

Neuroimaging Evidence of Altered Fronto-Cortical and Striatal Function after Prolonged Cocaine Self-Administration in the Rat

Alessandro Gozzi^{*,1,2}, Michela Tessari², Lisa Dacome², Federica Agosta², Stefano Lepore², Anna Lanzoni², Patrizia Cristofori³, Emilio M Pich², Mauro Corsi² and Angelo Bifone^{1,2}

¹Center for Nanotechnology Innovation, Istituto Italiano di Tecnologia @NEST, Pisa, Italy; ²GlaxoSmithKline Medicine Research Centre, Verona, Italy; ³GlaxoSmithKline Pathology Department, Hertford, UK

Cocaine addiction is often modeled in experimental paradigms where rodents learn to self-administer (SA) the drug. However, the extent to which these models replicate the functional alterations observed in clinical neuroimaging studies of cocaine addiction remains unknown. We used magnetic resonance imaging (MRI) to assess basal and evoked brain function in rats subjected to a prolonged, extended-access cocaine SA scheme. Specifically, we measured basal cerebral blood volume (bCBV), an established correlate of basal metabolism, and assessed the reactivity of the dopaminergic system by mapping the pharmacological MRI (phMRI) response evoked by the dopamine-releaser amphetamine. Cocaine-exposed subjects exhibited reduced bCBV in fronto-cortical areas, nucleus accumbens, ventral hippocampus, and thalamus. The cocaine group also showed an attenuated functional response to amphetamine in ventrostriatal areas, an effect that was significantly correlated with total cocaine intake. An inverse relationship between bCBV in the reticular thalamus and the frontal response elicited by amphetamine was found in control subjects but not in the cocaine group, suggesting that the inhibitory interplay within this attentional circuit may be compromised by the drug. Importantly, histopathological analysis did not reveal significant alterations of the microvascular bed in the brain of cocaine-exposed subjects, suggesting that the imaging findings cannot be merely ascribed to cocaine-induced vascular damage. These results document that chronic, extended-access cocaine SA in the rat produces focal fronto-cortical and striatal alterations that serve as plausible neurobiological substrate for the behavioral expression of compulsive drug intake in laboratory animals.

Neuropsychopharmacology (2011) **36**, 2431–2440; doi:10.1038/npp.2011.129; published online 20 July 2011

Keywords: cocaine; fMRI; phMRI; dopamine; addiction; rat

INTRODUCTION

Chronic cocaine use produces long-lasting neurobiological changes that are thought to underlie the loss of control over drug intake that defines cocaine dependence (Koob *et al*, 1998). Human neuroimaging studies have started to shed light on the nature of these changes and their relationship with specific behaviors or symptoms. Reduced frontostriatal perfusion and metabolism in abstinent cocaine abusers have been reported by multiple investigators (Strickland *et al*, 1993; London *et al*, 1999; Volkow *et al*, 1992). The disrupted function of frontal regions has been linked to the persistent neuropsychological deficits and impaired control over

drug taking that frequently triggers relapse (Strickland *et al*, 1993; Kalivas, 2004). Positron emission tomography (PET) studies with selective D₂ dopamine (DA) ligands have demonstrated that subjects with cocaine addiction show persistent reduction in D₂ DA receptor availability (Volkow *et al*, 1993; Martinez *et al*, 2004) and decreased dopaminergic responsivity in the nucleus accumbens and other components of the 'reward circuit' (Volkow *et al*, 1997), consistent with a decreased sensitivity to natural reinforcers observed in these subjects (Volkow *et al*, 2007). Recent research suggests that altered functional connectivity of catecholamine circuits may underlie the impaired inhibition of cortical function observed in cocaine abusers, a finding that portrays novel pathways for the neuroadaptational processes associated with addictive states (Tomasi *et al*, 2010; Gu *et al*, 2010).

Cocaine abuse is often modeled preclinically in experimental paradigms where rats are trained to self-administer (SA) the drug. By employing different SA patterns, experimenters have been able to reproduce several hallmark

*Correspondence: Dr A Gozzi, Istituto Italiano di Tecnologia, Center for Nanotechnology Innovation, IIT@NEST, Piazza San Silvestro, 12, 56127 Pisa, Italy, Tel: +39 050 509032, Fax: +39 050 509417, E-mail: alessandro.gozzi@iit.it

Received 2 February 2011; revised 12 May 2011; accepted 16 June 2011

features of drug addiction, including compulsive drug seeking (Vanderschuren and Everitt, 2004), uncontrolled drug use (Ahmed and Koob, 1998), and increased motivation to SA the drug (Paterson and Markou, 2003). These features make these models an experimental tool of excellent face validity to investigate the neuroplastic events associated with voluntary drug intake (Roberts *et al*, 2007). However, specific clinical correlates of cocaine addiction, such as the blunted DA responsivity of striatal areas observed in PET studies (Volkow *et al*, 1993; Martinez *et al*, 2004), do not appear to be adequately modeled by traditional short-term, limited-access cocaine SA paradigms, where instead 'sensitized' (ie, increased) dopaminergic responses are typically observed (Narendran and Martinez, 2008). Moreover, the extent to which these models replicate the multiple neurofunctional alterations observed in human neuroimaging studies remains unknown.

