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Brief Communication

PET Mapping of Neurofunctional Changes in a Post-traumatic Stress Disorder Model

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ABSTRACT

Post-traumatic stress disorder (PTSD) is an anxiety disorder that occurs following exposure to a traumatic event. This study aims to investigate the neurobiological changes before and after exposure-based therapy by positron emission tomography (PET) in a rat model of PTSD.

Methods: Serial ^{18}F-flurodeoxyglucose (^{18}F-FDG) PET imaging studies were performed under the control (tone presentation), fear conditioning and extinction retrieval phases. Neuroactivity marker c-Fos protein was used for immunostaining.

Results: Increased glucose metabolism was observed in the bilateral amygdale after fear conditioning ($P < 0.001$), and in the right posterior insular cortex under extinction retrieval ($P < 0.001$) compared with the control phase. Increased c-Fos expression in the posterior insular cortex under extinction retrieval was positively correlated to the glucose metabolism ($P < 0.01$).

Conclusion: Our results indicated that amygdala plays a key role in fear memory formation, and most importantly, insular cortex is related to the retrieval of extinction memory. ^{18}F-FDG PET may provide a promising in vivo approach for evaluation exposure-based therapy of PTSD.

Keywords: Positron emission tomography (PET), post-traumatic stress disorder (PTSD), fear conditioning, extinction

INTRODUCTION

Post-traumatic stress disorder (PTSD) is the most costly psychiatric disorder, affecting up to 40% of individuals over lifetime exposure to traumatic events (*1, 2*). Over the past decades, considerable studies have explored how fear memories are encoded in the brain. A neurocircuitry model of PTSD emphasized the importance of amygdala, as well as its interactions with the ventral/medial prefrontal cortex (vmPFC) and hippocampus (*3*). The hyperresponsivity within the amygdala to threat-related stimuli, with inadequate top-down governance over the amygdala by vmPFC and hippocampus (*3, 4*). The Pavlovian fear conditioning study have highlighted the key role of the amygdala in the acquisition and storage of conditioned fear memories (*5*). Electrophysiological recording and inactivation studies in rats suggest that fear extinction depended on increased neuroactivity in the medial PFC under extinction training (*6, 7*). Furthermore, amygdala has been found activated during fear acquisition (*8*) and positively correlated with the severity of PTSD symptoms (*9*). Failure to recall fear extinction memory is associated with lower activation in hippocampus and vmPFC in PTSD patients relative to trauma-exposed healthy subjects (*10*).

Although exposure-based therapy (conceptually based upon fear extinction) has been widely used in the treatment of PTSD (*1*), its underlying mechanism has not been completely elucidated. Since positron emission tomography (PET) has been increasingly used to characterize neural activities, we hypothesized that ^{18}F-flurodeoxyglucose (^{18}F-FDG) PET could be applied for evaluating cerebral glucose metabolism before and after exposure-based therapy, and provide a potential translational tool

for future clinical applications. Thus, the present study aims to investigate the neurobiological changes by ^{18}F-FDG PET in a rat model of PTSD.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (n = 25, body weight: 260 - 280 g) were housed under standard laboratory conditions with food and water *ad libitum*. All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee at Zhejiang University (Protocol No. ZJU201407-1-02-067).

Behavioral Procedures

Pavlovian fear conditioning and extinction procedures were conducted in standard operant chambers (AniLab Software & Instruments Co., Ltd.) (*11*). Prior to training, rats were habituated to handling and to the conditioning chamber for 2 days prior to training. Each rat was presented with 10 tones (30 s, 75 dB) only on Day 1 (serves as the control phase), and fear conditioned with 10 sets of tone (30 s, 75 dB) that co-terminated with a footshock (1 s, 0.5 mA) on Day 2 in the conditioning chamber (Fig. 1 A and B). Then the rat was moved to the extinction chamber and presented with 20 tones on Day 3 and 4, and tested for recall of extinction memory with 10 tones alone on Day 5. All the procedures were video-taped and freezing % was calculated as the percentage of time of the total tone duration when the rat remained immobile (frozen).

Image Acquisition and Analysis

PET imaging studies were done on Dy 1, 2 and 5 (Fig. 1A). Each rat performed the behavior training for 30 min, and PET images were acquired in the micro-PET R4 scanner (Siemens Medical Solutions) at 40 min after intraperitoneal injection of ^{18}F-FDG (18.5 MBq). Images were analyzed by statistical parametric mapping (SPM) (*12*).

