

Alteration of Neuroinflammation Detected by ^{18}F -GE180 PET Imaging in Place-Conditioned Rats with Morphine Withdrawal

Junpeng Li

Huashan Hospital Fudan University <https://orcid.org/0000-0001-6775-8375>

Da Shao

Collaborative Innovation Center for Brain Science, School of Basic Medical Science and Institutes of Brain Science, Fudan University

Donglang Jiang

Huashan Hospital Fudan University

Qi Huang

Huashan Hospital Fudan University

Yihui Guan

Huashan Hospital Fudan University

Bin Lai

Collaborative Innovation Center for Brain Science, School of Basic Medical Sciences and Institutes of Brain Science, Fudan University

Jun Zhao

Dongfang Hospital Tongji University

Fengchun Hua

Longhua Hospital Shanghai University of Traditional Chinese Medicine

Fang Xie (✉ fangxie@fudan.edu.cn)

Huashan Hospital Fudan University

Research Article

Keywords: Drug addiction, Neuroinflammation, Conditioned place aversion, Translocator protein 18 kDa, Positron emission tomography

Posted Date: June 21st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-621858/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Accumulating evidence indicates that neuroinflammation significantly contributes to drug addiction, but the conversion of neuroinflammation after drug withdrawal is not clear. Here, we conducted ^{18}F -flutriciclamide (GE180) positron emission tomography (PET) imaging to investigate the conversion of neuroinflammation during drug withdrawal and conditioning-induced aversion by measuring the change in microglial activation with ^{18}F -GE180.

Methods: Twelve male adult Sprague-Dawley rats were subjected to morphine withdrawal by the administration of naloxone, and six of them were used to model conditioned place aversion (CPA). ^{18}F -GE180 PET imaging was performed for 11 rats on the last day of the morphine treatment phase and for 10 rats on the response assessment phase of the behavior conditioning procedure. A GE180 template was established for spatial normalization of each individual image, and the differential ^{18}F -GE180 uptakes between the drug withdrawal (DW) group and the drug addiction (DA) group, the CPA group and the DA group, and the CPA group and the DW group were compared by a voxel-wise two-sample t-test using SPM8.

Results: Both the DW group and the CPA group spent less time in the conditioning cage during the posttest phase compared with the pretest phase, but only the difference in the CPA group was significant (63.2 ± 34.6 vs. -159.53 ± 22.02 , $P < 0.005$). Compared with the DA group, the uptake of ^{18}F -GE180 increased mainly in the hippocampus, visual cortex, thalamus and midbrain regions and decreased mainly in the sensory-related cortices after the administration of naloxone in both the DW and CPA groups. Increased ^{18}F -GE180 uptake was only observed in the mesolimbic regions after conditioned aversion compared with the DW group.

Conclusion: In morphine-dependent rats, neuroinflammation became more severe in the addiction-involved brain regions but remitted in the sensory-related brain regions after the administration of naloxone, and this neuroinflammation induced by withdrawal was further aggravated after conditioned aversion formation thus may help to consolidate the withdrawal memory.

Background

Drug addiction is mainly characterized by the repeated use of addictive drugs. Recently, accumulating evidence has indicated that glial activation, including microglia and astrocytes, and the associated neuroinflammatory signals significantly contribute to drug addiction[1,2], while modulation of glial activation shows some drug withdrawal symptoms[3]. However, the evolution of neuroinflammation after drug withdrawal is not clear.

Place conditioning is commonly used in cue-elicited drug craving and the treatment of addiction since the rewarding/aversive effects of the drug administered with the environment play a key role in addiction treatment[4]. Steven et al. conducted a conditioned place preference (CPP) assay to detect the efficacy of

dexamethasone in attenuating the augmentation of the behavioral response to cocaine and suggested that anti-inflammatory agents may be effective in normalizing the rewarding effects of cocaine[5]. Conditioned place aversion (CPA) was more commonly used in investigating the mechanism of withdrawal. Indeed, CPA induces several brain function changes such as learning-induced synaptic plasticity[6], brain derived neurotrophic factor (BDNF) and immediate early genes (IEGs) expression[7], protein kinase A (PKA) and kappa opioid receptor (KOR) changes[8]. But the effect of CPA training on neuroinflammation is less investigated.

Translocator protein 18 kDa (TSPO), formerly known as the peripheral benzodiazepine receptor, is highly expressed in phagocytic cells of the immune system, such as microglia and macrophages[9]. The radioligands of TSPO for positron emission tomography (PET) can detect neuroinflammation, mainly microglial activation and proliferation, *in vivo*[10]. The development of ^{11}C -PK11195 as the first-generation TSPO PET ligand made TSPO a surrogate marker of neuroinflammation[11,12]. However, the short half-life of ^{11}C , poor signal-to-noise ratio due to high nonspecific binding and rather problematic radiochemistry limit its clinical application[12]. Due to the issues of ^{11}C -PK11195 and the increased interest in neuroinflammation, improved Fluorine labeled TSPO PET tracers, such as ^{18}F -DPA-714 and ^{18}F -GE180, have been developed[13,14]. These second-generation tracers possessed improved binding affinity and better kinetics. Here, we chose ^{18}F -GE180 (Flutriciclamide) to detect the neuroinflammation in conditioned behavior *in vivo* for better understanding of the mechanism of neuroinflammation in drug addiction and aid in the search for treatments for addiction.

In this study, we constructed a digital ^{18}F -GE180 template for automated whole-brain analysis of ^{18}F -GE180 PET images. Then, we aimed to measure the change in neuroinflammation after naloxone treatment for morphine addiction and naloxone-CPA using ^{18}F -GE180 PET imaging with a new digital ^{18}F -GE180 template. We hypothesized that naloxone-induced morphine-withdrawal will arise neuroinflammation which account for aversion effects, and CPA training may aggravate the neuroinflammation thus help to consolidate withdrawal memory.

Methods

Animals and radiopharmaceuticals

Twelve male adult Sprague-Dawley rats (220-250 g) were housed singly in a 12 h light/dark cycle with free access to water and food and controlled temperature and humidity. All experimental procedures were performed according to protocols approved by Fudan University, Shanghai, China, and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals as well as the ARRIVE guidelines, original version. All efforts were made to minimize animal suffering and reduce the number of animals use. ^{18}F -GE180 was prepared at the PET Center, Huashan Hospital, Fudan University, under the requirement of GMP.

