantly, enhanced bilateral hippocampal neurogenesis (Kuipers et al., 2016). Chronic treatment with fluoxetine also increased the expression of FGF2 in hippocampus (Bachis, Mallei, Cruz, Wellstein, & Mochetti, 2008; Evans et al., 2004). In addition, the concentrations of FGF2 were negatively correlated with fear expression in both humans and rats (Graham, Dong, & Richardson, 2018; Graham, Zagic, & Richardson, 2017). Notably, the antidepressant effect of fluoxetine is partially due to an increase in the concentration of FGF2, which in turn promotes nerve regeneration and increases the survival rate of new cells in the hippocampus (Perez, Clinton, Turner, Watson, & Akil, 2009). In addition, the tissue levels of 5-HT were consistently decreased in fear circuit areas (Lin, Tung, & Liu, 2016), but it could be adjusted by treatment with fluoxetine. Fluoxetine increased the extracellular serotonin levels in the brain by blocking the reuptake of serotonin. A study showed that chronic fluoxetine treatment enhanced excitatory synaptic transmission in the hippocampus by slowly elevating serotonin accumulation in vivo (Van Dyke, Francis, Chen, Bailey, & Thompson, 2019). The reduced freezing behaviour was related to increased concentrations of 5-HT in the hippocampus. After chronic treatment with fluoxetine, glucose metabolism in the hippocampus was significantly increased than before, suggesting that the hippocampus is a particular target for PTSD therapy.

In our study, elevated [^{18}F]FDG uptake was found in the left striatum after the extinction training in both the EXT and EXT + FLX groups. The striatum is the terminal field of dopamine (DA) which is related to the reward and motivational processes (Schultz, 2001). Evidence suggests that PTSD involves the reward circuitry in the brain (Enman, Arthur, Ward, Perrine, & Unterwald, 2015). The DA and dopamine transporter

levels, dopamine receptor (DR) density and DA metabolites were decreased in the striatum in a rat model of PTSD (Lee, Shim, Lee, & Hahm, 2018). Emotional numbness is a manifestation of the alteration in moods observed in those with PTSD. An fMRI study revealed a significant negative correlation between emotional numbness and the activation of the right ventral striatum in PTSD patients (Felmington et al., 2014). Moreover, the release of DA in the striatum is involved in putative dopamine signalling during fear extinction (Kobayashi et al., 2010). Behavioural extinction training of fear has neurobiological mechanisms similar to those of exposure therapy. We performed a CS-no-US association that consisted of repeated presentations of the CS without the US to the rats in the EXT group and EXT + FLX group, and the memory of extinction learning was tested on the following day (called extinction retrieval) (Milad, Rosenbaum, & Simon, 2014). The disappearance of an expected US could be considered a reward-like safety signal during extinction training. A rodent study suggested that extinction must be mediated by DA signalling via the D1R (El-Ghundi, O'Dowd, & George, 2001). DR-mediated signal transduction involves metabolic changes in neurons. Consequently, our findings highlight that the increase in glucose metabolism in the left striatum is strongly consistent with the above conclusions.

The glucose metabolism in the right insular cortex was increased in the EXT + FLX group. Only a few studies indicated that the insular cortex participates in the extinction of conditioned fear. The insular cortex is involved in the extinction of conditioned taste aversion (CTA), which is delayed by blocking protein translation (Hadamitzky et al., 2016). The synaptic plasticity of insular neurons could be enhanced by extinction training and BDNF. Acute BDNF infusions into the insular cortex were able to promote the extinction of CTA (Rodriguez-Serrano, Ramirez-Leon, Rodriguez-Duran, & Escobar, 2014). In addition, studies have demonstrated that chronic treatment with fluoxetine had a positive effect on the increase in BDNF. Thus, we speculate that the activation of the insular cortex was the result of the combination of extinction training and chronic fluoxetine treatment.

Interestingly, we observed decreased glucose metabolism in the anterior cingulate cortex only in the EXT group. The anterior cingulate cortex, which belongs to the prefrontal cortex, participates in the dysregulation of emotion control and cognitive function in PTSD (Wisdom et al., 2014; Woodward et al., 2015). Previous clinical studies have shown that the symptom severity and grey matter volume in PTSD patients were negatively correlated with the level of decrease in anterior cingulate cortex activity (Bromis et al., 2018). PTSD is the result of persistent energy metabolism disorders that ultimately lead to chronic low-grade inflammation (Naviaux, 2012). Gene clusters in inflammation pathways following fear conditioning are enriched in the anterior cingulate cortex. Studies have shown that chronic fluoxetine treatment after trauma could reduce PTSD-like symptoms by altering the expression of inflammatory genes in the anterior cingulate cortex (Kao et al., 2016). However, only the EXT group showed metabolism changes in the anterior cingulate cortex in our study. Using c-Fos as a marker of neuronal activation, we confirmed that the expression of c-Fos in the anterior cingulate in the EXT group was decreased. Therefore, the effect of chronic fluoxetine treatment in the anterior cingulate cortex warrants further investigation.