Immunohistochemistry

The neuronal activation marker c-Fos was immunostained in the specific brain regions where increased ^{18}F-FDG accumulations were found. Immunohistological procedures were performed as described previously (*13*). Slides were incubated with a polyclonal antibody against c-Fos (1:1000; Santa Cruz Biotechnology) overnight at 4 °C, and c-Fos-positvie cells were calculated. Data are presented as means ± SEM, Differences were considered significant if $P < 0.05$.

RESULTS

Fear conditioning induced significant increases of freezing% compared with the control tone phase (peak Freezing% of 87.2% vs 23.6 %) (Fig. 1C). Freezing % decreased gradually after the early and late extinction training, and during extinction retrieval, freezing% were stabilized within a lower range.

^{18}F-FDG accumulations were increased in the bilateral amygdale ($P < 0.001$), but decreased in the bilateral secondary motor cortex, left primary somatosensory cortex, left ventroposterior medial (VPM) nucleus of the thalamus ($P < 0.001$) under fear conditioning compared with the control phase (Table 1,

Fig. 2 and Supplemental Fig. 1). After extinction retrieval, ^{18}F-FDG accumulations were increased ($P < 0.001$) in the right primary visual cortex and right posterior insular cortex, but decreased ($P < 0.001$) in a cluster comprising the right orbital cortex, lateral septum and bilateral bed nucleus of the stria terminalis (BNST) compared with the control (Table 2, Fig. 3 and Supplemental Fig. 2).

After extinction retrieval, c-Fos-positive cells in the posterior insular cortex were significantly increased compared with the fear conditioning ($P < 0.01$, Fig. 4A), and was positively correlated with the ^{18}F-FDG accumulation ($P < 0.01$, Fig. 4B).

DISSCUSSION

The present study investigated the changes of brain metabolism and neuroactivity during fear conditioning and extinction retrieval. We found increased glucose metabolisms in the bilateral amygdale under fear conditioning; and in the right posterior insular cortex after extinction retrieval. Other areas with decreased glucose metabolism were observed, including the bilateral secondary motor cortex, left primary somatosensory cortex, left VPM under fear conditioning; and the lateral septum and bilateral BNST after extinction retrieval compared with the control phase. Moreover, over-expression of the neuroactivity marker c-Fos was associated with increased glucose metabolism in the posterior insular cortex after extinction retrieval.

Interpreted in the context of other clinical PET imaging studies, amygdala is activated during fear acquisition in PTSD patients (*8*), and is involved in processing fearful faces in healthy subjects (*14*). Functional magnetic resonance imaging (fMRI) revealed exaggerated amygdala responses to fearful

faces, and were positively correlated with the severity of PTSD symptoms (*9*). Our finding of activated bilateral amygdale under fear conditioning is consistent with the above literatures, and confirmed our hypothesis of using PET technology to investigate neurofunctional changes in the rat model of PTSD. Interestingly, we observed decreased glucose metabolism in the bilateral secondary motor cortex, left primary somatosensory cortex (forelimb and jaw area) and left VPM under fear conditioning. Since VPM is a somatosensory relay station that relays input sensory information from individual whiskers and projects to the somatosensory cortex (*15*) in rats, decreased glucose metabolism in this region was associated with increased freezings and reduced exploring behaviors during fear conditioning, (*15*).

The most important finding of the present study was the increased glucose metabolism and activated c-Fos expression in the right posterior insular cortex after extinction retrieval. To our knowledge, this is the first to show that the posterior insular cortex is involved in the retrieval of fear extinction memory in rodents. Insular cortex is important for the acquisition and extinction of conditioned taste aversion (CTA) (*16*), and the extinction rate is positively correlated with c-fos mRNA expression in rats (*17*). Bilateral inhibition of the posterior insular cortex during stress exposure prevented the stress-mitigating effect of safety signals (*18*). The posterior insular cortex projects strongly to GABAergic neurons in the lateral subdivision of central amygdala (CeL) in rats (*19*), which serves as a switch capable of orchestrate the activity of projection neurons in the medial subdivision of central amygdala (CeM) and consequently regulates conditioned fear responses (*20, 21*). Therefore, we speculate that inhibitory CeL neurons were activated by projection neurons in the posterior insular cortex during

extinction retrieval, leading to inhibition of CeM projection neurons and thus suppression of fear responses.