Chronic morphine treatment

Male adult Sprague-Dawley rats were treated with morphine according to procedures described previously[15]. Briefly, morphine dependence was induced in rats by repeated intraperitoneal (i.p.) injections of morphine twice daily at 8:00 A.M. and 7:00 P.M. The morphine dose was progressively increased from 10 mg/kg to 40 mg/kg: day 1, 2 × 10 mg/kg; day 2, 2 × 20 mg/kg; day 3, 2 × 30 mg/kg; and days 4 and 5, 2 × 40 mg/kg. Control rats were treated with saline following the same procedure.

Conditioned place aversion

The procedure for CPA was similar to that described previously[16,17]. CPA took place in a three-compartment place conditioning apparatus (Med Associates, United States) with distinct visual and tactile contexts, which are readily discriminated by rats. The CPA procedure consisted of three phases: preconditioning, drug treatment, and conditioning phases. In the preconditioning phase (Day 1), rats were given an initial assessment of their baseline preference to determine whether rats had a preexisting preference for any of the three compartments. They were placed in the central neutral area of the apparatus for 2 min and allowed to freely explore the apparatus for 15 min. Rats showing strong unconditioned aversion or preference for any compartment were eliminated (i.e., $\leq 20\%$ or $\geq 80\%$ of the session time, the number of excluded rats did not exceed 10% of the sample). During the drug treatment phase (days 2-6), rats were treated with morphine for 5 consecutive days as described in the “Chronic morphine treatment” section and assigned to the drug addiction (DA) group. In the conditioning phase (days 7-10), rats were divided into drug withdrawal (DW) group and CPA group randomly. The CPA group received a naloxone injection (0.3 mg/kg) (to precipitate morphine withdrawal) after a morphine injection (40 mg/kg) before being placed in the minor preference compartment on days 7 and 9, and on the alternate days (days 8 and 10), they received a saline injection after a morphine injection (40 mg/kg) before being placed in the opposite compartment. To distinguish conditioned responses, rats in the withdrawal group received a naloxone injection (0.3 mg/kg) after a morphine injection (40 mg/kg) in the home cage on days 7 and 9 and a saline injection after a morphine injection (40 mg/kg) in the home cage on days 8 and 10. After one day of retrieval since the conditioning phase, each rat was placed blind in the same apparatus for 15 min to assess the place aversion response on day 12 (the response-assess phase). The time that rats spent in the compartment on the preconditioning phase was recorded as the pretest time, and that on the response-assess phase was recorded as the posttest time. The CPA score was defined as the time in the minor preference compartment minus the time in the opposite compartment. Since withdrawal/aversion conditioning was performed in the minor preference compartment, the opposite compartment became a more preferred compartment at retrieval after conditioning in the conditioned withdrawal/aversion group. Therefore, the CPA score was positive in the pretest session but was negative at retrieval in the conditioned withdrawal group.

PET scanning

Micro-PET/CT imaging was conducted as described previously using a micro-PET/CT scanner (Siemens Inc., United States)[18,19]. Briefly, static PET/CT imaging was collected for 20 min at 40 min post intravenous injection[14] of ^{18}F -GE180 (296-444 MBq/kg body weight) and at 50 min post intravenous

injection of ^{18}F -FDG (370-555 MBq/kg body weight). Then, the rats were positioned in a spread-supine position on the imaging bed and anesthetized by 2–3% isoflurane in medical oxygen (1-2 L/min) at room temperature with an isoflurane vaporizer (Molecular Imaging Products Company, United States) during the PET/CT procedure. PET/CT images were reconstructed using the ordered subsets expectation maximization 3D algorithm, and data were reviewed using Inveon Research Workplace software (Siemens). GE180 imaging was performed on the last day of the drug treatment phase (day 6, defined as the drug addiction group) and the response assessment phase (day 12), while FDG imaging was only performed on day 6 to assist in the establishment of the GE180 template. One animal died during the GE180 imaging on day 6, and one died during CPA training, so the final sample size of DA, DW and CPA groups was 11, 6 and 5, respectively.

Construction of the ^{18}F -GE180 PET template

The rat ^{18}F -GE180 PET template was reconstructed by a method described previously using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; <http://www.fil.ion.ucl.ac.uk/sp>)[20,21]. Briefly, we first coregistered all of the GE180 images to their individual FDG images obtained on day 6 due to the low cerebral uptake of GE180. Then, the individual FDG images were normalized to a rat FDG template of a toolbox of SPM8 named *spmatHEP*[22], and the transformation parameters were used for the normalization of all of the individual GE180 images. After that, the matrix value of the normalized GE180 images was rescaled to [0 255]. Finally, the normalized GE180 images were arithmetically averaged to create the template (Fig. 1). Since the averaged image after multiple iterations was distorted, all of the steps, including chorister, spatial normalization, and arithmetic average, were repeated only once.

Statistical analysis

Statistical analysis was conducted by GraphPad Prism 8. The normality of the distribution of the CPA scores were tested by Shapiro-Wilk normality test and Kolmogorov-Smirnov normality test. The difference of the CPA scores between multi-groups were analyzed by one-way ANOVA following by Bonferroni post hoc analysis, statistical significance was determined as $p < 0.05$.

To detect the change in the activation of glia after morphine withdrawal and CPA training, a voxel-wise two-sample t-test was performed by SPM8 with global mean intensity calculation to compare the different ^{18}F -GE180 uptake between the DW group, the DA group and the CPA group in pairs using the toolbox *spmatHEP*. A significance threshold was set as $P < 0.001$ with nonmultiple correction, and $K > 20$ voxels was set as the cluster extent threshold.

Results

Construction of the ^{18}F -GE180 template in rats

A ^{18}F -GE180 template was established (Fig. 2) for the spatial normalization of each individual image, and the coordinate of the template fitted the atlas image in Paxinos & Watson space[23]. The uptake of

GE180 was mainly concentrated in the area around the ventricle and was low in the cortex area, which was consistent with previous research[24].

Establishment of the CPA animal model

As shown in Fig. 3B. After CPA training, the CPA score in the CPA group rats was significantly decreased compared with that in the withdrawal group (CPA score, -159.53 ± 22.02 vs. 30.18 ± 28.89 , $P < 0.0001$). The difference in the CPA group between the pretest and posttest was also statistically significant (CPA score, 63.2 ± 34.6 vs. -159.53 ± 22.02 , $P < 0.005$).

