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ABSTRACT

The chronic and relapsing nature of addiction suggests that drugs produce persistent adaptations in the brain that make addicts particularly sensitive to drug-related cues and stress and incapable of controlling drug-seeking and drug-taking behavior. In animal models, several long-lasting neuroadaptations have been described. However, few studies have used brain-imaging techniques to provide a complete picture of brain functioning in the course of withdrawal from cocaine. In this study, we allowed rats to self-administer cocaine under short-access (1-h/day) or long-access (6-h/day) conditions and used 2-deoxy-2-(^{18}F)fluoro-d-glucose (^{18}FDG) positron emission tomography (PET) scanning to investigate the longitudinal changes in metabolic activity 1 and 4 weeks after discontinuation of cocaine self-administration. We found that compared to naive rats, both long-access and short-access rats showed significant disruptions in basal brain metabolic activity. However, compared to short-access, long-access rats showed more intense and long lasting neuroadaptations in a network of brain areas including the anterior cingulate cortex, the dorsolateral striatum, the mesencephalon, the amygdala and the hippocampus. This pattern is strikingly similar to that described in humans that has led to the proposal of the I-RISA (Impaired Response Inhibition and Salience Attribution) model of addiction. These results demonstrate that extended access to cocaine leads to persistent neuroadaptations in brain regions involved in motivation, salience attribution, memory, stress, and inhibitory control that may underlie increased risks of relapse.

Introduction

Brain imaging approaches are powerful tools to identify differences in brain structure and function in drug addicts (Goldstein and Volkow, 2002; Volkow et al., 2003; Volkow et al., 1997b). Some of these modifications persist long after discontinuation of drug use and may underlie the persistent risk of relapse over time whereas others appear recovered after withdrawal from the drug (Goldstein and Volkow, 2002; Parvaz et al., 2011). For example, using 2-deoxy-2-[^{18}F]fluoro-d-glucose (^{18}FDG) positron emission tomography (PET) imaging, it has been found that during early withdrawal cocaine addicts show global increase in functional brain activity whereas after protracted periods of withdrawal, brain activity decreased compared to naive subjects (Goldstein and Volkow, 2002; Parvaz et al., 2011; Volkow et al., 1991; Volkow et al., 1997b). Based on neuroimaging studies and the preclinical literature, Goldstein and Volkow proposed the I-RISA (Impaired Response Inhibition and Salience Attribution) model of addiction which postulates that addiction depends on neuroadaptations in brain areas such as the frontal cortex, the mesolimbic dopamine system, the extended amygdala and the striatum, involved in motivation, salience attribution, stress and cognition (Goldstein and Volkow, 2002; Koob and Volkow, 2010).

Whereas brain imaging in humans have greatly contributed to our understanding of the neurobiological dysfunctions associated with drug addiction, rodent models could be invaluable tools to rapidly test novel hypotheses that could be then translated back to humans. In animals, several studies have used brain imaging to investigate the changes in brain activity induced by drugs (Caprioli et al., 2013; Gould et al., 2014; Hanlon et al., 2013; Howell, 2008). Using fMRI (functional Magnetic Resonance Imaging), Gozzi et al. found that after 10 days of abstinence rats with a history of cocaine self-administration show decreases in basal cerebral blood flow in fronto-cortical regions such as the orbitofrontal (OFC), prefrontal and anterior cingulate (ACC) cortices, and the nucleus accumbens (NAc) (Gozzi et al., 2011). In

another study, Calipari et al. used *ex vivo* 2-[^{14}C]deoxyglucose methods and found a decrease in the activity of several brain areas 48 h after 5 days of cocaine self-administration, including the NAc and the ACC (Calipari et al., 2013). However until now, no longitudinal studies have been performed to assess changes in brain activity that occur after short and long periods of withdrawal from cocaine. Importantly, investigating changes in brain activity after long periods of withdrawal could enable obtaining information about brain recovery processes and about mechanisms underlying incubation of drug craving (Grimm et al., 2001; Hanlon et al., 2013; Pickens et al., 2011).

In the present study, we used PET with ^{18}FDG to evaluate metabolic activity changes after short (1 week) and long (4 weeks) periods of withdrawal from cocaine in rats with a history of cocaine self-administration using the escalation model (Ahmed and Koob, 1998). In this model, rats are given either short-access or long-access to cocaine self-administration for several weeks, allowing comparing recreational use in short-access rats to extended addiction-like escalated intake in long-access rats. Thus, the comparison of the brain metabolic maps obtained for short-access, long-access and naive rats, may provide insights into the short- and long-term cerebral metabolic changes after withdrawal after exposure to different regimens of cocaine intake.

Materials and methods

For detailed material and methods see *supplementary information*.

Subjects and housing conditions

Adult (8-9 weeks of age) male Sprague-Dawley rats (Janvier Labs, France), experimentally naive at the beginning of the study, were used in this study. All experiments were conducted during the light phase and in accordance with European Union directives (2010/63/EU) for the care of laboratory animals and approved by the local ethics committees (COMETHEA).

General experimental design

Our general experimental design is schematized in Figure 1 and is based on the escalation protocol (Ahmed and Koob, 1998). During the first 7 days, rats were allowed to self-administer cocaine in 2-h training sessions. On the 8th day, rats were divided into two groups: one group had access to cocaine for 1 h (short-access, n = 8) and the other group had access to cocaine for 6-h (long-access, n = 8) to 20 sessions. At the end of the last self-administration session (day 20), rats were transferred from the animal facility at the University of Poitiers to the animal facility at University of Tours by an authorized transporter and underwent abstinence for 4 weeks. Metabolic imaging using ^{18}FDG was performed after 6-8 days (1 week) and 27-29 days (4 weeks) of abstinence. Naive rats of the same age and with analogous housing conditions were used as controls (n =8).