As expected, we found increased glucose metabolism in brain areas involved in the audiovisual pathway, including the visual cortex, cochlear nucleus and posterior parietal cortex. When the rats were transferred from a dark chamber to the bright microPET scanning room, the light stimulation resulted in increased glucose metabolism in the visual cortex. The cochlear nucleus is not only the primary auditory information processing centre but also the only nucleus in the central auditory system (Schofield & Coomes, 2005). In addition, the posterior parietal cortex potentially integrates information from the visual, somatosensory and auditory cortices (Harvey, Coen, & Tank, 2012; Wilber et al., 2014). Thus, unsurprisingly, these brain areas showed increased glucose metabolism. We also observed decreased glucose metabolism in the primary somatosensory cortex, left primary/secondary motor cortex, cerebellar lobule and cuneiform nucleus. Environmental information obtained from whiskers could be sent the barrel field of the somatosensory cortex (Diamond, von Heimendahl, Knutsen, Kleinfeld, & Ahissar, 2008). In addition, the cerebellum receives facial sensory information and participates in cortical sensorimotor integration (Popa et al., 2013; Proville et al., 2014). Moreover, optogenetic stimulation of the dorsal periaqueductal grey increased glucose metabolism in the cuneiform nucleus in a rat model of panic disorder (He et al., 2019). Decreased glucose metabolism in the cuneiform nucleus was observed in our results, which was related to the decrease in freezing in the behavioural test.

Our study found that chronic fluoxetine enhanced the subsequent effects of fear extinction in a PTSD rat model. Our behavioural results were consistent with these findings, providing additional evidence for the evaluation of the treatment efficacy of fluoxetine combined with extinction training for PTSD. Extinction training combined with fluoxetine treatment were more effective in eradicating persistent fear memory (Karpova et al., 2011). Furthermore, [^{18}F]FDG microPET imaging could not only visualize glucose metabolism in various brain areas across different behavioural stages but also help evaluating the effects of fluoxetine and extinction training; Thus, we recommend using this non-invasive in vivo imaging technique for future research concerning PTSD.

Conclusions

Our results suggest that chronic fluoxetine enhanced the effect of extinction training in a rat model of PTSD. In vivo [^{18}F]FDG microPET imaging found increased glucose metabolism in the hippocampus after the chronic fluoxetine treatment.

Declarations

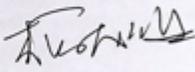
Ethical Approval

浙江大学实验动物福利伦理委员会

研究项目伦理审批件

项目批准号: ZJU20200003

有效期: 4 年

项目名称	创伤后应激障碍中单胺类神经环路的分子影像机理研究	
申请单位	浙江大学医学院附属第二医院	项目负责人: 张莺
		参与人: 吴爽
合作单位	无	
报送材料	<input checked="" type="checkbox"/> 伦理审查申请表 (申请编号: 16521) <input type="checkbox"/> 课题申报书 <input type="checkbox"/> 其它_____	
所用动物	大鼠 (SD): SPF 级, 30 只	
投票结果	参会 14 人, 同意 14 票, 不同意 0 票, 弃权 0 票。 • 是否启动紧急程序: <input type="checkbox"/> 是 <input checked="" type="checkbox"/> 否	
审查结果	<input checked="" type="checkbox"/> 同意 <input type="checkbox"/> 修改后同意 <input type="checkbox"/> 不同意 (项目终止或暂停)	
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Consent to Participate

Not applicable

Consent to Publish

All authors agree to publish this article in the Journal of Brain Imaging and Behavior.

Author contributions

Jing Liu: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization.

Jun Yu: Investigation, Writing - review & editing.

Hong Biao Liu: Conceptualization, Methodology, Investigation, Writing - review & editing.

Qiong Yao: Investigation, Writing - review & editing.

Ying Zhang: Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Conflict of interest

All authors declare no competing financial interests.

Availability of data and materials

All data generated or analysed during this study are included in this article and its supplementary information files.