The involvement of amygdala and insular cortex has also been demonstrated in previous human studies. Especially, repeated exposure to traumatic memory (used as an exposure-based treatment) could Increase functional connectivities between right amygdala and bilateral anterior insular corte, as well as between left amygdala and right anterior insular cortex in PTSD patients (*22*). Repeated presenting negative images to healthy subjects could increase bilateral posterior insular cortex activity, and was associated with increased functional connectivity between left posterior insular cortex and amygdala (*23*). In addition, smaller insular cortex was found in PTSD patients compared with trauma exposed healthy subjects, which indicated deficient extinction processes and an uncontrollable state of fear (*24, 25*). In consistent with those results, our PET imaging findings combined with immunohistological data indicated that the insular cortex plays a critical role in the retrieval of extinction memory.

CONCLUSION

In conclusion, our results support a key role for the amygdala in fear memory formation. The PET imaging findings combined with immunohistological data provide compelling evidence that the posterior insular cortex is involved in the retrieval of extinction memory. PET imaging of fear circuitry in animal models may provide a valuable translational approach to better characterize pathophysiological

mechanisms of PTSD. Future studies are required to better delineate the contribution of the insular cortex in extinction retrieval and its functional connectivity with other brain regions.

DISCLOSURE

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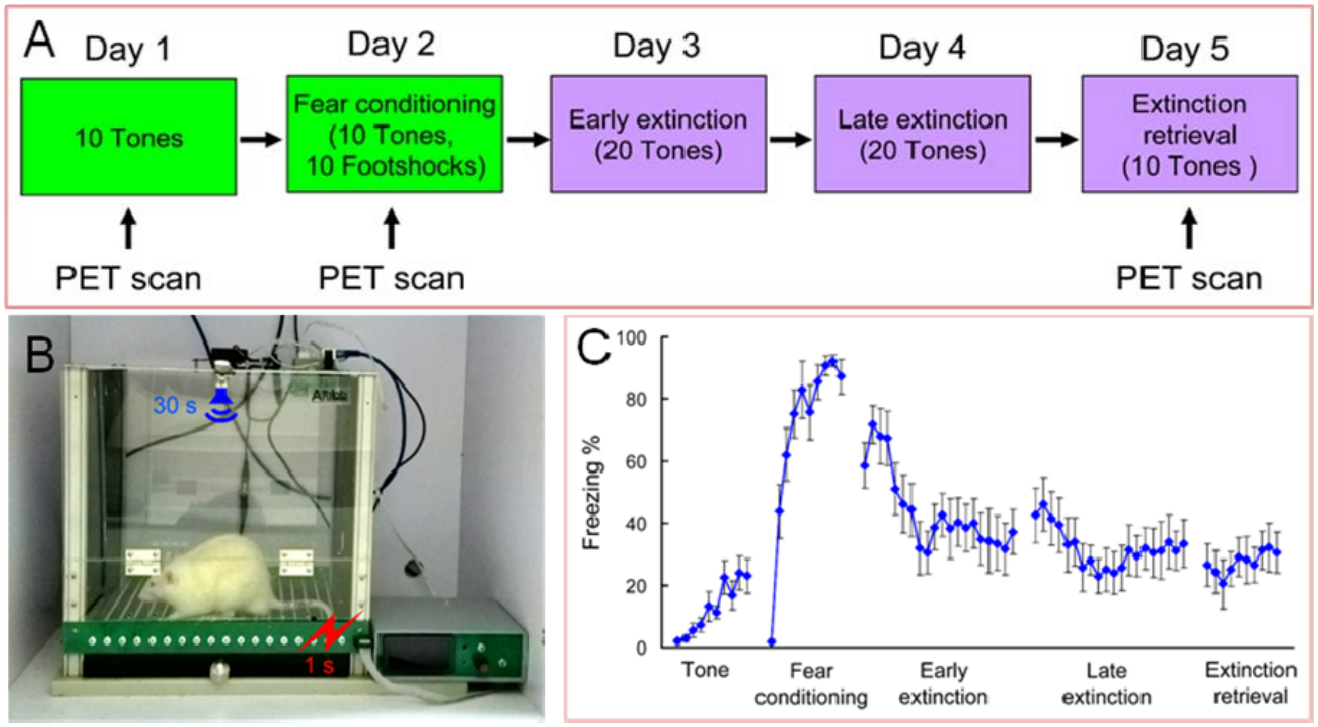

FIGURE 1. Experimental design and behavioral results. (A) Schematic of behavioral paradigm. (B) The rat is fear conditioned with 30s tone that co-terminated with a footshock. (C) Freezing% in different phases. Data are presented as mean ± SEM (n = 10).