Brain Imaging

Local uptake of ^{18}FDG reflects cerebral metabolic rates of glucose utilization and therefore allows the investigation of regional brain metabolic status (Phelps et al., 1979; Sokoloff, 1977). Metabolic imaging using ^{18}FDG was performed on freely moving rats under

basal conditions. Rats were habituated to the PET experimental procedures for 4 days before each scan, and fasted overnight before the scan. The day of brain-imaging acquisition, awake rats were injected with ^{18}FDG (18.5 MBq/100g i.p.; Cyclopharma, Tours), and placed in the habituation cage for 45 min. Then, they were anesthetized using isoflurane 4% (Baxter, Maurepas, France), placed on a heating pad (Minerve, Esternay, France) and centered in the field of view of the Explore VISTA-CT microPET camera (GE Healthcare, Velizy, France). A CT-scan was performed for attenuation correction of PET images and a list-mode PET acquisition of 30 minutes started 60 min after ^{18}FDG injection. After data reconstruction using a 2-D OSEM algorithm, all images were co-registered and normalized for tissue activity in the whole brain. Quantitative results were expressed as mean ± standard deviation (SD) and were presented on Z-score maps. Analyses were focused on brain areas known to be key nodes in addiction: 1) the ACC, OFC, Prelimbic (PrL), Infralimbic (IL), insular and motor cortices, in addition to the Dorsal Striatum (DStr), NAc, Ventral Pallidum (VP), Substantia Nigra/Ventral Tegmental Area (SN/VTA), Amygdala (Amyg), and Hippocampus (Hipp) (Volkow and Baler, 2014). Z-score values from these regions were obtained using Z-score maps and a modified version of the regions of interest in PMOD v3.2 software (PMOD Technologies Ltd, Switzerland) (Schiffer et al., 2006). It should be noted that areas that were too small to be identified using microPET ^{18}FDG imaging such as the habenula, were not included in our analysis.

Statistical Analyses

For self-administration, two-way repeated measures ANOVA with time as a within-subject factor and cocaine exposure (long-access or short-access) as a between-subject factor was used. Results showing significant overall changes were subjected to a Student-Newman-Keuls post-hoc test. Differences were considered significant when $p<0.05$.

For micro-PET data, a voxel-based analysis was also used to assess the differences in cerebral ^{18}FDG uptake between the averaged brains of long-access/short-access *vs.* control rats at each stage of withdrawal. This was performed using unpaired Student's two-tailed *t*-test with *p*-values corrected for multiple comparisons using the Benjamini-Hochberg control of false discovery rate (Benjamini and Hochberg 1995). However, all the individual voxel comparisons missed significance, as described in other PET studies with low degrees of freedom (Endepols et al., 2010). Therefore, Z-score maps with a threshold of p=0.01 for uncorrected *p*-values were generated to minimize the type I error rate, as previously proposed (Genovese et al., 2002). The regions of interest were derived from Schiffer's templates (Schiffer et al., 2006) using PMOD v3.2 software and applied to Z-score maps to obtain the Z-score values in these areas. Inter-group comparison was performed using a two-tail unpaired student *t*-test.

Results

Self-administration

Cocaine self-administration started with seven 2-h sessions (Figure 2, left in all graphs A-D). After the initial training, rats were divided into two groups and were allowed to self-administer cocaine for 1-h (short-access) and 6-h (long-access) for 20 additional sessions (Figure 2, right in all graphs A-D). In the short-access group, active nose-pokes (Figure 2A) and cocaine intake (Figure 2C) during the 20 sessions were stable whereas in the long-access group, the number of active nose-pokes (Figure 2A) and cocaine intake (Figure 2C) significantly increased over time. Figure 2D shows the cocaine intake during the first hour of access to the drug in short-access and long-access. During the first 7 days of self-administration short-access and long-access rats had similar levels of cocaine intake. In the short-access group the cocaine intake was stable over time, from 8.0±1.6 at day 8 to 8.8±1.3 mg/kg of cocaine at day 27. In contrast, in the long-access group, the cocaine intake significantly escalated over time, from 10.4 ±1.7 at day 8 to 14.8 ± 0.9 mg/kg of cocaine at day 27. Statistical analysis revealed a significant effect of time [$F(20,280)=2.83$, $p<0.0001$] and a significant GroupXTime interaction [$F(20,280)=2.14$, $p<0.01$].

Brain metabolic activity in long-access and short-access rats during abstinence in areas involved in reward, salience, motivation and drive

Nucleus accumbens: In the NAc, ^{18}FDG uptake was not altered in the short-access group (Figure 3) but was reduced in long-access rats *vs.* controls after short periods of abstinence (Z-Score=-3.7±0.4) and recovered after 4 weeks of abstinence (Figure 4). The direct comparison between long-access and short-access rats confirmed that the metabolic activity

of the NAc was reduced after 1 but not after 4 weeks of abstinence in long-access rats compared to short-access rats (Figure 5, $p<0.0001$).

Ventral pallidum: No significant differences between groups were found in the ventral pallidum (VP) at any time point (Figures 3-4).

Dorsal Striatum: In the DStr, ^{18}FDG uptake was increased in both short-access and long-access rats in the medial part (DMStr) after short periods of abstinence (Z-Scores=3.9±1.0 and 3.6±0.2, respectively) and decreased in the lateral part (DLStr) after long periods of abstinence compared to controls (Figures 3-4, Z-Scores=-2.9±1.6 and -4.0±1.3, respectively). Importantly, when we compared directly long-access to short-access rats, the metabolic activity of the DStr was similar after 1 week of abstinence but it was lower in long-access compared to short-access rats after 4 weeks (Figure 5, $p=0.015$), suggesting that extended access to cocaine produces quantitatively bigger disruptions in the activity of this brain area.