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Tables

Table 1 Significant Metabolic Changes under Fear Conditioning—Control phase vs. Fear conditioning

Brain region	Coordinate [mm]			Cluster size	<i>T</i> value	<i>Z</i> score	<i>P</i> _[uncorrected]
	x	y	z				
Increased							
Right amygdala	5	8	-4	2002	4.82	3.53	<0.001
Left primary visual cortex	-5	1	-6	927	3.97	3.47	<0.001
Decreased							
Left primary somatosensory cortex	-5	4	3	601	5.33	3.75	<0.001

Table 2 Significant Metabolic Changes under Extinction Retrieval—Control phase vs. Extinction retrieval

Brain region	Coordinate [mm]			Cluster size	<i>T value</i>	<i>Z score</i>	<i>P</i> _[uncorrected]
	x	y	z				
Increased							
Left striatum	-4	7	-1	744	4.90	3.56	<0.001
Left cochlear nucleus	-3	7	-11	1993	6.84	4.29	<0.001
Right secondary visual cortex	2	1	-5	272	4.34	3.3	<0.001
Decreased							
cingulum	0	1	-1	59	4.10	3.18	<0.001

Table 3 Significant Metabolic Changes under Extinction Retrieval [Control phase vs. Extinction retrieval]

Brain region	Coordinate [mm]			Cluster size	<i>T value</i>	<i>Z score</i>	<i>P</i> _[uncorrected]
	x	y	z				
Increased							
Left hipopocampus	-5	3	-6	4839	7.49	4.48	<0.001
Right primary visual cortex	3	1	-6	2700	7.97	4.62	<0.001
Decreased							
Left primary somatosensory cortex	-5	4	3	253	5.33	3.75	<0.001
Right primary somatosensory cortex	5	4	3	483	4.55	3.41	<0.001
Left primary motor cortex	-4	3	4	121	4.16	3.21	<0.001
Left secondary motor cortex	-4	2	5	109	4.33	3.29	<0.001
cuneate nucleus	-2	7	-14	53	4.41	3.33	<0.001

Table 4 Significant Metabolic Changes under Extinction Retrieval [Control phase vs. Extinction retrieval]

Brain region	Coordinate [mm]			Cluster size	<i>T</i> value	<i>Z</i> score	<i>P</i> _[uncorrected]
	x	y	z				
Increased							
Left hippocampus	-5	2	-4	3762	5.45	3.85	<0.001
Left striatum	-4	7	-1	1855	5.09	3.71	<0.001
Right insular cortex	5	8	-2	4103	7.95	4.72	<0.001
Right secondary visual cortex	2	2	-5	2209	6.54	4.28	<0.001
Left parietal association cortex	-3	1	-4	546	8.01	4.73	<0.001
Decreased							
Right copula of the pyramis	2	6	-14	78	3.87	3.1	<0.001
Left copula of the pyramis	-2	6	-14	859	5.04	3.69	<0.001
Right paramedian lobule	3	6	-14	99	4.02	3.18	<0.001

