

# Brain Glucose Metabolism and Dopamine Transporters Change in Addiction and Environmental Cue-Induced Memory

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## Original research

**Keywords:** Morphine, Memory retrieve, Striatum, Hippocampal and neocortical circuits

**Posted Date:** August 10th, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-55732/v1>



# Abstract

**Purpose:** Drug addiction is a chronic brain disease and abuse of morphine is compulsive. Several brain regions were involved in drug stimulus learning and drug seeking behaviors. Dopaminergic signaling pathway plays a key role in drug seeking behaviors. In this study, we focus on the regions of brain metabolism and dopamine transporters alternation, and figure out their role in drug seeking behaviors, and environmental cue-induced craving and retrieval of drug withdrawal memory by morphine-induced conditioned place preference (CPP) rats.

**Methods:** Rats with CPP training ( $n = 6$ ) were established by intraperitoneal injection of morphine (6 mg/kg body weight) in male SD rats (250 - 300g) during three-day conditioning, and rats with only morphine injection were used as addictive group ( $n = 6$ ). Micro-PET/CT scans were performed at 3 different time points by intravenous injection of  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -CFT. Regions of interests were collected by PMOD, voxel-wise analysis were performed by SPM8 software (uncorrected,  $P < 0.001$ ,  $k = 20$ ) on MATLAB platform.

**Results:** SUVr of FDG declined significantly in medial prefrontal cortex (mPFC), cingulate in short term addiction compared with baseline. Glucose metabolism alternation in somatosensory cortex, hippocampus, cingulate were also found in addictive rats by voxel-wise analysis. Striatum, thalamus, medial prefrontal cortex, primary motor cortex and many regions in cortex also involved in CPP rats. Striatum, primary somatosensory cortex and some cortical regions play key roles in memory retrieval of addiction compared with baseline. DAT expression alternation were only observed in long term addiction compared with short term addiction.

**Conclusion:** This study shows that the cerebral glucose metabolism in addiction and CPP is significantly different from control group mainly in mPFC, striatum and hippocampus. Hippocampal and neocortical circuits for episodic memories could also involve in memory retrieval of addiction, and primary somatosensory cortex, primary motor cortex could be the neocortical structure together with PFC.

## Introduction

Drug addiction is a brain disorder by repeated use of addictive drugs, such as heroin, morphine, methamphetamine etc. [1]. The major issue of treatment of drug abuse is the relapse following abstinence. Therefore, association between cues and the rewarding effects of drugs is one of the main reasons for drug relapse. A feelings of craving and conditioned physiological response always occur when abstinent drug abusers have cues of the rewarding effects of the used drugs [2]. Craving can't be measured directly by animal models, although we knew drug abuse strengthened by conditional associations with environmental cues. Conditioned place preference (CPP) is always used to investigate cue-elicited drug craving since it associated with the rewarding effects of the drug administered with the environment [3, 4].

Memory retrieval and episodic memory are very important for craving. Many brain structures involved in memories, such as hippocampus, neocortical structures. Hippocampus is the region for initial information stored and distributed to cortical networks for long-lasting memory and in reward retrieval [5, 6]. Episodic memories are transformed from hippocampus to neocortical structures, such as the medial prefrontal cortex (mPFC) [7]. mPFC also involved in withdrawal memory retrieval [8], and thalamus contributes to fear memory retrieval [9]. The function of dopamine system also involve several neurotransmitters in craving and memory retrieval, such as dopamine transporters (DAT), dopamine receptors (such as D2 receptor) [10, 1]. Decreased expression of DAT, dopamine receptors, and reduction of DA level as well was observed in drug addiction [11, 12]. And dopaminergic systems involved in learning, motivation, and reward related behaviors [13, 14]. Degeneration of the dopamine system contributed to drug craving and addictive behaviors.

Some of these neurotransmitters regulated dopaminergic activity within reward or withdrawal circuits. Dopamine is important for rewarding stimuli. Therefore, the study of chronic morphine-induced adaptive changes in the brain function, metabolism, transporter expression etc. is critical to investigate mechanism of addiction. In the previous study from our lab indicated morphine addiction induced increased expression of dopamine receptors at presynaptic terminals to basolateral amygdala, which to be relevant to environmental cue-induced retrieval of withdrawal memory [15].

Positron emission tomography (PET) has been shown to be an effective imaging technique to study neurometabolic and neurochemical processes involved in addiction.  $^{18}\text{F}$ -FDG, an analogue of glucose, could measure brain regional glucose metabolism. In clinic, FDG is widely used in evaluation of brain disorder or neurodegeneration, such as AD, PD and depression [16–18]. FDG PET is also an approach to figure out the function changes in brain region. DAT is highly expressed in striatum, radioligand targeting DAT are widely used as presynaptic markers binding to DAT for assessing dopamine [19, 20]. [ $^{11}\text{C}$ ] 2- $\beta$ -carbomethoxy-3- $\beta$ -(4-fluorophenyl)tropane ( $^{11}\text{C}$ -CFT) is a classic ligand to evaluate the expression, location and alternation of DAT in brain, which is widely used in evaluation of degeneration of dopamine [21, 22, 20]. Both tracers were widely used in brain study to investigate the brain function of different brain disorders.

In this study, we try to investigate the change of glucose metabolism and DAT alternation between short term addiction and long term addiction, and between CPP and environmental cue-induced memory. We want to figure out the role of dopamine system, memory of hippocampus in craving. We also intent to find the brain regions involved in memory of drug seeking behaviors.

## Materials And Methods

### Animals and Radiopharmaceuticals

Male Sprague Dawley (SD) rats were purchased from Shanghai Laboratory Animal Center (Shanghai, China) and housed in an animal housing facility with free access to food and drinking water.  $^{18}\text{F}$ -FDG

and  $^{11}\text{C}$ -CFT was prepared at the PET Center, Huashan Hospital, Fudan University according previous report and under GMP condition [21]. All small animal experiments were conducted according to a protocol approved by Fudan University, Shanghai, China.