In the present study, we used magnetic resonance imaging (MRI) to map basal and evoked brain function in a rat model of cocaine SA. A prolonged (52 days), extended-access (12 h) SA protocol was employed to model the characteristics of high-dose, chronic cocaine abuse in humans (Gawin and Ellinwood, 1988; Briand *et al*, 2008). Repeated abstinence periods were introduced to minimize the acute toxic effects of the drug and to ensure sustained motivation to self-administer high doses of cocaine (Roberts *et al*, 2007). After a 10-day detoxification period, we measured microvascular basal cerebral blood volume (bCBV), an indirect indicator of resting brain function (Gaisler-Salomon *et al*, 2009; Small *et al*, 2004), and assessed the reactivity of dopaminergic system by mapping the functional response elicited by the DA-releaser amphetamine using a CBV-based pharmacological MRI (phMRI) protocol (Gozzi *et al*, 2010; Schwarz *et al*, 2004). Correlation analyses between resting (bCBV) and amphetamine-evoked (rCBV) responses were performed in an attempt to identify dysregulation in circuits that control the recruitment and functional responsiveness of specific brain areas. Finally, *post-mortem* histopathological examinations were carried out to assess the potential contribution of direct vascular and neurotoxic effects of prolonged cocaine SA to the imaging findings.

MATERIALS AND METHODS

Experiments were carried out in accordance with the Italian regulations governing animal welfare and protection. Protocols were also reviewed by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985).

Cocaine SA

Apparatus for cocaine SA. Rats that underwent cocaine SA were tested in operant chambers as previously described (Moretti *et al*, 2010). Each experimental chamber (Med Associates, St Albans, VT) was fitted with a cue light placed above each lever, and with a 2900-Hz tone module. An infusion pump was connected via an external catheter to a single-channel liquid swivel (Instech Laboratories, Plymouth Meeting, PA). Data acquisition and operant-

schedule parameters were controlled by a Med-PC software (Med Associates).

Cocaine SA procedure. A total of 30 male Lister-Hooded rats (Charles-River, Margate, Kent, UK) weighing 275–300 g were individually housed in a temperature- and humidity-controlled room with water available *ad libitum*. Animals were food restricted throughout the experiment to maintain a constant body weight of 300 g (± 10 g).

After their arrival, rats were acclimatized for 1 week and subsequently implanted with a catheter in the jugular vein as previously described (Moretti *et al*, 2010). After a 7-day recovery period, rats were transported to the operant chamber. Cocaine SA procedure was initiated under a fixed ratio (FR) 1 schedule of reinforcement. Each press on the active lever was associated with a 0.1 ml infusion of a cocaine hydrochloride solution (3 mg/ml, corresponding to 300 μ g per infusion and 1 mg/kg in rats weighing 300 g) plus the simultaneous illumination of the stimulus (cue) light and extinction of the chamber light for 20 s. Presses on the 'inactive' lever had no programmed consequences. Each drug infusion ('reward delivery') was followed by a 20-s lever retraction. The first three 'training' sessions were terminated after either 50 infusions or 2 h from the start of the session. In the subsequent 30 sessions, the cocaine access time was extended to 12 h (1800–0600 h), the unit dose reduced to 0.150 μ g/infusion (0.1 ml of 1.5 mg/ml cocaine solution, corresponding to 0.5 mg/kg in rats weighing 300 g), and FR gradually increased to 3 (sessions 4–6) and eventually to 5 (remaining 27 sessions).

Subjects that lost catheter patency or appeared unhealthy (ie, showed signs of infection) were removed from the study (11 subjects altogether). Repeated 48–72 h abstinence periods were introduced on days 16 (session 14, 72 h), 23 (session 18, 72 h), and 31 (session 23, 48 h) to minimize the risk of acute cocaine-induced intoxication. Session 30 was followed by a longer (5 days) binge abstinence followed by two additional sessions. Such intervals were introduced because of the necessity to harmonize the timing of MRI scan and SA protocol over the relatively large number of subjects employed. A 10-day detoxification period within the home cage was introduced before the imaging experiment.

Vehicle SA procedure. A group of 14 rats was used as baseline reference group. The subjects were implanted with a jugular catheter and subjected to the same training and SA procedures (including number, duration of SA sessions, and abstinence) as described above, except for the use of vehicle (saline, 0.1 ml) instead of cocaine during operant sessions.