Substantia Nigra/Ventral Tegmental Area: In the SN/VTA, increased ^{18}FDG uptake was observed after 1 week of abstinence in both short-access and long-access rats compared to controls (Figures 3-4, Z-Scores=2.0±0.3 and 3.7±0.4, respectively) but this effect was stronger in long-access than in short-access rats (Figure 5, $p=0.0024$). After 4 weeks of abstinence, increased ^{18}FDG uptake was still observed in the SN/VTA of long-access rats (Figure 3, Z-Score=4.2±0.7), whereas a decreased uptake was observed in short-access rats (Figure 4, Z-Score=-3.8±1.5). Direct comparison of long-access and short-access rats confirmed that the metabolic activity of the SN/VTA was higher in long-access compared to short-access rats (Figure 5, $p<0.0001$).

Orbitofrontal Cortex: In the OFC, a decreased metabolic activity compared to controls was found in short-access rats after 1 week of abstinence but this returned to control levels after 4 weeks of abstinence (Figure 3, Z-Score=-3.9±0.5). No change in the metabolic activity of this region was detected in long-access rats (Figure 4). Direct long-access/short-access

comparison showed that the metabolic activity in the OFC of long-access rats was increased compared to short-access rats after 1 week of abstinence (Figure 5, $p<0.0001$) but this effect was due to metabolic hypoactivity in short-access rats rather than a change compared to control.

Motor Cortex: In the Motor cortex, increased ^{18}FDG uptake was found in short-access (Figure 3, Z-Score=-3.8±1.4) but not long-access rats compared to controls after 1 week of abstinence (Figure 4), but the metabolic activity of this region returned to control levels after 4 weeks of abstinence (Figures 3-4). Direct long-access/short-access comparison highlighted a lower metabolic activity in the Motor cortex of long-access compared to short-access rats after 1 week of abstinence (Figure 5, $p<0.0001$) but this effect was due to metabolic hyperactivity in short-access rats rather than a change compared to controls.

Brain metabolic activity in long-access and short-access rats after abstinence in areas involved in inhibitory control, executive functions, and interoception

Prelimbic and Infralimbic Cortices: No change in the metabolic activity of the PrL was observed in any group at any time point (Figures 3-4). On the other hand, the IL shows increases in ^{18}FDG uptakes in short-access (Figure 3, Z-Score=3.5±1.4) and decreases in long-access rats (Figure 3, Z-Score=-3.2±1.1) after 1 week of abstinence but both modifications disappeared after 4 weeks of abstinence (Figures 3-4). Direct long-access/short-access comparison confirmed that the IL of long-access rats show a hypoactive metabolism compared to short-access rats specifically after a short period of abstinence (Figure 5, $p<0.0001$).

Anterior Cingulate Cortex: The ^{18}FDG uptake was reduced in the ACC in both short-access and long-access rats compared to controls after both short (Z-Scores=-4.3±1.4 and -3.7±0.2, respectively) and long (Z-Scores=-2.0±1.0 and -3.3±1.2, respectively) periods of

abstinence (Figures 3-4). When we compared directly long-access to short-access rats, we found that the hypoactive metabolism was more pronounced in short-access rats after 1 week of abstinence ($p=0.016$), while the contrary was observed after 4 weeks of abstinence with long-access rats showing less activity of the ACC compared to short-access rats (Figure 5, $p=0.0093$). This suggests that extended self-administration of cocaine produced more intense and more persistent disruptions in the activity of this brain area.

Insular cortex: In the insula, the ^{18}FDG uptake was reduced in long-access, but not in short-access rats after short and long periods of abstinence (Figures 3-4, Z-Scores=-3.7±1.4 and -3.3±1.1, respectively). Direct long-access/short-access comparison confirmed that the metabolic activity in this area was consistently lower in long-access compared to short-access rats (Figure 5, $p<0.0001$ for both comparisons).

Brain metabolic activity in long-access and short-access rats after abstinence in areas involved in contextual and emotional memories

Hippocampus: After short periods of abstinence, metabolic activity was reduced in both short-access and long-access rats in the dorsal part of the Hipp (DHipp) (Figures 3-4, Z-Scores=-3.7±1.3 and -2.0±0.5, respectively) and in the ventral part (VHipp) (Figures 3-4, Z-Scores=-3.4±1.2 and -3.6±0.2, respectively).

After 4 weeks of abstinence, in short-access rats the metabolic activity of the DHipp was still reduced (Z-Scores=-3.4±1.2) whereas the VHipp became hyperactive (Figure 3, Z-Scores=3.8±1.6). In contrast, in long-access rats, after 4 weeks of abstinence, both the DHipp and the VHipp became hyperactive compared to controls (Figure 4, Z-Scores=3.6±2.0 and 4.2±0.7, respectively). When we compared directly long-access to short-access rats, the metabolic activity of the VHipp was lower in long-access rats compared to short-access rats after 1 week of abstinence suggesting that hypoactivity of this region was more pronounced in

long-access than in short-access rats (Figure 5, $p=0.0051$). In addition, after 4 weeks of abstinence, the metabolic activity of the DHipp was higher in long-access compared to short-access rats (Figure 5, $p<0.0001$).

Amygdala: In short-access rats, the metabolic activity of the Amyg, was increased compared to controls after short periods of abstinence (Z-Scores=3.5 ± 1.0) but recovered after 4 weeks of abstinence (Figure 3). Conversely, in long-access rats the metabolic activity of the Amyg was normal after short periods of abstinence but increased after 4 weeks of abstinence (Figure 4, Z-Scores=3.3±1.1). Direct comparison of long-access and short-access rats, confirmed that the metabolic activity of the Amyg in long-access rats was lower compared to short-access rats after 1 week of abstinence but became higher after 4 weeks of abstinence (Figure 5, $p<0.0001$ for both comparisons).