### **Conditioned place preference (CPP) apparatus**

The CPP apparatus consisted of two large compartments (30 cm × 30 cm × 40 cm) separated by a small compartment (10 cm × 30 cm × 40 cm). The two large compartments were distinguishable by distinct visual and tactile cues: one compartment had a white wall and a floor with many quadrate holes, and the other had a black wall and a floor with many hollow longitudinal lines. The middle section contained a transitional compartment with a grey wall and a solid grey polyvinyl chloride floor. Between the three compartments were two guillotine doors (8 cm × 8 cm) patterned to match the outer compartments, which were closed on the conditioning days and opened on the adaptation and testing days. In this manner, the rats were able to identify the black and white compartments not only by visual but also touch perception. Infrared cameras in each compartment were used to record time spent in each of the three compartments during CPP testing.

### **CPP procedure**

The CPP procedure was similar to that described previously [23]. Behavioral subjects were individually habituated to the investigator by handling for 5 min and placed in the experimental environment for adaptation for 3 days before the CPP session. A standard CPP protocol consisted of four consecutive phases: adaptation, pre-test, conditioning, and post-test phases. During the adaptation phase, the rats were put into the CPP apparatus for 30 min to allow them to adapt to the testing environment. During this phase, the doors were always open so that the rats could freely access all three compartments. During the pre-test phase, the rats were placed in the CPP apparatus for 15 min, and the time that the rats spent in each compartment was recorded. The rats were removed when the time spent in any of the three compartments exceeded 540 s. For all experimental rats, the large compartment that was occupied for the shorter time was designated as the drug-paired compartment, and the other large compartment was designated as the drug-unpaired compartment. Then, the conditioning sessions were conducted: during this phase, each group received alternating injections of either saline or morphine every day at 6-h intervals. Briefly, for the CPP group, during the morning of the first training day (at 08:00), the rats were intraperitoneally injected with morphine (20 mg/kg) and immediately confined to the drug-paired compartment. After the rats stayed quietly in the drug-paired compartment for 45 min with the doors closed, they were returned to their feeding cage. Similarly, 6 h later, in the afternoon (at 14:00), the rats were confined to the drug-unpaired compartment for 45 min after the injection of saline (2 mL/kg, i.p.). On the next day, the procedure was performed in reverse order in the morning and the afternoon. Additionally, the rats in the the addiction group received only morphine or saline injection but without conditioning in CPP apparatus. After the final conditioning session, the post-test phase was carried out 24 h later. For the post-test, the doors were raised, and the rats were permitted to access the entire apparatus for 15 min. CPP score, defined as the time spent in the drug-unpaired compartment minus the

time spent in the drug-paired compartment, was calculated. From our perspective, a post-test CPP score  $\neq$  0 indicated successful establishment of the CPP model.

### Micro-PET/CT imaging and Analysis

Micro-PET/CT imaging in these rats was conducted at the designed time point according to a method described previously [24, 21]. Briefly, the rats were anesthetized with 2% isoflurane in 100% oxygen (1 L/min) at room temperature using an isoflurane vaporizer (Molecular Imaging Products Company, USA). The rats were positioned in a spread-supine position on the imaging bed and subjected to 2% isoflurane in 100% oxygen (1 L/min) via inhalation during the PET/CT procedure. After the intravenous administration of  $^{18}\text{F}$ -FDG ( $\sim 10 \mu\text{Ci/g}$  body weight) and  $^{11}\text{C}$ -CFT ( $\sim 10 \mu\text{Ci/g}$  body weight), rats were allowed to move freely in 10 min, then these rats were permitted to access the CPP apparatus for 15 min and Static PET/CT imaging was obtained for 20 min at 60 min post-injection of  $^{18}\text{F}$ -FDG or 20 min at 40 min post-injection of  $^{11}\text{C}$ -CFT. PET/CT images were reconstructed using the ordered subsets expectation maximization 3D algorithm (OSEM3D), and the data were reviewed and processed using the IRW. Image processing was performed in PMOD software (version 3.4, PMOD Technologies Ltd., Zurich, Switzerland). Imaging was manually fused with the PMOD rat template, and 58 brain regions of interest (ROIs) were collected (Supplementary Fig. 1).  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -CFT uptakes were quantified as the SUV, and the SUV ratio (SUVr) was calculated by comparing the SUV uptake in the ROIs to that of the cerebellum.

Voxel-wise analysis was performed using spmratIHEP [25] based on Statistical parametric mapping 8 (SPM) software (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK). Firstly, the images were spatially normalized to the standard FDG-PET template in Paxinos & Watson space; then a Gaussian kernel of  $[2 \ 2 \ 4]$  in full width at half maximum was used to smooth the data for voxel-wise analysis. After that, two sample t test was performed to compare the group difference between addiction rats with CPP and baseline. The statistical level was set at  $P < 0.001$  with uncorrected multiple comparisons and a minimum cluster extent  $k_e \geq 20$  voxels.

### Study design

A longitudinal, sequential PET/CT study was performed to assess the glucose metabolism and DAT change in rat brains as shown in Fig. 1. All rats conducted CPP pre-test and the results were recorded.  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -CFT imaging were performed at the following 2 days after pre-test. After PET imaging, chronic morphine or saline treatment was added on these rats at the following 5 days. At the same time, one group of rats had CPP procedure as CPP group, and the other rats only had chronic morphine treatment without CPP procedure as additive group.  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -CFT imaging were performed one day after these treatments as short term addiction and short term CPP, also as cue-induced craving. All of these rats were housed with food and water available *ad libitum* without any training or drug administration in the next 14 days. post-test procedure was conducted and the results were recorded, which is to reflect environmental cue-induced retrieval of withdrawal memory. And then,  $^{18}\text{F}$ -FDG and  $^{11}\text{C}$ -

CFT imaging were performed as same at last time as long term addiction and long term CPP, long term CPP group also reflected memory of retrieval.