Magnetic Resonance Imaging

Animal preparation. Imaging studies were performed 10 days after the last SA session. Animal preparation and MRI acquisition parameters have been previously described in greater detail (Gozzi *et al*, 2010; Schwarz *et al*, 2004). Briefly, rats were anesthetized with 3% halothane, tracheotomized, and artificially ventilated with a mechanical respirator. The femoral artery and vein were cannulated and the animals were paralyzed with D-tubocurarine. After surgery,

halothane level was set to 0.8%. The body temperature of all subjects was maintained within physiological range and mean arterial blood pressure (MABP) was monitored continually through the femoral artery.

MR image acquisition. Anatomical and fMRI time series were acquired on a Bruker Avance 4.7 Tesla system. The animals were positioned prone in a custom-made holding support, and a 'Rat Brain' curved quadrature two-loop receive coil (Bruker, Ettlingen, Germany) was mounted on top of the animal skull and fixed to the animal holder. The animal holder was then fitted into a 72 mm birdcage resonator (Bruker) that was used for radiofrequency transmit only. Both coils are standard components provided by the manufacturer.

A T_2 -weighted anatomical volume was acquired using the RARE sequence (TR = 5461 ms, $TE_{\text{eff}} = 72$ ms, RARE factor 8, FOV 40 mm, 256×256 matrix, 20 contiguous 1 mm slices) followed by a time-series acquisition (TR_{eff} = 2700 ms, $TE_{\text{eff}} = 111$ ms, RARE factor 32, d.t. = 27) with same spatial coverage, yielding a functional pixel volume of $\approx 1 \text{ mm}^3$. Total MRI time-series acquisition time was 58 min (128 repetitions) for both groups.

Following five reference images, 2.67 ml/kg of the contrast agent Endorem (Guerbet, Roissy CdG Cedex, France) was injected to make the fMRI signal changes sensitive to cerebral blood volume (rCBV) (Mandeville *et al*, 1998; Schwarz *et al*, 2003). D-amphetamine (0.5 mg/kg) was administered intravenously 25 min after contrast agent injection, and MRI data were acquired over a period of 25 min following the challenge. The dose of D-amphetamine was chosen based on previous *in vivo* studies (Schwarz *et al*, 2004; Gozzi *et al*, 2011). The dose ensures robust brain activation, does not produce 'ceiling' rCBV responses (Micheli *et al*, 2007), and elicits transient MABP responses that are homeostatically compensated under halothane anesthesia (Gozzi *et al*, 2007; Zaharchuk *et al*, 1999).

Data Analysis

Basal CBV. The bCBV time-series image data for each experiment were analyzed within the framework of the general linear model (Worsley *et al*, 1992). Individual subjects were spatially normalized to a stereotaxic rat brain MRI template set (Schwarz *et al*, 2006a). Signal intensity changes were converted into bCBV(*t*) on a pixel-wise basis as previously described (Chen *et al*, 2001; Mandeville *et al*, 1998). bCBV time series were calculated over a 4.5-min time window starting 6.8 min after contrast agent injection. Mean bCBV volumes for individual subjects were created by averaging the 10 time points time-wise. Linear detrending was introduced to account for contrast agent washout (Schwarz *et al*, 2003). Voxel-wise group statistics was carried out using FSL (Smith *et al*, 2004) using multilevel Bayesian inference, with 0.7 mm spatial smoothing, a *Z* threshold > 1.6 , and a corrected cluster significance threshold of $p = 0.01$.

phMRI response to D-amphetamine. MRI signal intensity changes were converted into fractional CBV (rCBV) as previously described (Mandeville *et al*, 1998) and detrended to account for contrast agent elimination from the blood

pool (Schwarz *et al*, 2003). Unsmoothed, rCBV time series for amphetamine challenge were calculated covering 12.5-min prechallenge and 24-min postchallenge window. Voxel-wise statistics was carried out using FEAT with 0.7 mm spatial smoothing and using a model function (Supplementary Figure S1) capturing the temporal profile of amphetamine-induced rCBV response (Schwarz *et al*, 2006b). Higher-level group comparisons were carried out with multilevel Bayesian inference and thresholded at $Z > 1.6$ with a corrected cluster significance threshold of $p = 0.01$. In order to specifically test the hypothesis of an altered striatal reactivity to D-amphetamine in cocaine rats, a 3D binary mask of major subcortical area (striatum, thalamus, hippocampus, hypothalamus the striatum, ventral pallidum, BNST, and amygdala) was generated using a digital reconstruction of the rat brain atlas (Schwarz *et al*, 2006a) and used to prethreshold rCBV time series before higher-level FSL analysis. This procedure increases the statistical power of the analysis by reducing the number of multiple comparisons (Huettel *et al*, 2004). To investigate the regional specificity of the effect in a hypothesis-free manner and rule out generalized reductions in amphetamine response throughout the brain, the same analysis was repeated on non-masked rCBV data sets (Supplementary Figure S5). Volume of interest (VOI) mean bCBV values and time courses for the amphetamine challenge were extracted as previously described (Schwarz *et al*, 2006a; Gozzi *et al*, 2008). Statistical differences in mean bCBV were assessed using a one-way ANOVA test followed by Fisher's test for multiple comparisons.