Alternation of the brain uptake in different rats

Change in ^{18}F -GE180 uptake after the administration of naloxone and conditioning training

The primary analysis investigated the change in the activation of glia after drug withdrawal and conditioning training (Fig. 4). After the administration of naloxone, the DW group displayed increased ^{18}F -GE180 uptake in the bilateral visual and cingulate cortices, right hippocampus, thalamus and midbrain as well as decreased ^{18}F -GE180 uptake in the bilateral sensory, insular and piriform cortices, optic chiasm, left hippocampus, and pons compared with the DA group (Fig. 4A). Similar but wider results were found in the CPA group than in the DA group, as shown in Fig. 4B. The CPA group displayed increased ^{18}F -GE180 uptake in the bilateral visual cortex, left entorhinal cortex, left septal nucleus, bilateral caudate putamen, bilateral hippocampus, thalamus, midbrain and cerebellum and decreased uptake of ^{18}F -GE180 in the bilateral sensory, insular and piriform cortices, left caudate putamen, optic chiasm, olfactory, and medulla compared with the DA group.

Increased ^{18}F -GE180 uptake in the CPA group compared with the withdrawal group

Additional analysis aimed to investigate the conversion of neuroinflammation caused by withdrawal after conditioning training. Fig. 5 indicates aggravated neuroinflammation after conditioning training since only increased ^{18}F -GE180 uptake was obtained in the right sensory cortex, right medial entorhinal cortex, left hippocampus, left caudate putamen, left hypothalamus, and midbrain regions in the CPA group compared with the withdrawal group.

Discussion

In addition to numerous studies illustrating the contribution of neuroinflammation to drug addiction, we established a ^{18}F -GE180 PET template and further investigated the change in microglial activation during drug withdrawal and conditioning-induced aversion in a rodent model. This is the first study to set up a ^{18}F -GE180 PET template for small animals, although we did not perform any validation in the statistical analysis with this small sample size, we provided a method to set up a ^{18}F -GE180 PET template for small animals.

Previous studies found that microglial activation accounts for withdrawal symptoms, and blocking the activation of microglial cells can alleviate this symptom[25,26]. An *in vitro* experiment also revealed that the administration of naloxone resulted in increased expression of inflammatory mediators, including tissue necrosis factor-alpha (TNF- α), interleukin-1-beta (IL-1 β), interleukin-6 (IL-6), and interleukin-10 (IL-10), in brain tissues of morphine-dependent mice[27]. Both *in vivo* and *ex vivo* studies have demonstrated that TSPO was well parallel with the microglial activation[28,29], and PET imaging using TSPO ligands is a promising tool to visualize neuroinflammation induced by the activated microglial[30-32]. In our *in vivo* study, despite the wider regions the CPA group exhibited than the DW group in comparison with the DA group, both groups presented overexpression of ^{18}F -GE180 mainly in the hippocampus, visual cortex, thalamus, and midbrain. Similar results were reported in a methadone maintenance treatment of heroin-related addiction by fMRI that the protracted abstinence patients demonstrated significantly higher brain responses in mesolimbic regions and visuospatial-attention regions[33]. The hippocampus plays an important role in memory, and morphine exposure, regardless of acute or chronic exposure, impairs hippocampus-dependent spatial learning and memory[34,35]. The visual cortex has been demonstrated to be impacted by different behavior contexts[36] and was found to be activated during drug-cue-induced craving[37]. The thalamus, which is a vital sensory center, was found to be involved in the reinstatement, extinction, and expression of drug-seeking behaviors[38,39]. The midbrain is necessary for locomotion[40], and it has been reported that heightened midbrain activations may reduce the approach motivation for cocaine[41]. All of these findings suggested that addiction-involved regions may be vulnerable to neuroinflammation during the withdrawal stage.

More importantly, after CPA training, microglial activation was increased compared with that in the DW group. The regions mainly include the mesolimbic regions (right medial entorhinal cortex, left hippocampus, left caudate putamen, left hypothalamus, and midbrain). The reduced CPA score also supported this imaging result: after conditioning training, rodents were even less willing to stay in the compartment where they received naloxone injection. Furthermore, the medial entorhinal cortex could connect with the hippocampus for episodic memory transfer[42]. Hence, we considered that the aggravated neuroinflammation that occurred in the hippocampus and midbrain after CPA training may account for the reinforcement of withdrawal memory and escape behavior.

We also detected reduced ^{18}F -GE180 uptake mainly in the sensory-related cortices (the sensory, insular and piriform cortices) in both the DW and CPA group rats compared with the DA group. There are two possible reasons for this result. One is that naloxone might not only cause neuroinflammation but also have an antagonistic effect on morphine[43], and previous reports have verified naloxone can reduce the activation of microglia by different manners[44,45]. We hypothesized that the reduced microglia activation observed in this study might be due to the antagonistic effect on morphine. The second is that the specificity of TSPO for neuroinflammation and glial cells is not exclusive. TSPO is also expressed by endothelial cells[46]; in addition, the expression of TSPO may indicate the denseness of microglial cells instead of activation[28]. Further *in vitro* and longitudinal studies are needed to validate these hypotheses.

There are also some limitations in this study. First is that we only enrolled male rats in order to eliminate the differences in behavior and neurobiology of drug addiction caused by sex[47,48], but how the neuroinflammation is affected by drug addiction/withdrawal in female animals still needs to be illustrated in further research. Second is that in this animal experiment, we did not measure the affinity of brain microglia to GE180, because there is no significant difference in the affinity of brain microglia in rats. However, when applied in clinical practice, we consider that microglia with different affinities in human brain will have an impact on the uptake of GE180, which should be taken into account.

Conclusions

In this rodent study, we set up a ^{18}F -GE180 template to investigate the conversion of neuroinflammation by detecting changes in glial activation in the brains of naloxone-induced morphine withdrawal rats and CPA model rats. Specifically, after naloxone treatment, neuroinflammation became more severe in the addiction-involved regions but remitted in the sensory-related regions, and this neuroinflammation induced by withdrawal was further aggravated after conditioned aversion formation thus may help to consolidate the withdrawal memory. TSPO PET can well monitor the change of neuroinflammation during morphine withdrawal course.