## Statistical analysis

Statistical analyse was performed using Prism7 (GraphPad Software, CA, USA) software. All data are presented as mean  $\pm$  standard deviation (SD). To determine whether the difference of the between the CPP score in these 2 group of rats, we applied two-way analysis of variance (ANOVA). For data of SUV and the SUVr in the ROIs in these rats differed among different groups, we applied two-way ANOVA, followed by Bonferroni's corrections for multiple comparisons test. A *P* value of less than 0.05 was considered statistically significant.

# Results

## Changes of CPP score in addiction and CPP rats

CPP score was calculated to valid the success of establishing model of CPP. CPP score is the time difference between each rat spent on these two compartments. As shown in Fig. 2, more time in one compartment after CPP training, which means the rats prefer one of the compartment.

## Change of Brain glucose metabolism in addiction group

A significant decrease of glucose metabolism in whole brain was observed from baseline (SUV:  $6.83 \pm 0.30$ ) to short term addictive rats (SUV:  $5.04 \pm 0.74$ ,  $p < 0.05$ ) and long term addition (SUV:  $5.31 \pm 0.87$ ,  $p < 0.05$ ). Glucose uptake (SUV) in short term addiction were significant lower than baseline in all brain regions ( $p < 0.001$ ) except pituitary. No difference of FDG uptake observed between short term and long term addiction (Supplemental Table 1). SUVr in short term addiction decreased significantly compared with baseline in mPFC (SUVr:  $1.44 \pm 0.04$  vs  $1.34 \pm 0.07$ ,  $p = 0.040$ ) and cingulate Cortex (SUVr:  $1.41 \pm 0.02$  vs  $1.31 \pm 0.06$ ,  $p = 0.029$ ) (Supplemental Table 1, Table 2). No difference found between short and long term addiction.

As shown in Fig. 3, voxel-wise comparison revealed hypometabolism in brain regions, such as bilateral primary somatosensory cortex, hippocampus, cingulate cortex, right caudate putamen, hypermetabolism in lateral posterior thalamic nucleus, dorsal peduncular cortex, right secondary auditory cortex, primary somatosensory cortex in short term addiction compared with baseline. Compared with short term addiction, long term addiction displayed hypermetabolism in bilateral primary somatosensory cortex, right caudate putamen.

## Change of Brain glucose metabolism in rats with cue-induced memory

A significant decrease of glucose metabolism in whole brain was observed from baseline (SUV:  $6.83 \pm 0.30$ ) to rats with cue-induce craving (SUV:  $5.14 \pm 0.72$ ,  $p < 0.05$ ) and rats with memory retrieval (SUV:  $5.18 \pm 0.62$ ,  $p < 0.05$ ). The brain uptake of FDG decreased significantly from baseline to cue-induce

craving, and stayed in a low level in rats with memory retrieval in all brain regions. SUVr in many brain declined significantly from baseline to CPP group, such as in striatum (SUVr:  $1.33 \pm 0.05$  vs  $1.21 \pm 0.06$ ,  $p < 0.05$ ), thalamus (SUVr:  $1.39 \pm 0.05$  vs  $1.27 \pm 0.07$ ,  $p < 0.05$ ), medial prefrontal cortex (SUVr:  $1.44 \pm 0.04$  vs  $1.28 \pm 0.07$ ,  $p < 0.05$ ), and other regions of cortex, such as auditory cortex, cingulate cortex, frontal association cortex, insular cortex, motor cortex, orbitofrontal cortex, retrosplenial cortex, somatosensory cortex (Supplemental Table 3, Table 4). No difference was found between rats with cue-induced craving and retrieval of memory.

Similar results were found in voxel-wise analysis, hypometabolism in bilateral primary somatosensory cortex, right hippocampus, principal sensory trigeminal nucleus, hypermetabolism in bilateral primary somatosensory cortex were found in cue-induced craving rats compared with baseline. Only hypermetabolism in left laterodorsal tegmental nucleus, and hypometabolism in bilateral hippocampus were observed in rats with retrieval of memory compared with cue-induced craving (Fig. 4).

### **Difference of Brain glucose metabolism between addiction and CPP group**

No significant difference of SUV and SUVr of FDG in all of the collected regions between short term addiction and short term CPP, and between long term addiction and long term CPP. In voxel-wise analysis, hypermetabolism in left lateral septal nucleus and primary somatosensory cortex, hypometabolism in right external cortex of the inferior colliculus and secondary auditory cortex were present in short term addiction compared with cue-induced craving. And hypometabolism demonstrated in bilateral primary somatosensory cortex, primary motor cortex, right caudate putamen, central nucleus of the inferior colliculus, and secondary auditory cortex, hypermetabolism in nucleus of the inferior colliculus, and secondary auditory cortex in long term addiction rats compared with rats with retrieval of memory (Fig. 5).

### **Change of DAT expression in addictive rats**

DAT expression in striatum decreased significantly from baseline to short term addiction (SUV:  $2.31 \pm 0.67$  vs  $1.32 \pm 0.16$ ,  $p < 0.05$ ), and from baseline to short term CPP rats (SUV:  $2.31 \pm 0.67$  vs  $1.25 \pm 0.18$ ,  $p < 0.05$ ) and long term CPP (SUV:  $2.31 \pm 0.67$  vs  $1.07 \pm 0.45$ ,  $p < 0.05$ ). No difference of SUVr in striatum between any two groups of rats (Supplemental Table 5, 6). In voxel-wise analysis, only increased DAT expression in some part of ventral striatum in long term addiction compared with short term addiction (Fig. 6).