Figures

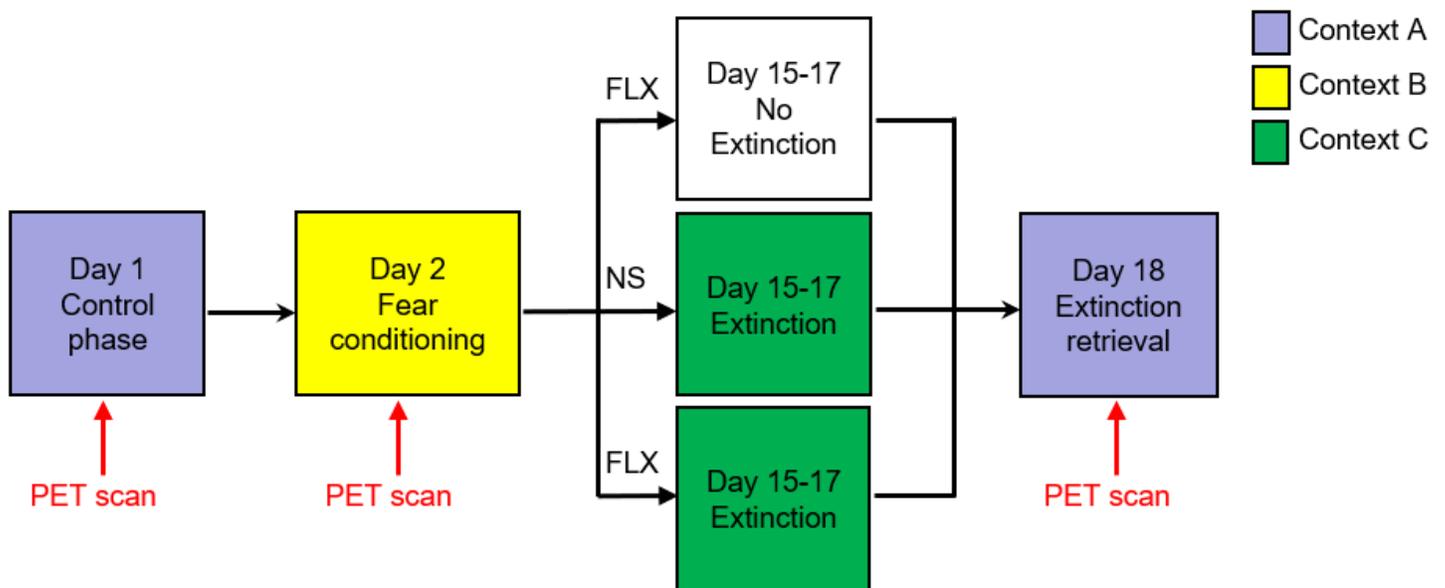


Figure 1

PET scans were performed on days 1, 2 and 18 .

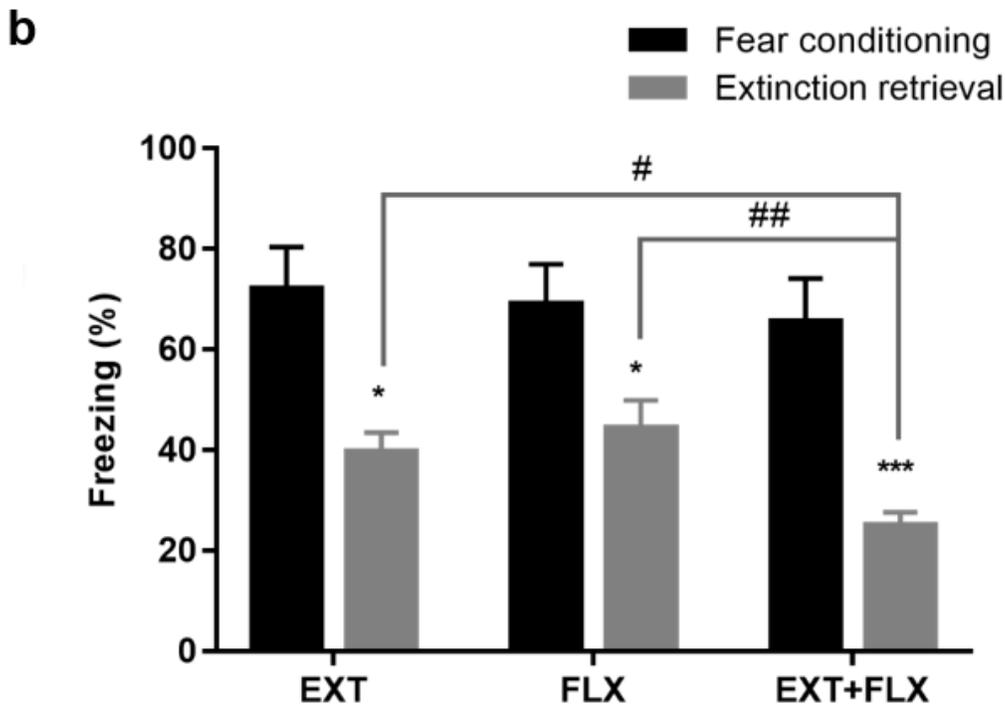
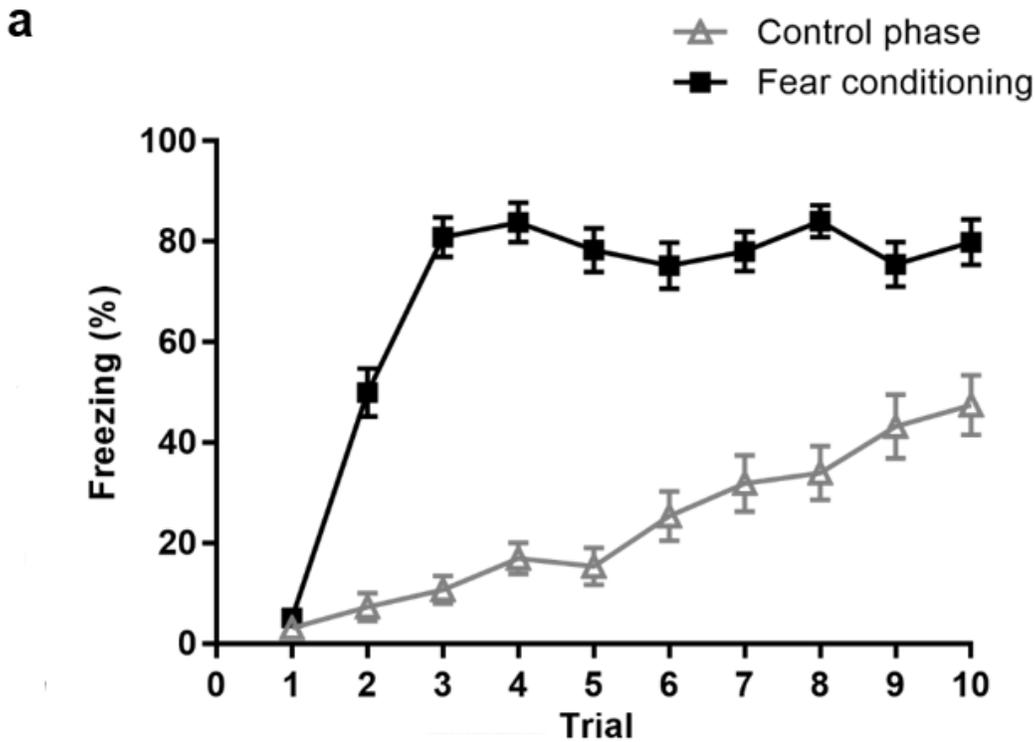