Discussion

We show that voluntary intake of cocaine produces changes in basal brain metabolic activity that depends on the intensity of cocaine self-administration and on the duration of abstinence. Indeed, escalation of cocaine self-administration produces cerebral changes that are quantitatively and qualitatively different from those found after short-access cocaine self-administration. Importantly, the neuroadaptations found in this study are consistent with data described in humans and support the idea that cocaine exposure is associated with specific disruptions in interconnected meso-striato-cortical, limbic and fronto-cortical circuits that are involved in reward, motivation, salience attribution, executive control, and stress reactivity (Goldstein and Volkow, 2002; Koob and Volkow, 2010; Parvaz et al., 2011; Volkow and Baler, 2014; Volkow et al., 2003).

Longitudinal brain dysregulations during abstinence from cocaine self-administration

Several animal studies have investigated the changes in brain functioning associated with drug exposure (Caprioli et al., 2013; Gould et al., 2014; Hanlon et al., 2013; Howell, 2008) but only two studies have focused on withdrawal from cocaine intake using brain imaging in rats (Calipari et al., 2013; Gozzi et al., 2011). Whereas those studies were limited to the early consequences of cocaine withdrawal on brain activity, here we investigated longitudinally both early and long-term changes in metabolic activity related to cocaine abstinence in rats exhibiting two different patterns of cocaine voluntary intake (stable low intake and escalated high intake). Although the direct comparison of the results is complicated because of the different experimental protocols and imaging approaches used (fMRI *vs.* PET), a common finding in all studies is that cocaine-exposed rats displayed decreased metabolic activities in the ACC, medial prefrontal cortex and the NAc after short periods of withdrawal (Calipari et al., 2013; Gozzi et al., 2011). It is important to note that some of these adaptations

were found both after limited and extended access to cocaine. However, the intensity of metabolic modification was significantly higher in long-access than in short-access. In addition, whereas some brain regions show spontaneous recovery of normal metabolic activity after protracted abstinence, in long-access rats, a few brain structures (the DStr, the ACC, the SN/VTA, the Amyg, the Hipp and the insula) show persistent changes in metabolic activity that may underlie long-lasting risks of relapse. These results are consistent with separate previous studies that have shown that withdrawal from cocaine self-administration is associated with neuroadaptations in the DStr (Hearing et al., 2008; Pomierny-Chamiolo et al., 2015), ACC (Hearing et al., 2008; Hemby et al., 2005; Pomierny-Chamiolo et al., 2015; Zavala et al., 2007; Zorrilla et al., 2001), VTA (mostly with no change in the SN) (Arroyo et al., 2000; Chen et al., 2008; Grimm et al., 2003; Lu et al., 2003), Amyg (Lee et al., 2013; Lu et al., 2005a; Zorrilla et al., 2001) and Hipp (Garcia-Fuster et al., 2012; Noonan et al., 2008; Pomierny-Chamiolo et al., 2015; Thompson et al., 2004). Importantly, each of these regions has been shown to be involved in cocaine craving and relapse in animal models (Cosme et al., 2015; Fuchs et al., 2006; Fuchs et al., 2005; Grimm and See, 2000; Lu et al., 2005b; McFarland and Kalivas, 2001; McLaughlin and See, 2003; Rogers and See, 2007; See et al., 2007; Torregrossa et al., 2013; Zavala et al., 2008). Thus, these results suggest that extended access cocaine self-administration is associated with simultaneous dysregulations in interconnected networks that can lead to increased risks of relapse.

Similarities between rat and human studies

Over a decade ago, on the basis of clinical and brain-imaging data in humans, Goldstein and Volkow proposed the I-RISA model of addiction that postulates that addiction is the result of a change in the balance of different functions and in the activity of corresponding brain areas (Goldstein and Volkow, 2002). In this model, the six main functions involved in drug addiction are: 1) reward/saliency involving the NAc, the VP and

the dopaminergic system; 2) memory/learning/habits involving the DStr, the Amyg and the Hipp; 3) inhibitory control/executive functions involving the prefrontal cortex and the ACC; 4) motivation/drive involving the OFC, the motor cortex and the dopaminergic system (SN/VTA); 5) interoception involving the insula and ACC and 6) aversion involving the Amyg (Goldstein and Volkow, 2002; Koob and Volkow, 2010; Parvaz et al., 2011; Volkow and Baler, 2014; Volkow et al., 2003). In particular, in this model, reductions in the activity of prefrontal and frontal cortical areas induce dysregulated top-down processes ultimately leading to difficulties in controlling and inhibiting drug-seeking behavior and increased risks of relapse (for review see (Goldstein and Volkow, 2002)). For example, long-term abstinent cocaine abusers (more than 6 weeks) show frontal hypo-metabolism (Volkow et al., 1992) and this disruption is supposed to favor compulsive drug taking (Volkow et al., 1993). This hypo-metabolism is associated with decreased dopamine D2 receptor availability (Volkow et al., 1993; Volkow et al., 1990), reflecting a dysregulation of dopamine receptors following alterations in the activity of the dopaminergic system, described in detoxified cocaine abusers (Volkow et al., 1997a; Volkow et al., 1997c). Our data demonstrate that rats that have extended access to cocaine show a profile of brain metabolic activity that is consistent with this model. In fact, we found a persistent reduction in the metabolic activity of ACC associated with an increase in the activity of the SN/VTA and a decrease in the activity of the DStr, which could be secondary to hyperactivity of the dopaminergic system and activation of dopamine receptors. In addition, after long periods of abstinence, we found that the Amyg and Hipp are hyperactive which is consistent with the critical role of memory and stress-related process in relapse (Koob and Volkow, 2010, 2016). Finally, we found a decrease in the activity of the insula that reveals a dysregulation in the functioning of this region that has been shown to be critical for interoceptive processes and relapse (Goldstein et al., 2009).