List Of Abbreviations

CPP: conditioned place preference

CPA: conditioned place aversion

DA: drug addiction

DW: drug withdrawal

GE180: ^{18}F -Flutriciclamide

TSPO: translocator protein 18 kDa

Declarations

Ethics approval and consent to participate

All experimental procedures were performed according to protocols approved by Fudan University, Shanghai, China, and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals as well as the ARRIVE guidelines, original version.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

All the raw data of this study, including PET images and behavior data, can be obtained through the corresponding authors on reasonable request.

Competing interest

The authors declare no competing interest in this article.

Funding

This research was sponsored by the National Science Foundation of China (82071962, 82001143), the Shanghai Sailing Program (19YF1405300, 20YF1404400), the Shanghai Municipal Science and Technology Major Project and ZJLab (No. 2018SHZDZX01), and the Shanghai Municipal Key Clinical Specialty (shslczdzk03402).

Author contributions

JL and FX contributed to the conception and design of the study. DS and DJ designed and performed the animal experiments. FX produced the radiotracers. JL collected the PET/CT data. JL and QH contributed to the analysis and interpretation of the data. JL wrote the draft of this manuscript. YG and BL reviewed the study design and helped to redesign this study. FX, FH and JZ contributed to critical review and revision of the manuscript for this article.

Acknowledgements

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

References

- [1] Bachtell RK, Jones JD, Heinzerling KG, Beardsley PM, Comer SD. Glial and neuroinflammatory targets for treating substance use disorders. *Drug Alcohol Depend.* 2017;180:156-70. doi:10.1016/j.drugalcdep.2017.08.003.
- [2] Lacagnina MJ, Rivera PD, Bilbo SD. Glial and Neuroimmune Mechanisms as Critical Modulators of Drug Use and Abuse. *Neuropsychopharmacology.* 2017;42:156-77. doi:10.1038/npp.2016.121.
- [3] Cooper ZD, Johnson KW, Pavlicova M, Glass A, Vosburg SK, Sullivan MA, et al. The effects of ibudilast, a glial activation inhibitor, on opioid withdrawal symptoms in opioid-dependent volunteers. *Addict Biol.* 2016;21:895-903. doi:10.1111/adb.12261.
- [4] Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nat Protoc.* 2006;1:1662-70. doi:10.1038/nprot.2006.279.

- [5] Merkel SF, Andrews AM, Lutton EM, Razmpour R, Cannella LA, Ramirez SH. Dexamethasone Attenuates the Enhanced Rewarding Effects of Cocaine Following Experimental Traumatic Brain Injury. *Cell Transplant*. 2017;26:1178-92. doi:10.1177/0963689717714341.
- [6] Pati D, Pina MM, Kash TL. Ethanol-induced conditioned place preference and aversion differentially alter plasticity in the bed nucleus of stria terminalis. *Neuropsychopharmacology*. 2019;44:1843-54. doi:10.1038/s41386-019-0349-0.
- [7] Garcia-Perez D, Ferenczi S, Kovacs KJ, Victoria Milanés M. Distinct regulation pattern of Egr-1, BDNF and Arc during morphine-withdrawal conditioned place aversion paradigm: Role of glucocorticoids. *Behavioural Brain Research*. 2019;360:244-54. doi:10.1016/j.bbr.2018.12.026.
- [8] Song XH, Li WQ, Shi YZ, Zhang JD, Li Y. Expression of protein kinase A and the kappa opioid receptor in selected brain regions and conditioned place aversion in morphine-dependent rats. *Oncotarget*. 2017;8:82632-42. doi:10.18632/oncotarget.19671.
- [9] Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, et al. Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci*. 2006;27:402-9. doi:10.1016/j.tips.2006.06.005.
- [10] Li F, Liu J, Liu N, Kuhn LA, Garavito RM, Ferguson-Miller S. Translocator Protein 18 kDa (TSPO): An Old Protein with New Functions? *Biochemistry*. 2016;55:2821-31. doi:10.1021/acs.biochem.6b00142.
- [11] Leung K. 1-(2-Chlorophenyl)-N-[(11)C]methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide. *Molecular Imaging and Contrast Agent Database (MICAD)*. Bethesda (MD): National Center for Biotechnology Information (US); 2004.
- [12] Chauveau F, Boutin H, Van Camp N, Dollé F, Tavitian B. Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. *Eur J Nucl Med Mol Imaging*. 2008;35:2304-19. doi:10.1007/s00259-008-0908-9.
- [13] Boutin H, Prenant C, Maroy R, Galea J, Greenhalgh AD, Smigova A, et al. [18F]DPA-714: direct comparison with [11C]PK11195 in a model of cerebral ischemia in rats. *PLoS One*. 2013;8:e56441. doi:10.1371/journal.pone.0056441.
- [14] Boutin H, Murray K, Pradillo J, Maroy R, Smigova A, Gerhard A, et al. 18F-GE-180: a novel TSPO radiotracer compared to 11C-R-PK11195 in a preclinical model of stroke. *Eur J Nucl Med Mol Imaging*. 2015;42:503-11. doi:10.1007/s00259-014-2939-8.
- [15] Desjardins S, Belkai E, Crete D, Cordonnier L, Scherrmann JM, Noble F, et al. Effects of chronic morphine and morphine withdrawal on gene expression in rat peripheral blood mononuclear cells. *Neuropharmacology*. 2008;55:1347-54. doi:10.1016/j.neuropharm.2008.08.027.