Figure 2

The rats received 10 sets of CS-US presentations in Context B to acquire fear memories. Compared with the freezing (%) in the control phase, the freezing (%) of the rats with conditioned fear memory was significantly increased (control vs. fear: $23.54 \pm 4.83\%$ vs. $69.06 \pm 7.74\%$, $p < 0.05$) (Fig. 2a). The repeated-measures ANOVA revealed that all rats with conditioned fear showed less freezing (%) following the treatment (all $p < 0.05$). In the extinction retrieval phase, the Bonferroni adjustment for multiple

comparisons showed that there were significant differences between the EXT+FLX group and the two other groups (EXT group and FLX group) (all $p < 0.05$), but there were no differences between these two groups ($p > 0.05$) (Fig. 2b).

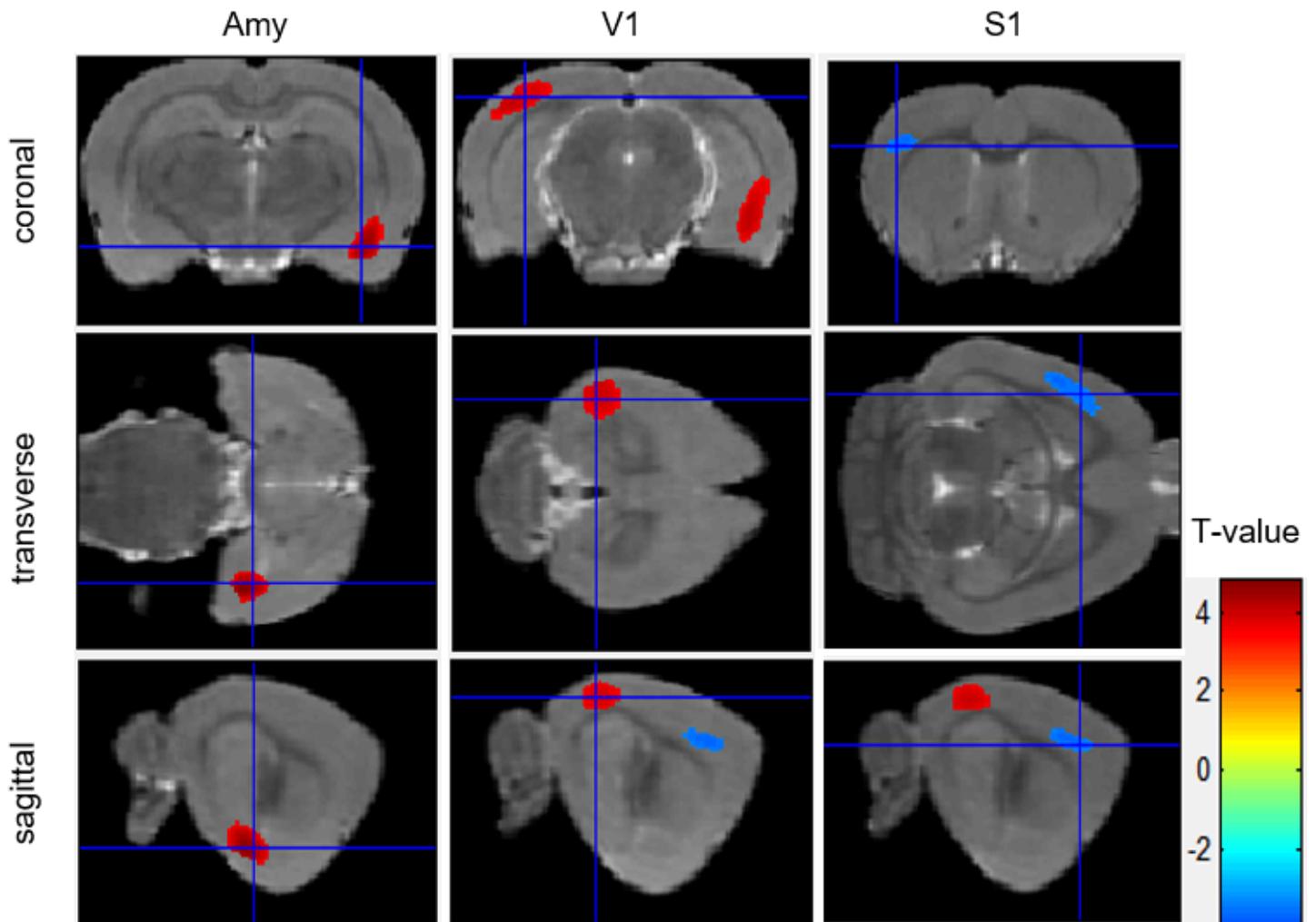


Figure 3

Compared with the level of glucose metabolism in the control phase, the rats exposed to conditioned fear showed increased glucose metabolism in the right amygdala and left primary visual cortex but decreased glucose metabolism in the left primary somatosensory cortex ($p < 0.001$)