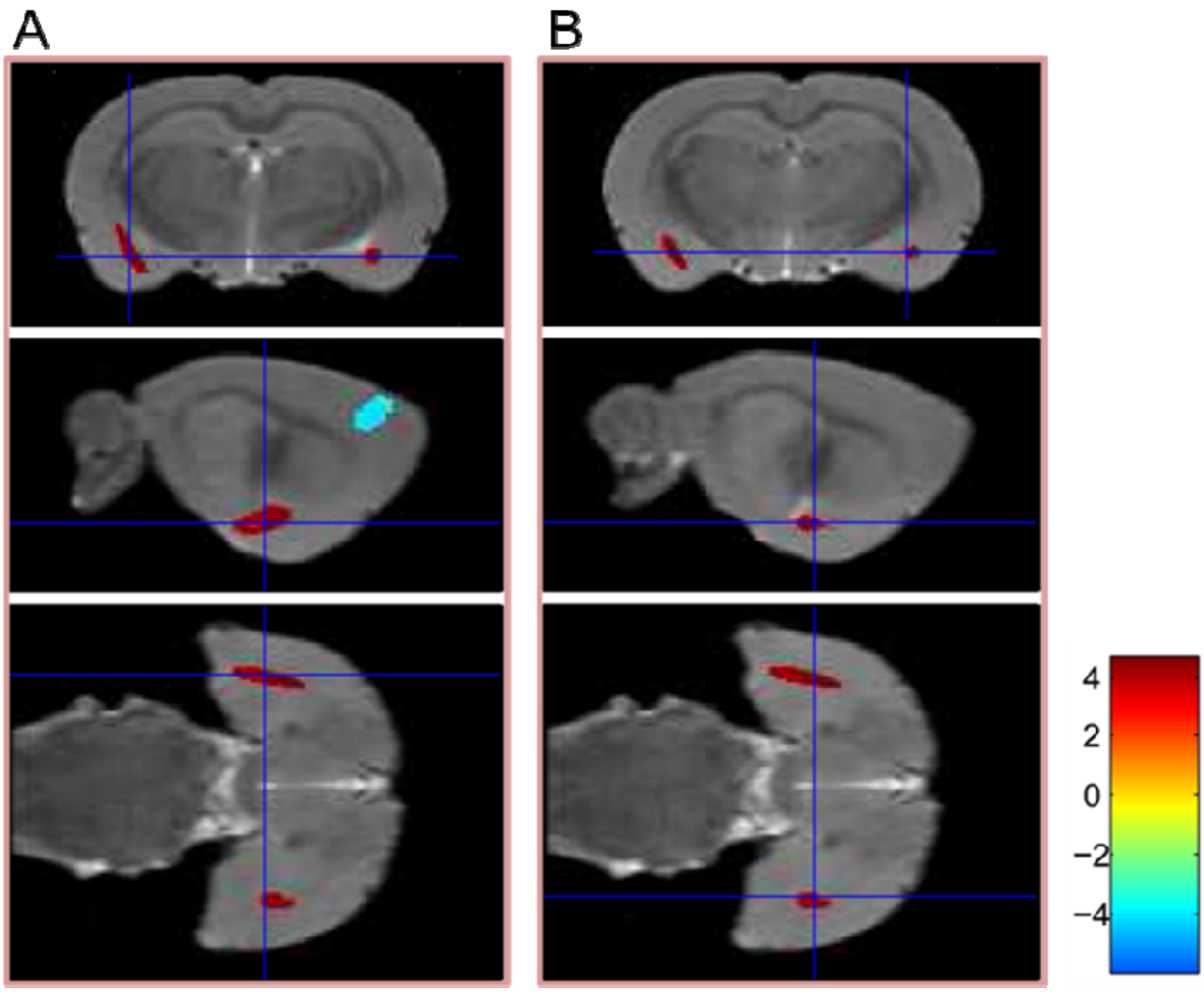

FIGURE 2. Coronal, sagittal and transverse images demonstrated increased glucose metabolism in the left (A) and right amygdala (B) under fear conditioning (n = 8, $P < 0.001$).