- [16] Zhao Y, Zhang J, Yang H, Cui D, Song J, Ma Q, et al. Memory retrieval in addiction: a role for miR-105-mediated regulation of D1 receptors in mPFC neurons projecting to the basolateral amygdala. *BMC Biol.* 2017;15:128. doi:10.1186/s12915-017-0467-2.
- [17] Chen M, Shao D, Fu Y, Ma Q, Chen M, Cui D, et al. Key determinants for morphine withdrawal conditioned context-induced increase in Arc expression in anterior cingulate cortex and withdrawal memory retrieval. *Exp Neurol.* 2019;311:234-46. doi:10.1016/j.expneurol.2018.10.009.
- [18] Xie F, Xi Y, Pascual JM, Muzik O, Peng F. Age-dependent changes of cerebral copper metabolism in Atp7b (-/-) knockout mouse model of Wilson's disease by [(64)Cu]CuCl(2)-PET/CT. *Metab Brain Dis.* 2017;32:717-26. doi:10.1007/s11011-017-9956-9.
- [19] Jiang D, Lu X, Li Z, Rydberg N, Zuo C, Peng F, et al. Increased Vesicular Monoamine Transporter 2 (VMAT2) and Dopamine Transporter (DAT) Expression in Adolescent Brain Development: A Longitudinal Micro-PET/CT Study in Rodent. *Front Neurosci.* 2018;12:1052. doi:10.3389/fnins.2018.01052.
- [20] Verhaeghe J, Wyffels L, Wyckhuys T, Stroobants S, Staelens S. Rat brain normalization templates for robust regional analysis of [11C]ABP688 positron emission tomography/computed tomography. *Mol Imaging.* 2014;13. doi:10.2310/7290.2014.00037.
- [21] Huang Q, Nie B, Ma C, Wang J, Zhang T, Duan S, et al. Stereotaxic (18)F-FDG PET and MRI templates with three-dimensional digital atlas for statistical parametric mapping analysis of tree shrew brain. *J Neurosci Methods.* 2018;293:105-16. doi:10.1016/j.jneumeth.2017.09.006.
- [22] Nie B, Liu H, Chen K, Jiang X, Shan B. A statistical parametric mapping toolbox used for voxel-wise analysis of FDG-PET images of rat brain. *PLoS One.* 2014;9:e108295. doi:10.1371/journal.pone.0108295.
- [23] Paxinos. G, Watson C. *The Rat Brain in Stereotaxic Coordinates - The New Coronal Set 5th Edition* ed: Academic Press; 2004.
- [24] Deussing M, Blume T, Vomacka L, Mahler C, Focke C, Todica A, et al. Data on specificity of [(18)F]GE180 uptake for TSPO expression in rodent brain and myocardium. *Data Brief.* 2018;19:331-6. doi:10.1016/j.dib.2018.04.133.
- [25] Hutchinson MR, Lewis SS, Coats BD, Skyba DA, Crysedale NY, Berkelhammer DL, et al. Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (ibudilast). *Brain Behav Immun.* 2009;23:240-50. doi:10.1016/j.bbi.2008.09.012.
- [26] Burma NE, Bonin RP, Leduc-Pessah H, Baimel C, Cairncross ZF, Mousseau M, et al. Blocking microglial pannexin-1 channels alleviates morphine withdrawal in rodents. *Nat Med.* 2017;23:355-60. doi:10.1038/nm.4281.
- [27] Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Amirgholami N, Houshmand G, Alboghobeish S. Venlafaxine inhibits naloxone-precipitated morphine withdrawal symptoms: Role of inflammatory

cytokines and nitric oxide. *Metab Brain Dis.* 2020;35:305-13. doi:10.1007/s11011-019-00491-4.

[28] Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry.* 2015;72:268-75. doi:10.1001/jamapsychiatry.2014.2427.

[29] Tyler RE, Kim SW, Guo M, Jang YJ, Damadzic R, Stodden T, et al. Detecting neuroinflammation in the brain following chronic alcohol exposure in rats: A comparison between in vivo and in vitro TSPO radioligand binding. *Eur J Neurosci.* 2019;50:1831-42. doi:10.1111/ejn.14392.

[30] Walker MD, Dinelle K, Kornelsen R, Lee NV, Miao Q, Adam M, et al. [11C]PBR28 PET imaging is sensitive to neuroinflammation in the aged rat. *J Cereb Blood Flow Metab.* 2015;35:1331-8. doi:10.1038/jcbfm.2015.54.

[31] Auvity S, Saba W, Goutal S, Leroy C, Buvat I, Cayla J, et al. Acute Morphine Exposure Increases the Brain Distribution of [18F]DPA-714, a PET Biomarker of Glial Activation in Nonhuman Primates. *Int J Neuropsychopharmacol.* 2017;20:67-71. doi:10.1093/ijnp/pyw077.

[32] Keller T, Lopez-Picon FR, Krzyczmonik A, Forsback S, Kirjavainen AK, Takkinen JS, et al. [(18)F]F-DPA for the detection of activated microglia in a mouse model of Alzheimer's disease. *Nucl Med Biol.* 2018;67:1-9. doi:10.1016/j.nucmedbio.2018.09.001.

[33] Farahmandfar M, Karimian SM, Naghdi N, Zarrindast MR, Kadivar M. Morphine-induced impairment of spatial memory acquisition reversed by morphine sensitization in rats. *Behav Brain Res.* 2010;211:156-63. doi:10.1016/j.bbr.2010.03.013.

[34] Han H, Dong Z, Jia Y, Mao R, Zhou Q, Yang Y, et al. Opioid addiction and withdrawal differentially drive long-term depression of inhibitory synaptic transmission in the hippocampus. *Sci Rep.* 2015;5:9666. doi:10.1038/srep09666.

[35] Froudarakis E, Fahey PG, Reimer J, Smirnakis SM, Tehovnik EJ, Tolias AS. The Visual Cortex in Context. *Annu Rev Vis Sci.* 2019;5:317-39. doi:10.1146/annurev-vision-091517-034407.

[36] Hanlon CA, Dowdle LT, Naselaris T, Canterberry M, Cortese BM. Visual cortex activation to drug cues: a meta-analysis of functional neuroimaging papers in addiction and substance abuse literature. *Drug Alcohol Depend.* 2014;143:206-12. doi:10.1016/j.drugalcdep.2014.07.028.

[37] Huang AS, Mitchell JA, Haber SN, Alia-Klein N, Goldstein RZ. The thalamus in drug addiction: from rodents to humans. *Philos Trans R Soc Lond B Biol Sci.* 2018;373. doi:10.1098/rstb.2017.0028.

[38] Millan EZ, Ong Z, McNally GP. Paraventricular thalamus: Gateway to feeding, appetitive motivation, and drug addiction. *Prog Brain Res.* 2017;235:113-37. doi:10.1016/bs.pbr.2017.07.006.