Methodological considerations

When interpreting the results of this study, several aspects should be considered. First, our measures reflect metabolic activity under resting states. Thus, it is possible that dysregulation in the ability of certain brain regions to respond to external or internal stimuli may have passed unnoticed in the present study. This is particularly important because most of the recent studies in humans have used fMRI approaches to highlight the changes in reactivity of the brain of addicts to several manipulations such as exposure to drug cues (Goldstein and Volkow, 2002; Volkow and Baler, 2014; Wilson et al., 2004). In addition, PET imaging allows a resolution of one millimeter (Caprioli et al., 2013). For this reason, some regions that were too small to be identified individually, such as the SN/VTA or different nuclei of the Amyg, had to be pooled. Thus, it is possible that certain changes attributed to one region may be actually due to changes in one of their sub-regions. Conversely, it is possible that lack of effects in some regions may be in part due to differences in the activity of the sub-regions. Whereas in this study we focused our attention to the abstinence phase of addiction, brain-imaging studies could be potentially used longitudinally to compare brain functioning in the same individual before, during, and after exposure to cocaine. Unfortunately, because the behavioral equipment and the PET scan were not in the same location, we were not able to perform such studies. Future studies will be needed to allow this additional important comparison. A final aspect to consider is that in our study, control naive animals did not undergo surgery and were not exposed to operant cages. While we cannot rule out the possibility that these manipulations may have contributed to differences in metabolic activity measured in short-access and long-access rats *vs.* controls, the contribution of these experimental differences is likely limited compared to exposure to cocaine. Furthermore, it should be considered that from a translational point of view, cocaine naive individuals do not share with cocaine addicts a wide variety of drug-related experiences and may indeed resemble in many ways to our naive control groups. Finally, whereas the

comparison to naive control is an important aspect of this work, our main focus concerns the differences between short-access and long-access rats that had very similar experimental histories.

Conclusions

We show that extended access intake of cocaine in the escalation model (Ahmed and Koob, 1998) produce a complex regional and temporal pattern of changes in basal brain metabolic activity that are strikingly similar to those reported in humans and framed into the I-RISA model of addiction (Goldstein and Volkow, 2002). These functional dysregulations would lead to increase sensitivity to drugs and drug-related cues, difficulties in regulating emotional responses and deficits in cognitive functions that render addicts persistently vulnerable to relapse. The combination of animal models of addiction and brain imaging approaches represents a unique tool to investigate the brain mechanisms underlying the effects of novel therapeutic interventions.

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Figures legends

Figure 1. Experimental design. Behavioral Experiments. After 7 days of cocaine self-administration training (2-h/day), rats were assigned to two groups Short Access (ShA), which have access to cocaine for 1-h/day and Long Access (LgA), which have access to cocaine for 6-h/day. At the end of the 20 days of cocaine self-administration, rats underwent a 4-week abstinence during which brain metabolic imaging using ^{18}FDG was performed at 1 week and 4 weeks of abstinence. Naive rats were used as controls.

Figure 2. Cocaine self-administration training. Number of active (A) and inactive (B) nose-pokes, total cocaine intake (C) and cocaine intake during the first hour of sessions (D) during the training phase in which all animals had access to cocaine for 2h (left part of the graphs) and during the escalation phase in which rats were divided into short access (ShA, n=8, 1-h sessions) and long access (LgA, n=8, 6-h sessions) groups (right part of the graphs). * $p<0.05$ and **, $p < 0.01$ compared to session 1 of LgA self-administration, ## $p<0.0001$ LgA compared to ShA group.

Figure 3. Changes in metabolic activity in ShA Rats. (A) Summary of the significant increases (red) and decreases (blue) in ^{18}FDG uptake observed in ShA rats *vs.* controls (n=8/group) after 1 week (upper part) and 4 weeks (lower part) of cocaine withdrawal presented on representative coronal plates of the Paxinos and Watson atlas (student's two-tailed *t*-test; $p<0.01$). (B) Examples of the significant differences in ^{18}FDG uptake observed between ShA and control rats after 1 week (upper part) and 4 weeks (lower part) of cocaine abstinence presented on coronal images of Z-score maps fused with an MRI template (increases in ^{18}FDG uptake from dark red to yellow, decreases in ^{18}FDG uptake from black to

light blue; student's two-tailed *t*-test; $p<0.01$). ACC: Anterior Cingulate Cortex, Amyg: Amygdala, DHipp: Dorsal Hippocampus, DMStr: Dorsomedial Striatum, DLStr: Dorsolateral Striatum , IL: Infralimbic Cortex, Ins: Insula, NAc: Nucleus Accumbens, Mot: Motor Cortex, OFC: Orbitofrontal Cortex, PrL: Prelimbic Cortex, VHipp: Ventral Hippocampus, VP: Ventral Pallidum, SN/VTA: Substantia Nigra/Ventral Tegmental Area.

Figure 4. Changes in metabolic activity in LgA Rats. (A) Summary of the significant increases (red) and decreases (blue) in ^{18}FDG uptake observed in LgA rats *vs.* controls (n=8/group) after 1 week (upper part) and 4 weeks (lower part) of cocaine abstinence presented on representative coronal plates of the Paxinos and Watson atlas (student's two-tailed *t*-test; $p<0.01$). (B) Examples of the significant differences in ^{18}FDG uptake observed between LgA and control rats after 1 week (upper part) and 4 weeks (lower part) of cocaine abstinence presented on coronal images of Z-score maps fused with an MRI template (increases in ^{18}FDG uptake from dark red to yellow, decreases in ^{18}FDG uptake from black to light blue; student's two-tailed *t*-test; $p<0.01$). ACC: Anterior Cingulate Cortex, Amyg: Amygdala, DHippo: Dorsal Hippocampus, DMStr: Dorsomedial Striatum, DLStr: Dorsolateral Striatum, IL: Infralimbic Cortex, Ins: Insula, NAc: Nucleus Accumbens, Mot: Motor Cortex, OFC: Orbitofrontal Cortex, PrL: Prelimbic Cortex, VHipp: Ventral Hippocampus, VP: Ventral Pallidum, SN/VTA: Substantia Nigra/Ventral Tegmental Area.