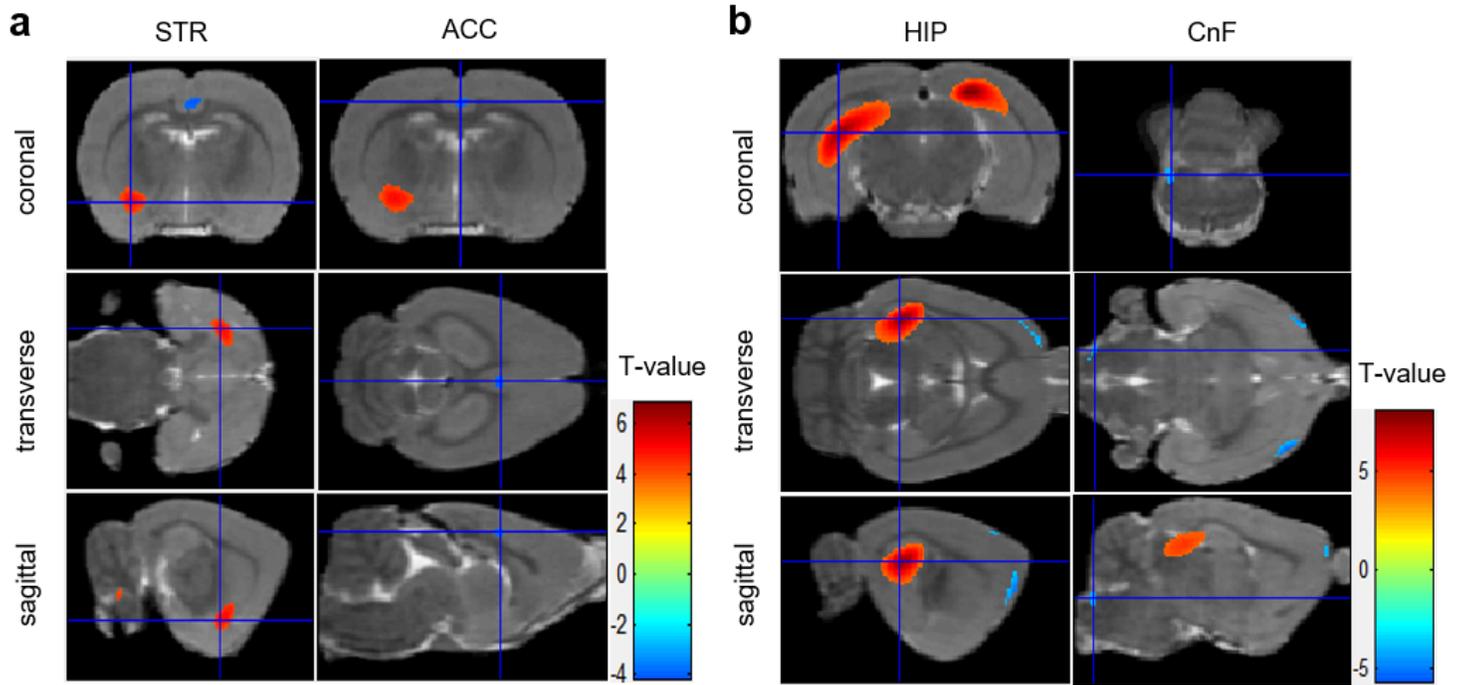


Figure 4

After the extinction retrieval, the rats in the EXT group presented an increased [18F]FDG uptake in the left striatum, left cochlear nucleus and right primary visual cortex but decreased uptake in the anterior cingulate cortex ($p < 0.001$) (Table 2, Fig. 4a). [18F]FDG accumulation was significantly increased in the left hippocampus and right primary visual cortex but decreased in the bilateral primary