## **Discussion**

Drug abuse is a serve social problem resulting in heavy financial burden, and deleterious consequences to individuals, families and society. Development of addictive therapies were urgent for financial and public-health perspective. PET is a suitable tool with quantitative capabilities for measuring the neuroanatomical distribution of specific receptors and the dynamic properties of these receptors, which contributed tremendously to the understanding of the underlying biology of drug addiction and treatment



development. Drug abuse were considered to stimulate the central reward circuit and the mesolimbic reward system. Self-administration animal models showed that animals possessed strong positive reinforcement of drugs, such as cocaine and morphine. CPP models show animals prefer environments associated with abused drugs, this model could help us to study the reward system in drug abuse [3, 4, 26].

In previous study, relative FDG uptake increased in cerebellum, thalamus, frontal, sensory and motor cortices, decreased in insular cortex and throughout the hippocampus in rats with place preference to methamphetamine addiction compared with controls, and FDG uptake correlated with individual differences in preference [2]. But no memory retrieval and dopamine activity were investigated. these study will be very helpful for drug abuse therapy.

In this study, we used FDG to investigate the glucose metabolism change in brain regions, we found PFC and cingulate cortex displayed hypometabolism in short term addiction compared with baseline. This result was consistent with previous report. An acute single cocaine injection in rats induced elevations of cerebral blood flow in the dorsolateral PFC by  $^{15}\text{O}\text{-H}_2\text{O}$  PET [27]. Glucose hypometabolism in PFC and striatum was observed in monkey with a single non-contingent cocaine compared with cocaine-naive monkeys [28]. Self-administration of cocaine in rhesus monkeys during PET image acquisition extend active metabolism in the PFC and striatum to anterior cingulate cortex [29]. And also, SUVr in PFC, striatum and thalamus demonstrated hypometabolism in cue-induced craving compared with baseline, PFC plays a key role in episodic memories, withdrawal memory retrieval, thalamus contributes to memory retrieval [7, 9], that means memory and memory retrieval is important to cue- induce craving.

We also noticed hypometabolism in hippocampus in voxel-wise analysis in rats with retrieval of memory compared with cue-induced craving. Hippocampus is the main region for memory and cognition, it has been considered as message initially learnt in the hippocampus is stored in distributed cortical networks [6]. We knew episodic memories are transformed from neocortical structures to being dependent on the hippocampus, and back again. Functional differentiation and connectivity of hippocampus to neocortex make it a hub for memory formation and transformation. mPFC is one of these structure which has been studied well [7, 6].

Primary somatosensory cortex alternation was observed in almost all of these group comparisons. Primary somatosensory cortex is responsible for processing somatic sensations and cross-modal working memory, it also contributes to deficits in motor dysfunction and involved in several types of motor skill learning, motor memory [30–34]. In this study, alternation in primary somatosensory cortex may mainly contribute by the behavior of addiction and anesthesia in PET study. Primary somatosensory cortex could be another structure connected to hippocampus playing a key role in long-lasting memories and episodic memories encoding. Long-lasting memories could last at least two weeks in rodents, which independently retrievable by cortical structure. Glucose hypometabolism in primary motor cortex also found in rats with memory retrieval compared with long term addiction. It could also be one terminal of

this circuit as PFC and primary somatosensory cortex. And both of them were involved in motor functions, which implicated motor function could play a role in memory of craving.

Striatum is another region play a key role in addiction, episodic memories. Only small part of ventral striatum displayed elevated DAT expression in long term addiction compared with short term addiction. Dopamine is a common molecular target for specialized treatments for each abused compound or behavior. Such as D2-like receptors is one of the most important receptors in drug addiction, which located pre- and post-synaptically. Decreased D2-like receptor expression enhanced vulnerability to drug use, and chronic drug abuse further decreases D2-like receptor function with developing a cycle toward addiction [35]. And there was no association between D2-like receptor binding potential and drug dose, but a significant relationship between D2-like function and duration of drug use [35].

## Conclusion

PFC, striatum and hippocampus were the main regions in addiction and memory retrieval of cue-induced craving. Primary somatosensory cortex and primary motor cortex could be neocortical structure in hippocampal and neocortical circuits for episodic memories with PFC in memory retrieval of cue-induced craving. DAT expression could involve in behaviors of addiction but not in cue-induced memory.

## Declarations

**Compliance with Ethical Standards:**

### Funding

This research was sponsored by the National Science Foundation of China (81801752, 81571345), the Shanghai Sailing Program (18YF1403200, 19YF1405300, 20YF1404400), startup fund of Huashan Hospital, Fudan University (2017QD081), Shanghai Municipal Key Clinical Specialty (shslczdzk03402), Shanghai Municipal Science and Technology Major Project (No.2018SHZDZX03) and ZJLab.

### Conflict of interest

The authors declare no conflict of interest.

**Ethical approval:** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

### Acknowledgments

The authors want to thank Zhiwei Pan, Yue Qian for their generous assistance for this study.