Figure 5. Comparisons of the changes in metabolic activity in LgA vs ShA rats. Summary of the significant increases (red) and decreases (blue) in ^{18}FDG uptake observed in LgA *vs.* ShA rats (n=8/group) after 1 week (upper part) and 4 weeks (lower part) of cocaine

abstinence presented on representative coronal plates of the Paxinos and Watson atlas (student's two-tailed *t*-test; $p<0.01$).

Figure 1

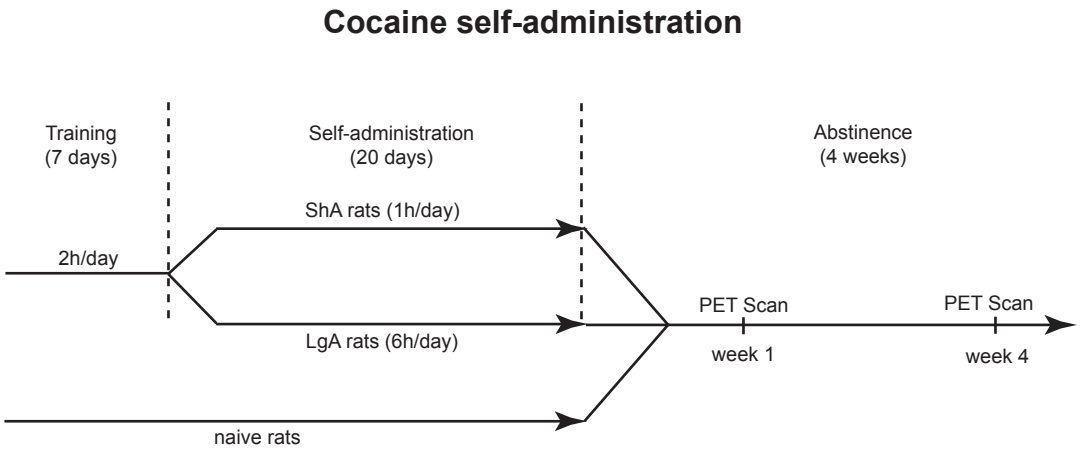

Cocaine self-administration
Training
(7 days)
Self-administration
(20 days)
Abstinence
(4 weeks)
ShA rats (1h/day)
2h/day
LgA rats (6h/day)
PET Scan
week 1
PET Scan
week 4
naive rats