- [39] Caggiano V, Leiras R, Goñi-Erro H, Masini D, Bellardita C, Bouvier J, et al. Midbrain circuits that set locomotor speed and gait selection. *Nature*. 2018;553:455-60. doi:10.1038/nature25448.
- [40] Moeller SJ, Tomasi D, Woicik PA, Maloney T, Alia-Klein N, Honorio J, et al. Enhanced midbrain response at 6-month follow-up in cocaine addiction, association with reduced drug-related choice. *Addict Biol*. 2012;17:1013-25. doi:10.1111/j.1369-1600.2012.00440.x.
- [41] Stinus L, Cador M, Zorrilla EP, Koob GF. Buprenorphine and a CRF1 antagonist block the acquisition of opiate withdrawal-induced conditioned place aversion in rats. *Neuropsychopharmacology*. 2005;30:90-8. doi:10.1038/sj.npp.1300487.
- [42] Chao OY, de Souza Silva MA, Yang YM, Huston JP. The medial prefrontal cortex - hippocampus circuit that integrates information of object, place and time to construct episodic memory in rodents: Behavioral, anatomical and neurochemical properties. *Neurosci Biobehav Rev*. 2020;113:373-407. doi:10.1016/j.neubiorev.2020.04.007.
- [43] Li H, Sagar AP, Keri S. Translocator protein (18kDa TSPO) binding, a marker of microglia, is reduced in major depression during cognitive-behavioral therapy. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;83:1-7. doi:10.1016/j.pnpbp.2017.12.011.
- [44] Jiang X, Ni Y, Liu T, Zhang M, Ren H, Xu G. Inhibition of LPS-induced retinal microglia activation by naloxone does not prevent photoreceptor death. *Inflammation*. 2013;36:42-52. doi:10.1007/s10753-012-9518-6.
- [45] Anttila JE, Albert K, Wires ES, Mätlik K, Loram LC, Watkins LR, et al. Post-stroke Intranasal (+)-Naloxone Delivery Reduces Microglial Activation and Improves Behavioral Recovery from Ischemic Injury. *eNeuro*. 2018;5. doi:10.1523/eneuro.0395-17.2018.
- [46] Biswas L, Farhan F, Reilly J, Bartholomew C, Shu X. TSPO Ligands Promote Cholesterol Efflux and Suppress Oxidative Stress and Inflammation in Choroidal Endothelial Cells. *Int J Mol Sci*. 2018;19. doi:10.3390/ijms19123740.
- [47] Bobzean SA, DeNobrega AK, Perrotti LI. Sex differences in the neurobiology of drug addiction. *Exp Neurol*. 2014;259:64-74. doi:10.1016/j.expneurol.2014.01.022.
- [48] Fattore L, Melis M. Sex differences in impulsive and compulsive behaviors: a focus on drug addiction. *Addict Biol*. 2016;21:1043-51. doi:10.1111/adb.12381.

Figures

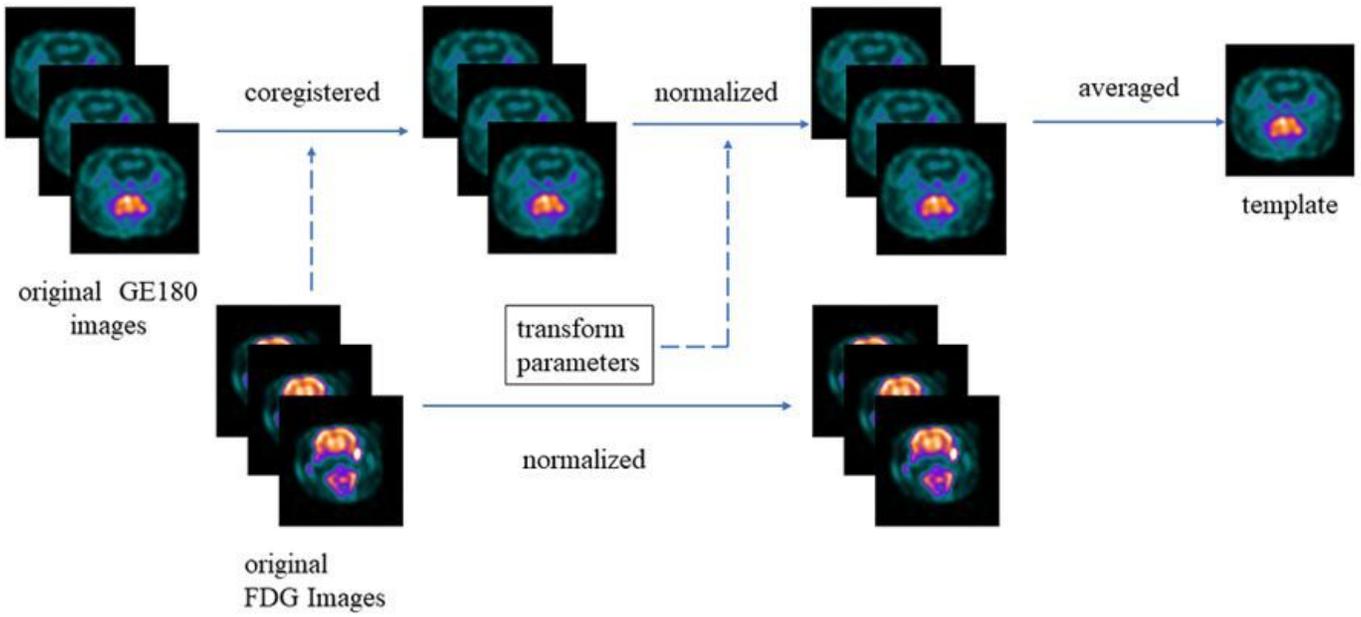


Figure 1

Flowchart for 18F-GE180 template construction. The solid arrows represent the following procedure, while the dashed arrows indicate the target for coregistration and special normalization. All of the steps were repeated only once.