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## Figures

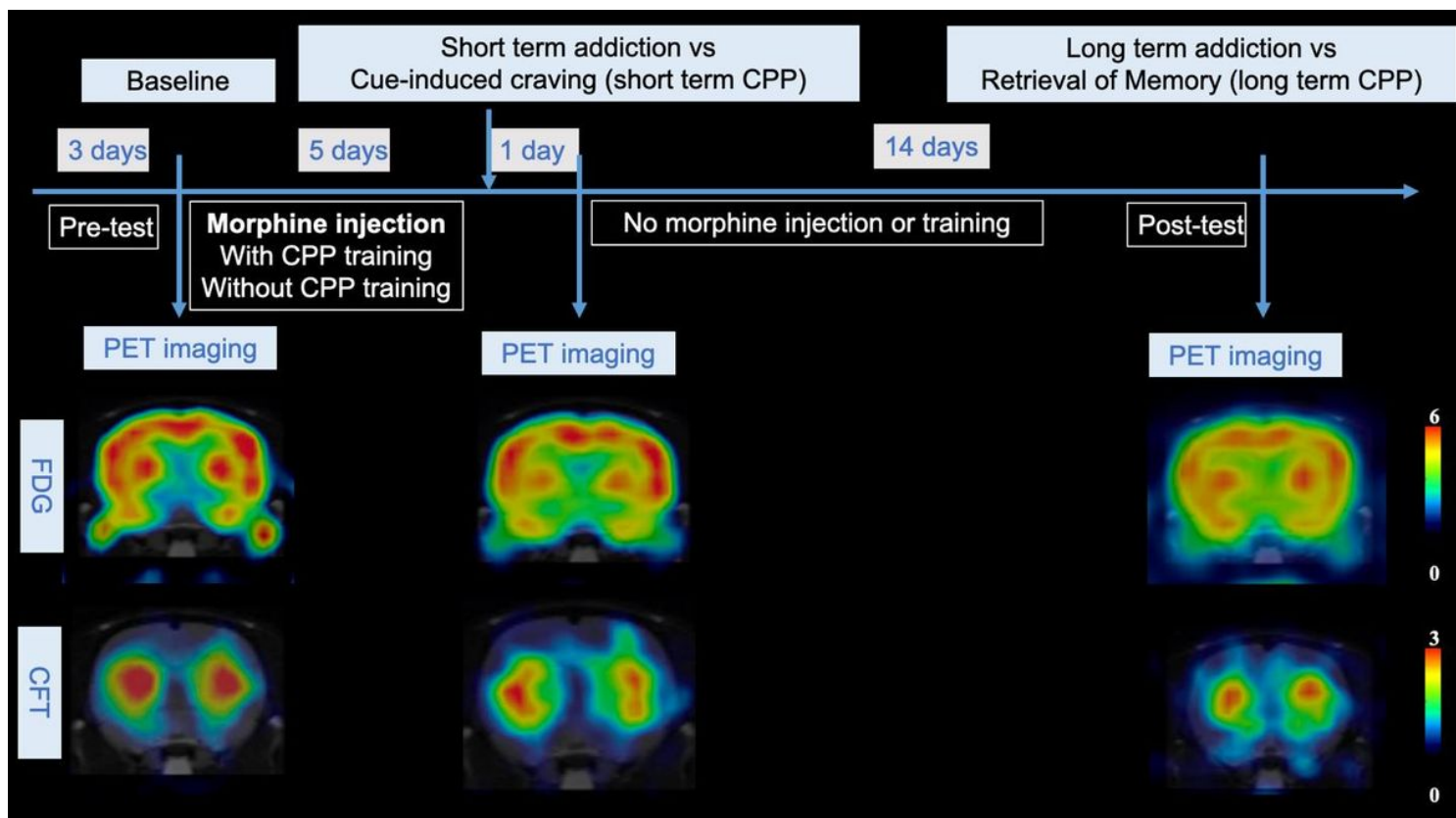
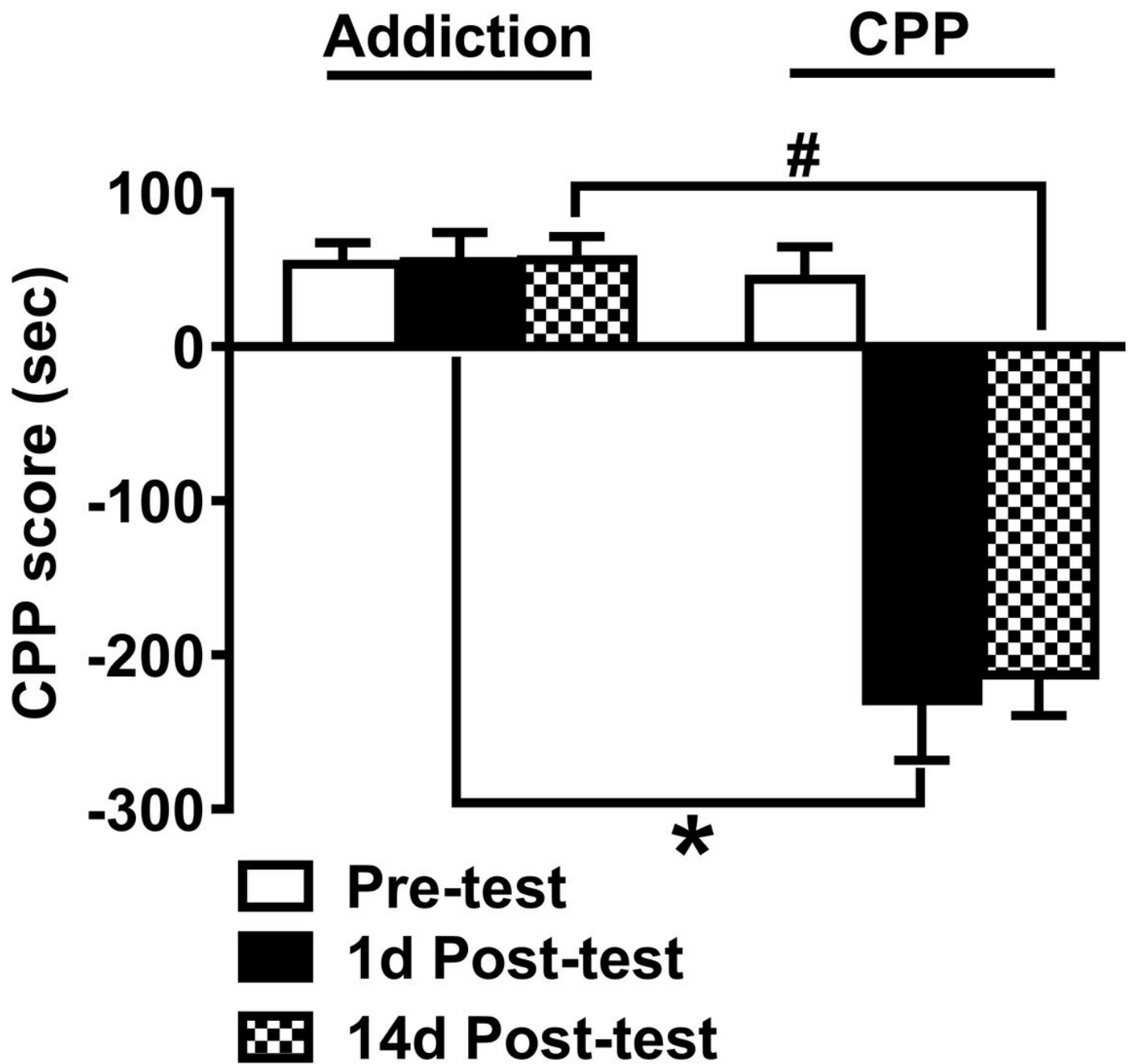