Figure 2

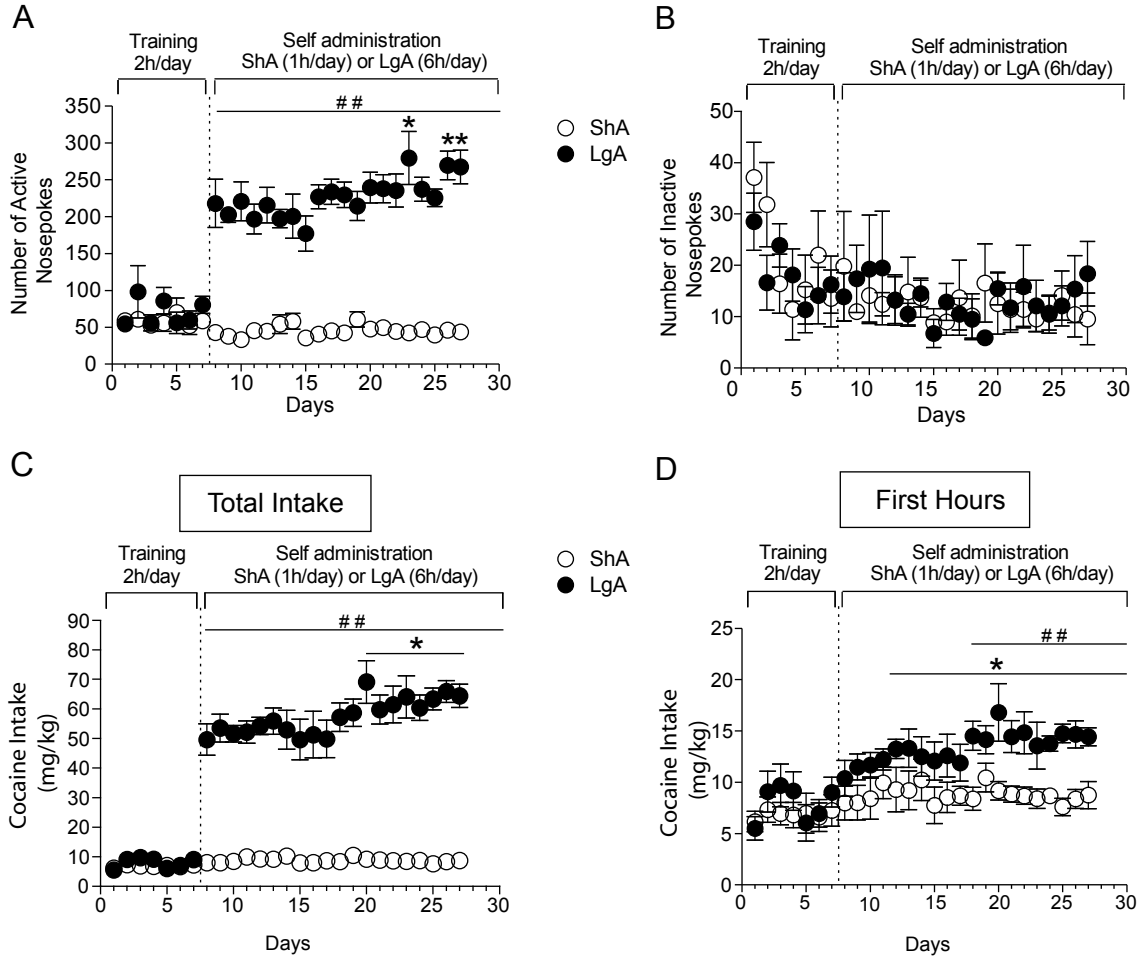

A
Training
2h/day
Self administration
ShA (1h/day) or LgA (6h/day)
##
*
**
Number of Active Nosepokes
Days
ShA
LgA
B
Training
2h/day
Self administration
ShA (1h/day) or LgA (6h/day)
Number of Inactive Nosepokes
Days
C
Total Intake
Training
2h/day
Self administration
ShA (1h/day) or LgA (6h/day)
##
*
Cocaine Intake (mg/kg)
Days
ShA
LgA
D
First Hours
Training
2h/day
Self administration
ShA (1h/day) or LgA (6h/day)
##
*
Cocaine Intake (mg/kg)
Days

Figure 3

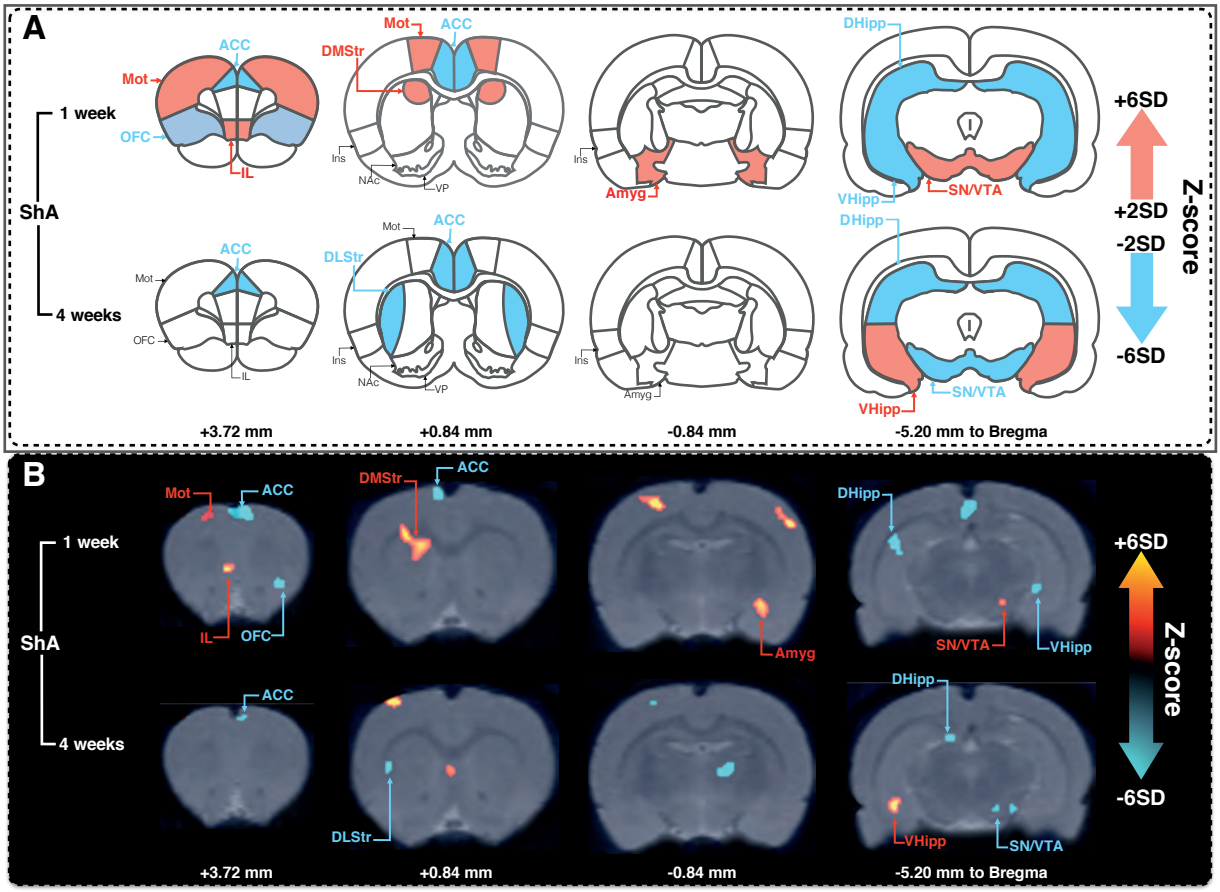
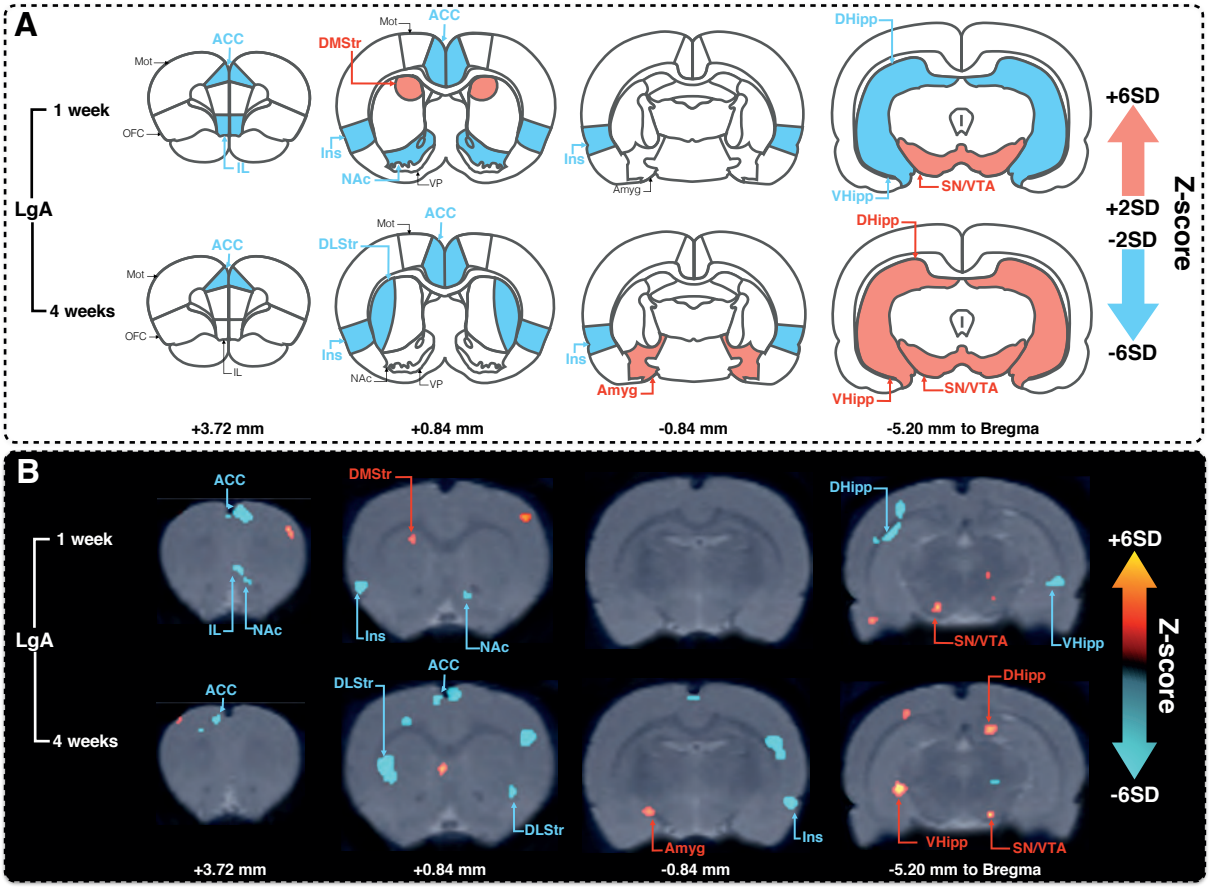