somatosensory cortex, left primary/secondary motor cortex and cuneiform nucleus of the rats in the FLX group ($p < 0.001$) (Table 3, Fig. 4b).

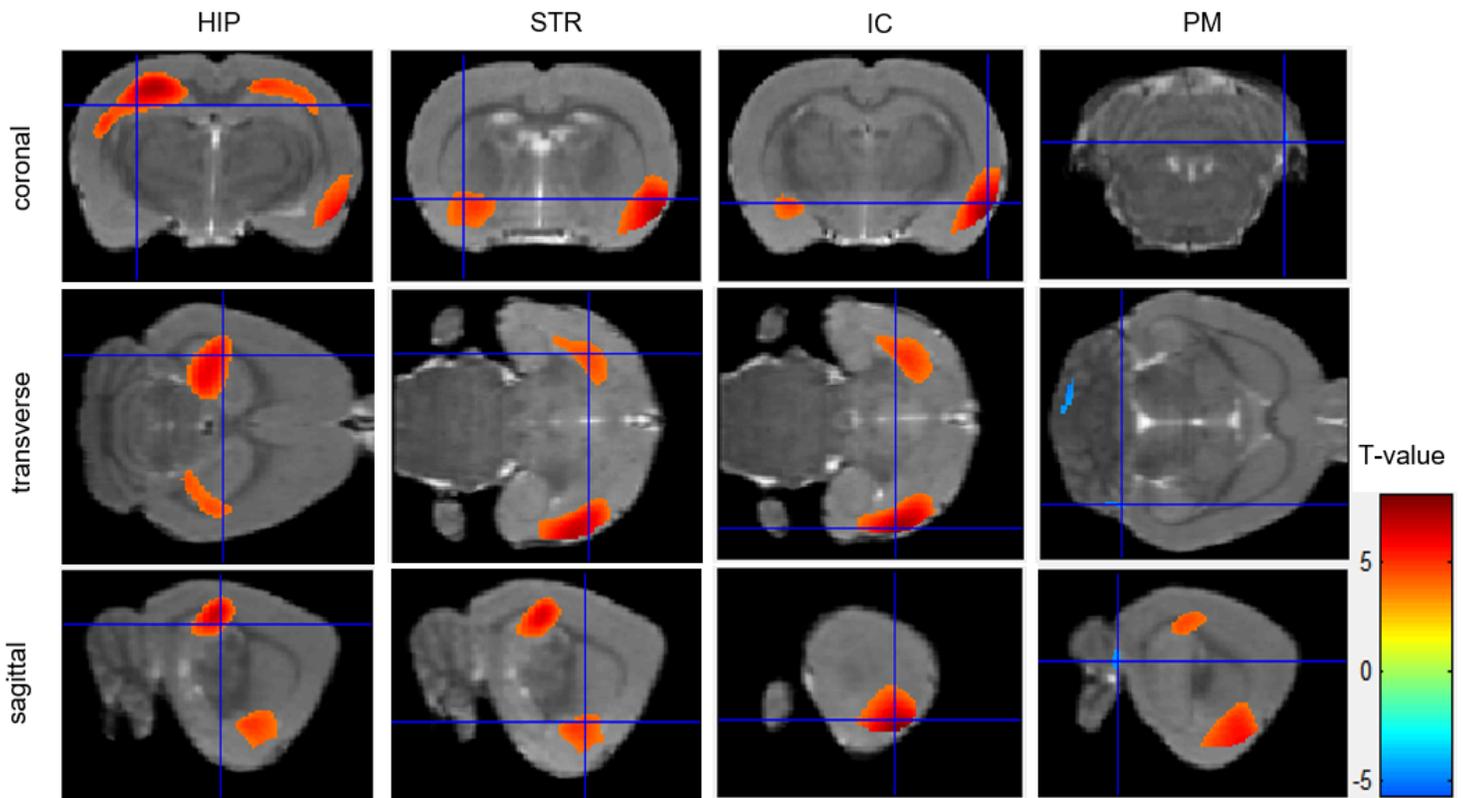


Figure 5

The EXT+FLX rats exhibited increased [18F]FDG uptake in the left hippocampus, left striatum, right insular cortex, left posterior parietal cortex and right secondary visual cortex but reduced [18F]FDG uptake in the cerebellar lobule ($p < 0.001$) (Table 4, Fig. 5)