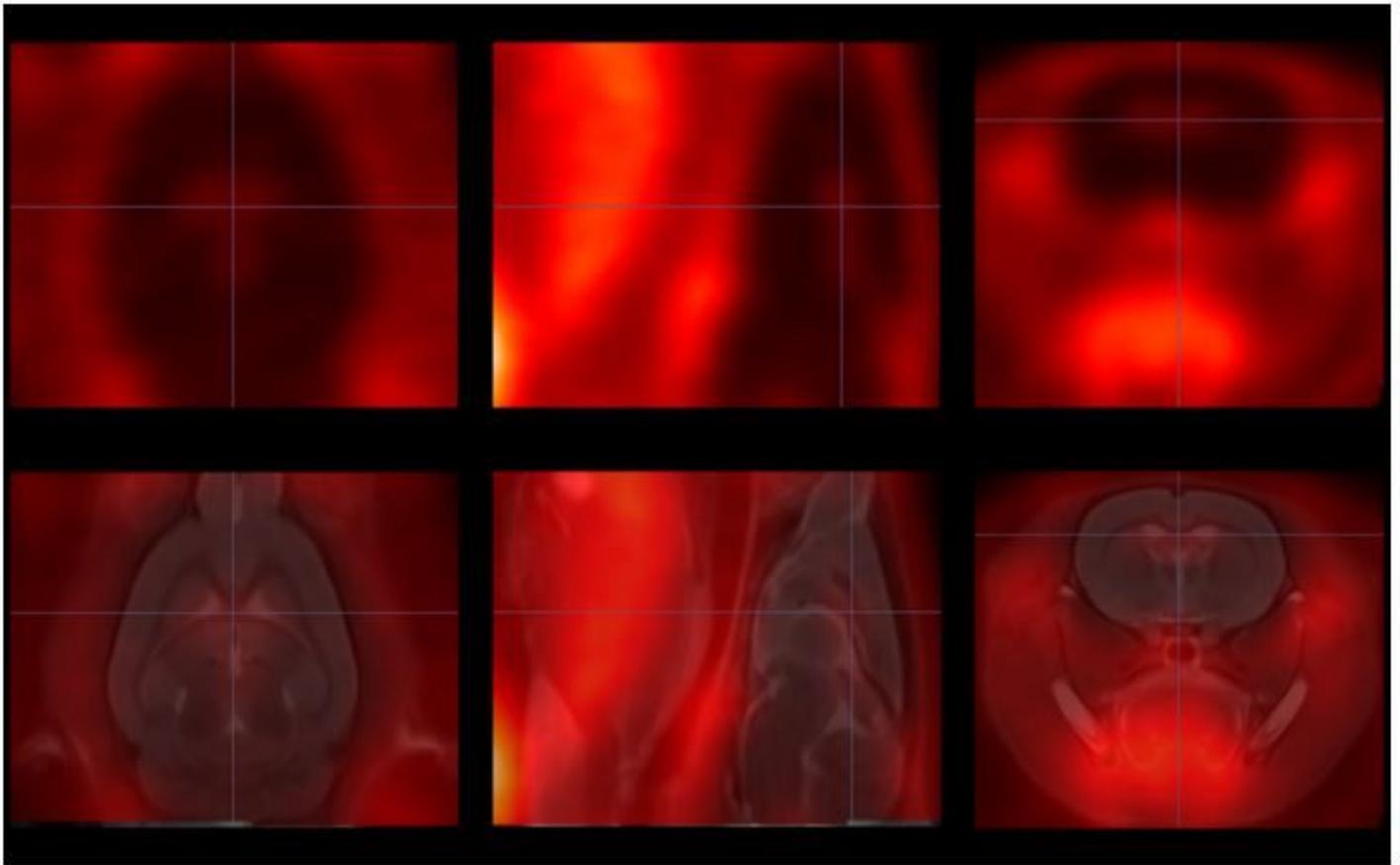


Figure 2

The rat GE180 template. The upper row of images are the coronal plane, sagittal plane, and cross-section of the template. The lower row of images are the overlaid images of the template on an MRI T2 template. The cross curve was focused on the area around the ventricle.

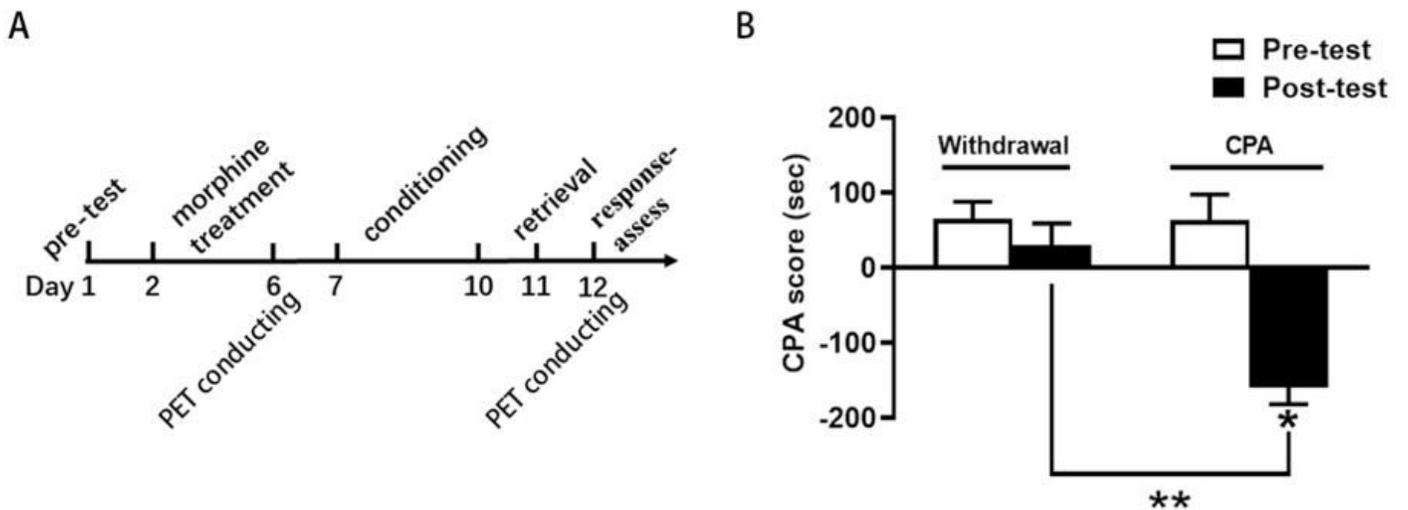


Figure 3

The establishment of the behavior model. (A) The timeline of the behavior produced. (B) The average CPA scores of the withdrawal group and CPA group. *P < 0.005, **P < 0.0001