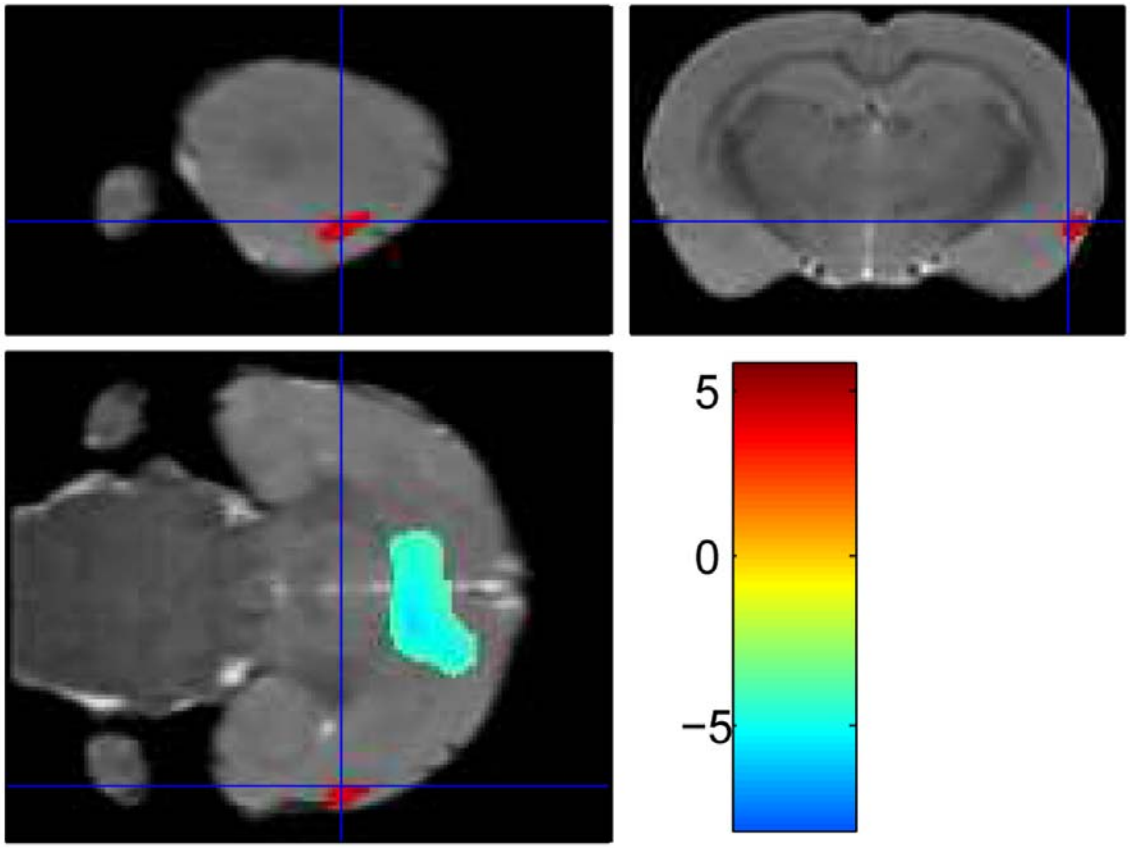

FIGURE 3. Sagittal, coronal and transverse images demonstrated increased glucose metabolism in the right posterior insular cortex under extinction retrieval (n = 8, $P < 0.001$).