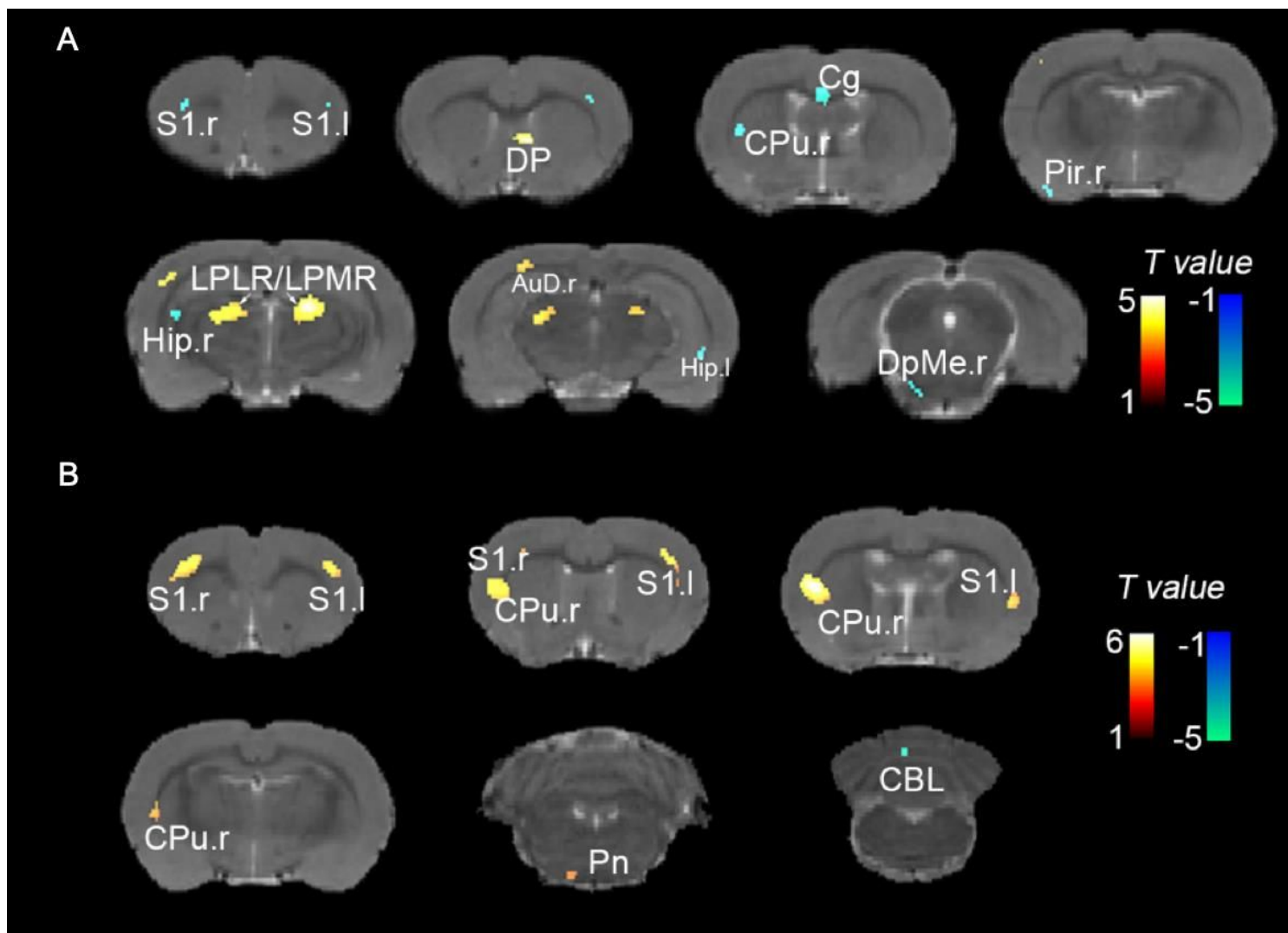
Figure 1

Study design of this longitudinal study of change of DAT, and glucose metabolism in rat brains with morphine addition in CPP condition.