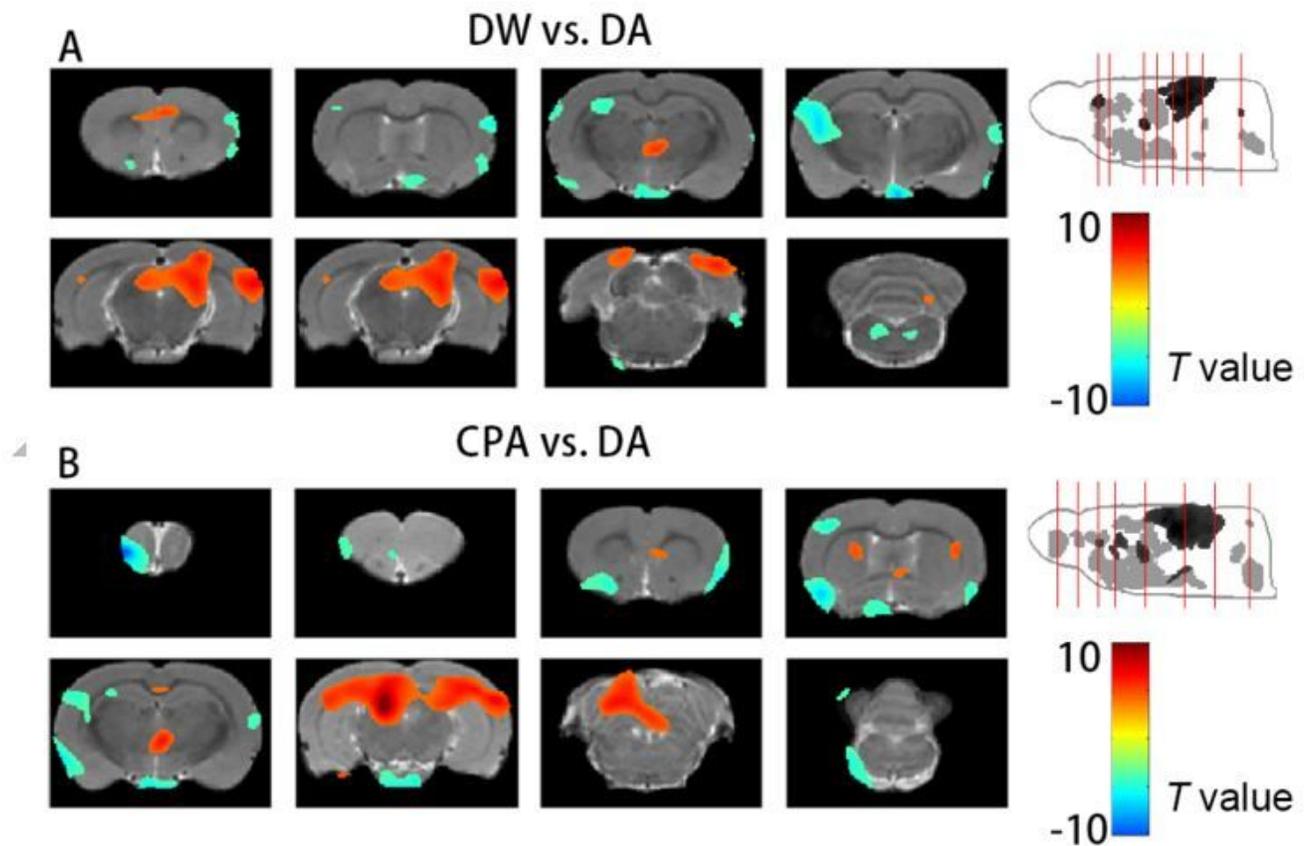


Figure 4

The result of the changed ¹⁸F-GE180 uptake after drug withdrawal and CPA formation. (A) The changed GE180 uptake in the DW group compared with the DA group. (B) The changed GE180 uptake in the CPA group compared with the DA group. The increased uptake regions are shown in red, while the decreased uptake regions are shown in blue. The red lines on the maximum intensity projection (MIP) image refer to the location of the cross-section images on the left, and the cross-section images are arranged from front to back, left is left. The color bar represents the T value of each significant voxel.

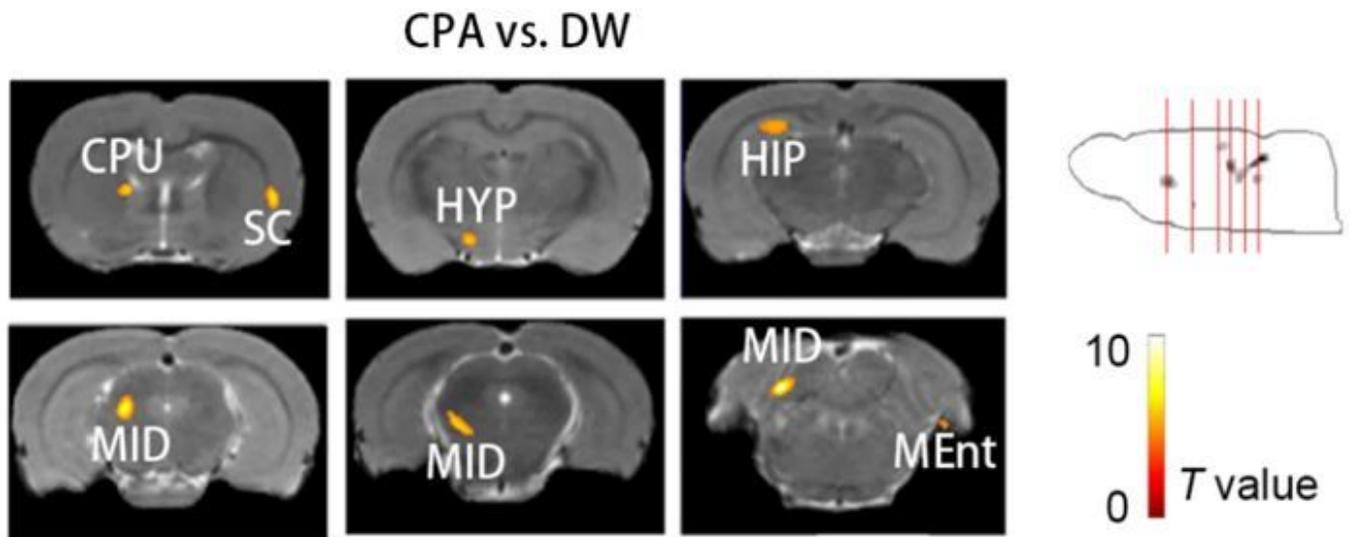


Figure 5

The result of the increased ^{18}F -GE180 uptake in the CPA group rats compared with the withdrawal rats. The red lines on the MIP image refer to the location of the cross-section images on the left, and the cross-section images are arranged from front to back, left is left. The color bar represents the T value of each significant voxel. The abbreviation of each brain region is marked on the cross-section images. CPU caudate putamen, SC sensory cortex, HYP hypothalamus, MID midbrain regions, MEnt medial entorhinal cortex,