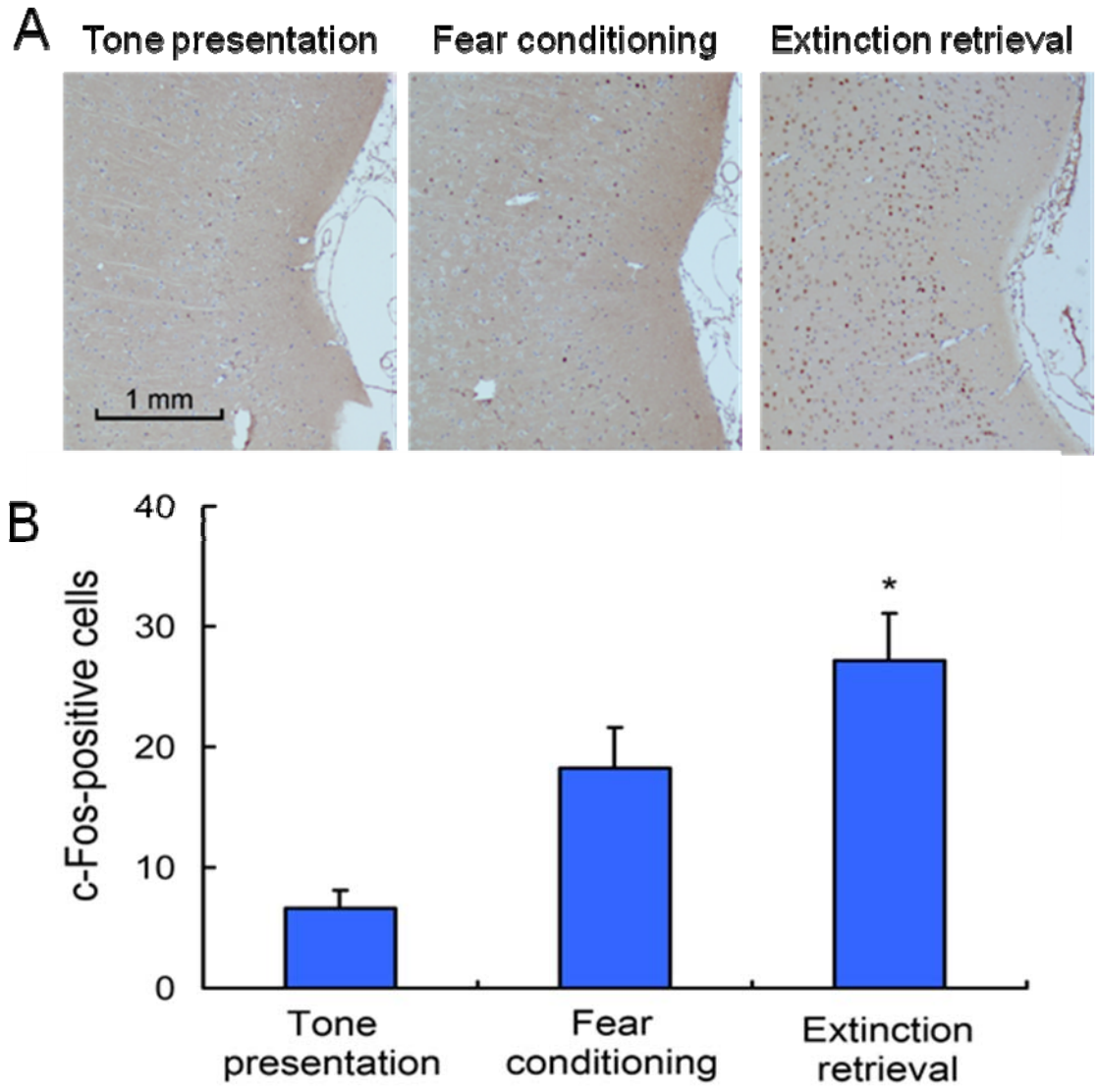

FIGURE 4. Expression of c-Fos in the posterior insular cortex. (A) Representative photomicrographs of immunolabeled c-Fos neurons in a tone presentation, fear conditioning and extinction retrieval. (B) Quantification of c-Fos-positive neurons in the posterior insular cortex (n = 5 in each phase, * $P < 0.01$).

TABLE 1. Significant Glucose Metabolic Changes under Fear Conditioning (Control vs. Fear conditioning)

	Coordinate (mm)			Cluster level		
Region	x	y	z	*t* value	*z* score	$P_{uncorrected}$
Increased						
Left amygdala	-5	8	-3	4.65	3.56	< 0.001
Right amygdala	5	8	-3	4.32	3.39	< 0.001
Decreased						
Right secondary motor cortex	2	2	5	5.90	4.11	< 0.001
Left secondary motor cortex	-2	2	4	5.35	3.88	< 0.001
Left primary somatosensory cortex (forelimb and jaw area)	-5	2	2	5.15	3.80	< 0.001
Left ventroposterior medial nucleus of the thalamus	-3	6	-4	4.22	3.33	< 0.001

TABLE 2. Significant Glucose Metabolic Changes under Extinction Retrieval (Control vs. Extinction retrieval)

	Coordinate (mm)			Cluster level		
Region	x	y	z	*t* value	*z* score	$P_{uncorrected}$
Increased						
Right primary visual cortex	3	2	-6	5.75	4.05	< 0.001
Right insular cortex	6	7	-2	4.15	3.30	< 0.001
Decreased						
Right orbital cortex	1	5	4	8.00	4.83	< 0.001
Left lateral septum	-1	6	1	8.00	4.83	< 0.001
Right lateral septum	1	6	1	8.00	4.83	< 0.001
Right bed nucleus of the stria terminalis	-1	7	0	5.74	4.05	< 0.001
Left bed nucleus of the stria terminalis	1	7	0	5.56	3.98	< 0.001