**Figure 2**

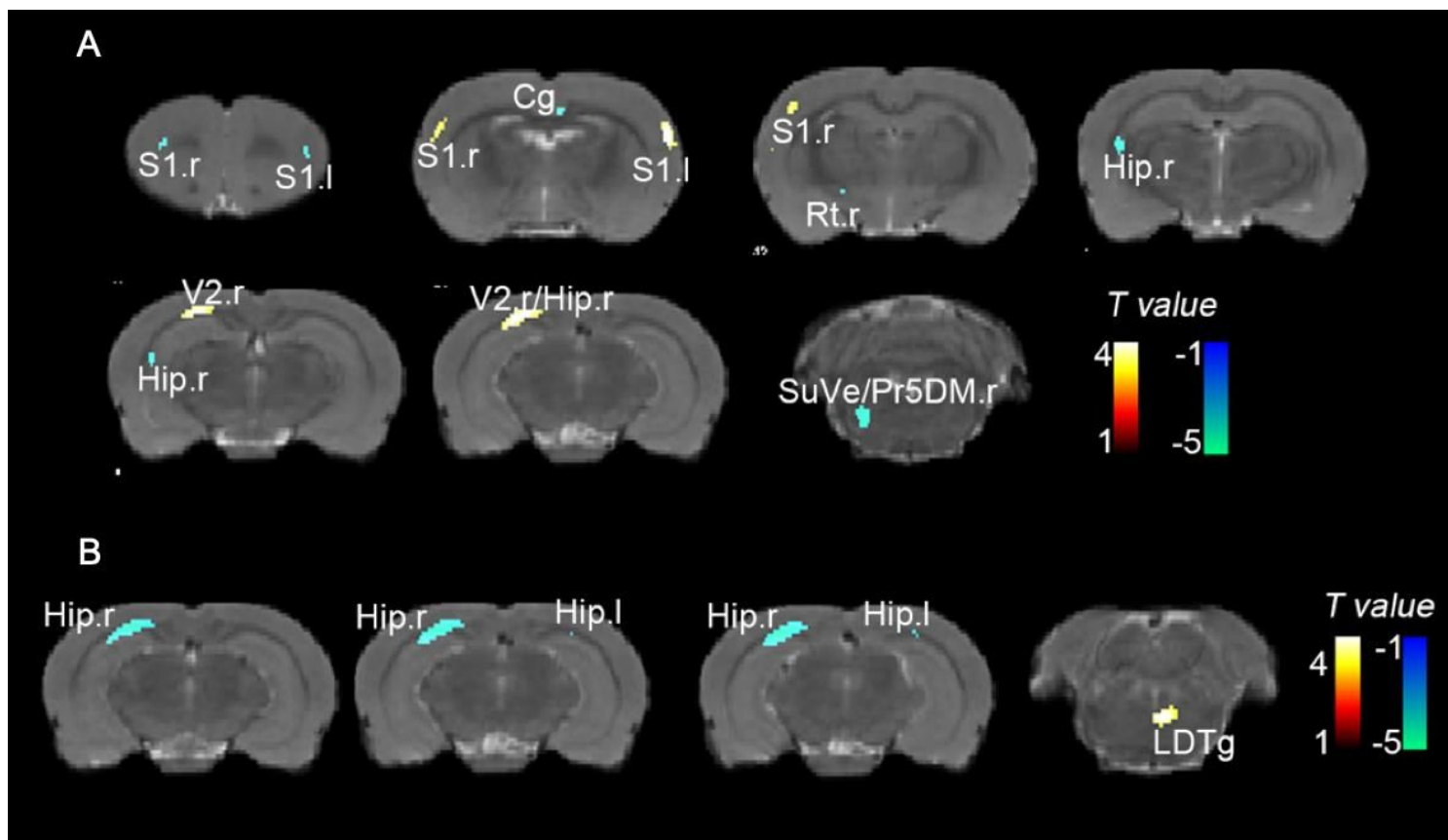
CPP score before (pre-test) and after (post-test) CPP training in rats with addition. The difference of CPP scores was significantly higher after 1 day and 14 days of CPP training.