A
1 week
ShA
4 weeks
ACC
Mot
OFC
IL
DMStr
DLStr
Ins
NAc
VP
Amyg
DHipp
VHipp
SN/VTA
+6SD
+2SD
-2SD
-6SD
Z-score
+3.72 mm
+0.84 mm
-0.84 mm
-5.20 mm to Bregma
B
1 week
ShA
4 weeks
ACC
Mot
IL
OFC
DMStr
DLStr
Amyg
DHipp
SN/VTA
VHipp
+6SD
Z-score
-6SD
+3.72 mm
+0.84 mm
-0.84 mm
-5.20 mm to Bregma

Figure 4


A
1 week
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LgA
ACC
Mot
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VHipp
SN/VTA
+6SD
+2SD
-2SD
-6SD
Z-score
+3.72 mm
+0.84 mm
-0.84 mm
-5.20 mm to Bregma
B
1 week
4 weeks
LgA
ACC
IL
NAc
DMStr
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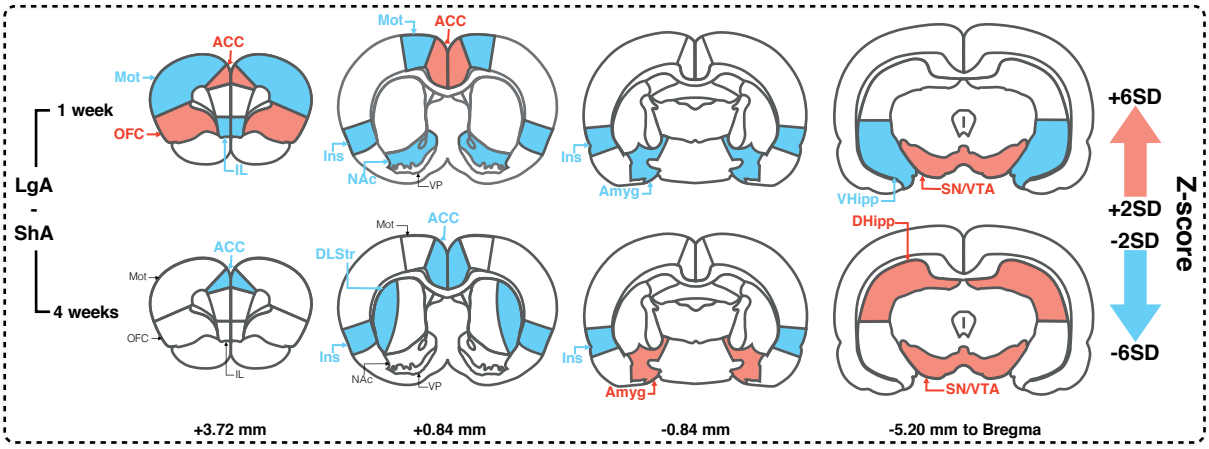
DLStr
Amyg
DHipp
SN/VTA
VHipp
+6SD
Z-score
-6SD
+3.72 mm
+0.84 mm
-0.84 mm
-5.20 mm to Bregma

Figure 5


1 week
LgA
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ShA
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ACC
Mot
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IL
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DLStr
Amyg
VHipp
DHipp
SN/VTA
+6SD
+2SD
-2SD
-6SD
Z-score
+3.72 mm
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