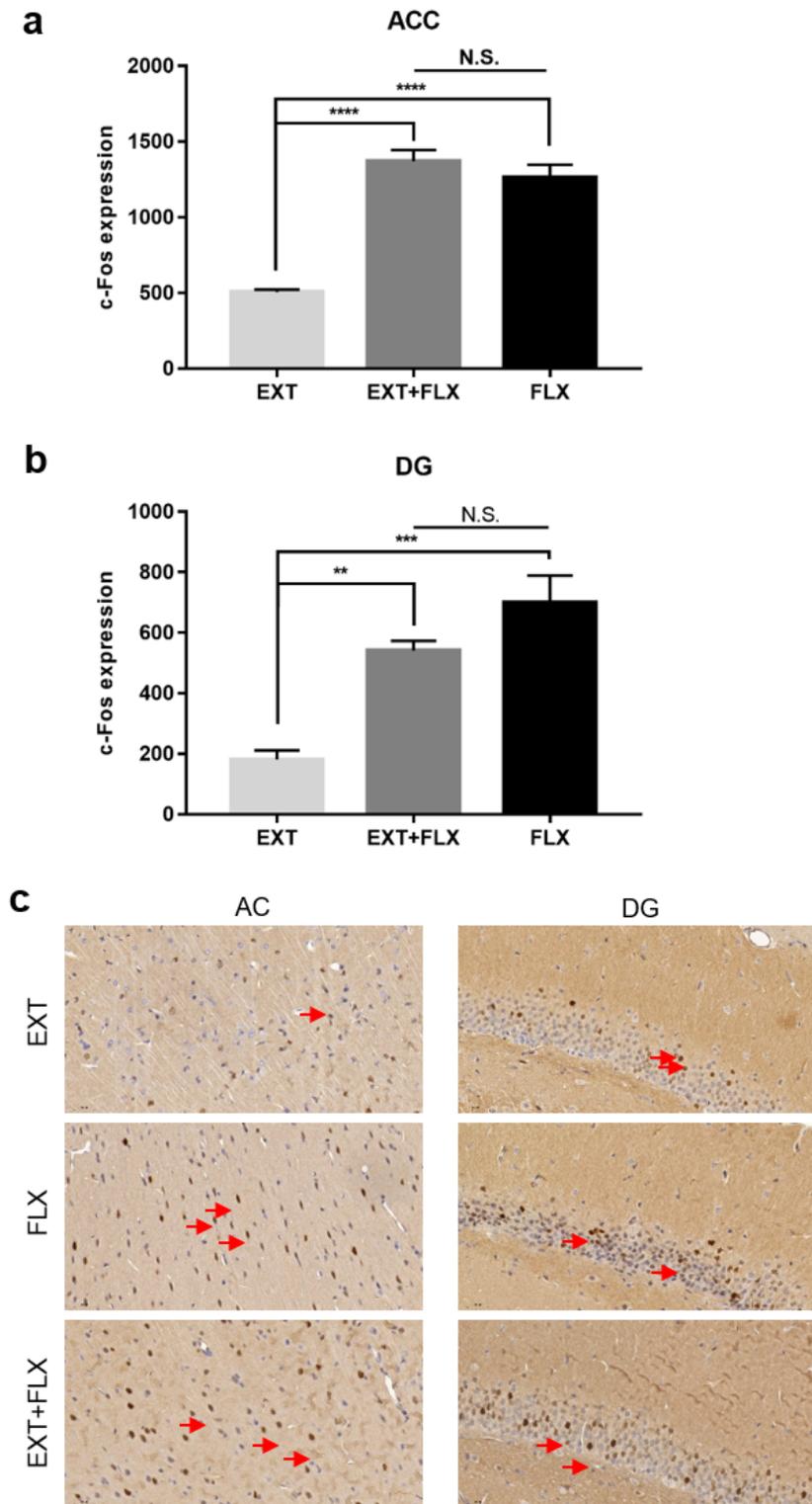


Figure 6

After the extinction retrieval, compared with the EXT group, c-Fos expression in the dentate gyrus of the hippocampus and anterior cingulate cortex was significantly increased in the FLX and EXT+FLX groups (all $p < 0.05$), whereas no significant difference was found between the FLX and EXT+FLX groups ($p > 0.05$) (Fig. 6).

Supplementary Files

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