**Figure 3**

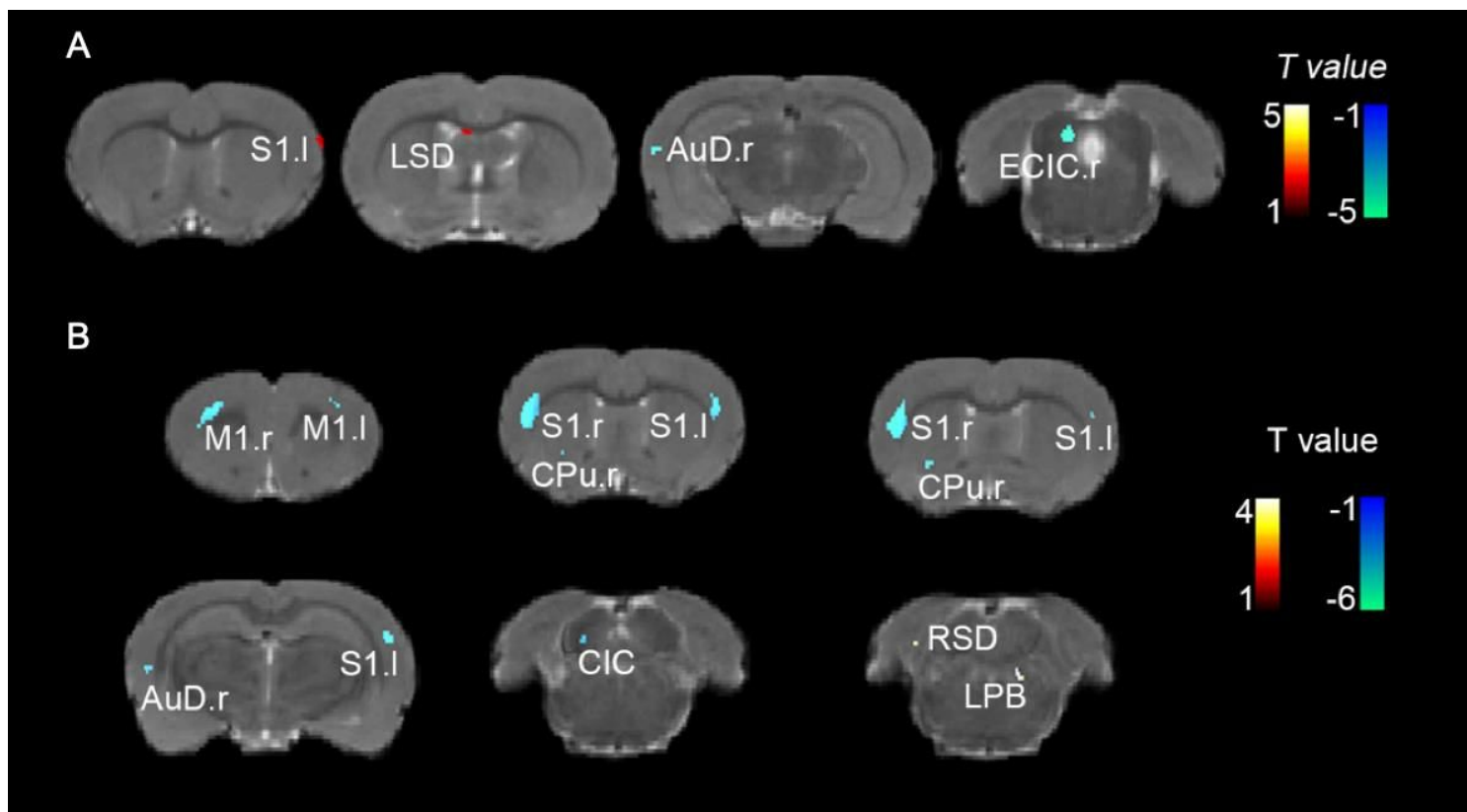
Brain regional glucose metabolism alternation in addiction. A: Brain regional glucose metabolism alternation between baseline and short term addiction; B: Brain regional glucose metabolism alternation between addition in short term and long term. The color bar stands for the T value.





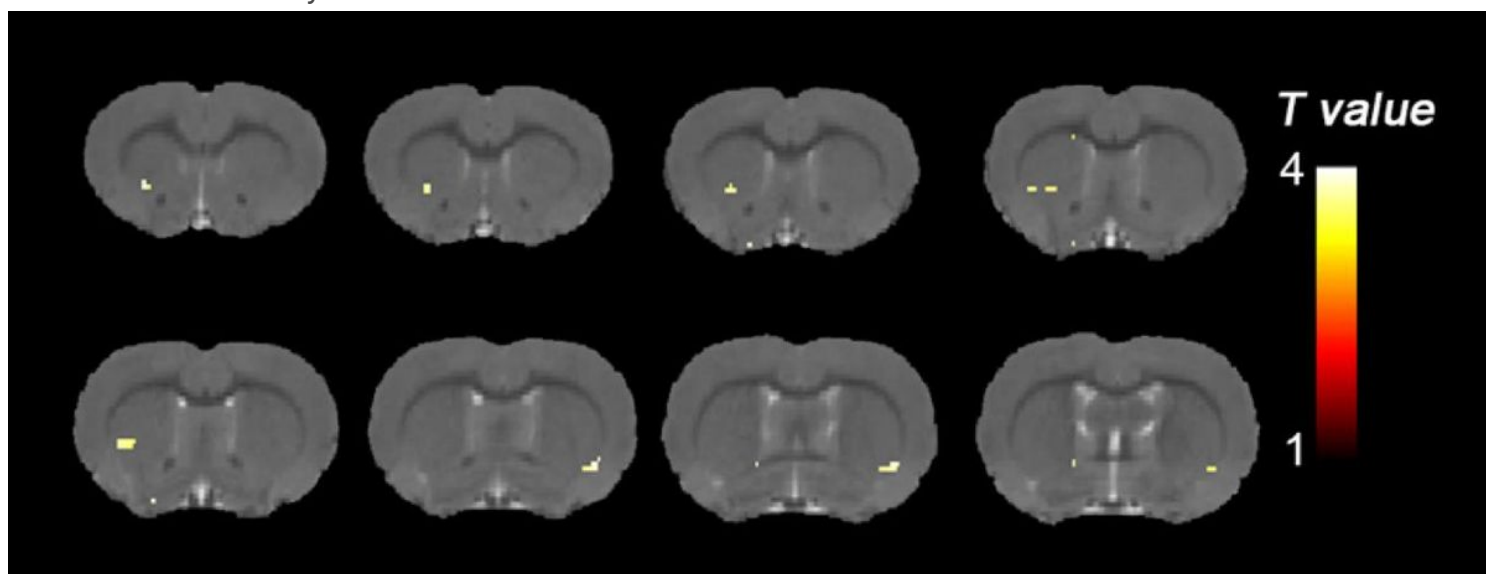
**Figure 4**

Brain regional glucose metabolism alternation in addiction with CPP and environmental cue-induced memory. A: Brain regional glucose metabolism alternation between baseline and addiction with short term CPP; B: Brain regional glucose metabolism alternation between short term CPP and environmental cue-induced memory. The color bar stands for the T value.



**Figure 5**

Brain regional glucose metabolism alternation in addiction and CPP. A: Brain regional glucose metabolism alternation between short term addiction and short term CPP; Right: Brain regional glucose metabolism alternation between long term addiction and long term CPP. The color bar stands for the T value from SPM analysis.



**Figure 6**

Brain regional DAT expression alternation in addiction. Brain regional DAT expression alternation between addition in short term and long term. The color bar stands for the T value.

## Supplementary Files

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