Correlation analysis. Maps of correlated bCBV and D-amphetamine-induced rCBV responses across subjects were calculated within the GLM framework at the group level with reference to bCBV in representative regions using FSL (Schwarz *et al*, 2007a, b). A number of representative VOIs were selected based on the results of the intergroup bCBV maps (medial prefrontal, insular, orbitofrontal, somatosensory cortex, caudate putamen, nucleus accumbens, reticular thalamus, and posteroventral thalamus). For each VOI, the design matrix comprised a regressor capturing the group mean bCBV signal in the anatomical structure and another containing the zero-mean bCBV vector across the *N* subjects in the group from the selected reference structure. The *Z*-statistic images were calculated via contrasts capturing positive and negative correlations with the reference response, and were thresholded with $Z > 1.6$ and a corrected cluster significance threshold of $p = 0.01$. Linear regression plots of correlated bCBV and rCBV responses were calculated by plotting bCBV and mean rCBV response to amphetamine across individual subjects, the latter being expressed as mean response over a 20 min (4–24 min postinjection) time window.

Histopathology. Histopathological evaluation was performed on 10 cocaine subject and 8 randomly chosen controls as previously described (Barroso-Moguel *et al*, 2002). After the MRI experiment, rats were maintained under deep anesthesia (halothane 5%), and a 15-min aortic perfusion of fixative media (10% buffered formalin) was performed, preceded by a 5-min infusion of saline. Perfused brains were removed and stored in fixative solution for

further 24–72 h. Brain trimming was then performed using a brain matrix (ASI Instruments) designed for rats weighing 200–400 g. Tissue samples were paraffin embedded, sectioned into 5- μ m-thin slices, and stained with a combination of hematoxylin–eosin and Luxol Fast Blue (Scholtz, 1977). The slice and brain regions analyzed were the cingulate and prefrontal cortex, caudate putamen, corpus callosum, hippocampus (C2), cerebellum (Purkinje cells), and substantia nigra. The examination was performed by two study-blind veterinary pathologists.

RESULTS

Chronic Cocaine SA

All the subjects completed the 33 cocaine SA sessions successfully over a time period of 52 days. The SA schedule used ensured a prolonged and sustained intake of cocaine throughout the study (Figure 1). The average cumulative intake of SA cocaine per subject was 1138.4 ± 33.3 mg/rat. Both active level presses and cocaine intake appeared to be rather stable throughout the course of the experiment, although linear regression highlighted a weak but significant ($p < 0.03$, $F = 4.62$) trend toward an overall increased cocaine intake over time when all homogeneous sessions were compared (sessions 4–31, FR 3–5, binge abstinence intervals 48–72 h) (Supplementary Figure S2).

Basal CBV

In order to investigate the effect of chronic cocaine administration on basal brain function, we measured bCBV in cocaine SA and control subjects and mapped the regions

exhibiting statistically significant differences between groups. Rats with the SA cocaine showed significantly reduced bCBV in several brain areas compared with control rats (Figures 2 and 3). The effect was prominent in the medial-prefrontal, cingulate, orbitofrontal cortex, septum, ventral hippocampus, core region of the nucleus accumbens, as well as in raphe nuclei and reticular thalamic areas. No difference in total CBV between groups was observed ($p = 0.23$, Student's *t*-test). No correlation was found between bCBV and total cocaine intake in all the VOIs examined ($P > 0.16$, all VOIs).

Functional Response to D-Amphetamine

In order to probe striatal dopaminergic reactivity, cocaine SA and control rats were challenged with the DA-releaser amphetamine, and the presence of functional alterations in the magnitude of the rCBV response elicited by the drug was assessed via voxel-wise statistics. Consistent with previous studies (Schwarz *et al*, 2004), amphetamine produced robust activation of subcortical and cortical areas in both groups of subjects (Supplementary Figure S3). Rats chronically exposed to cocaine exhibited an attenuated functional response to amphetamine in the striatum compared with control rats (Figure 4 and Supplementary Figure S3). The effect was also apparent in un-detrended rCBV time profiles (Supplementary Figure S4). In rats that self-administered cocaine, the magnitude of the striatal response to amphetamine was found to be inversely correlated with cumulative cocaine intake ($p = 0.03$, Figure 4). Additional foci of reduced functional response to amphetamine were observed in sensory-motor and orbitofrontal cortex (Supplementary Figures S3 and S5).

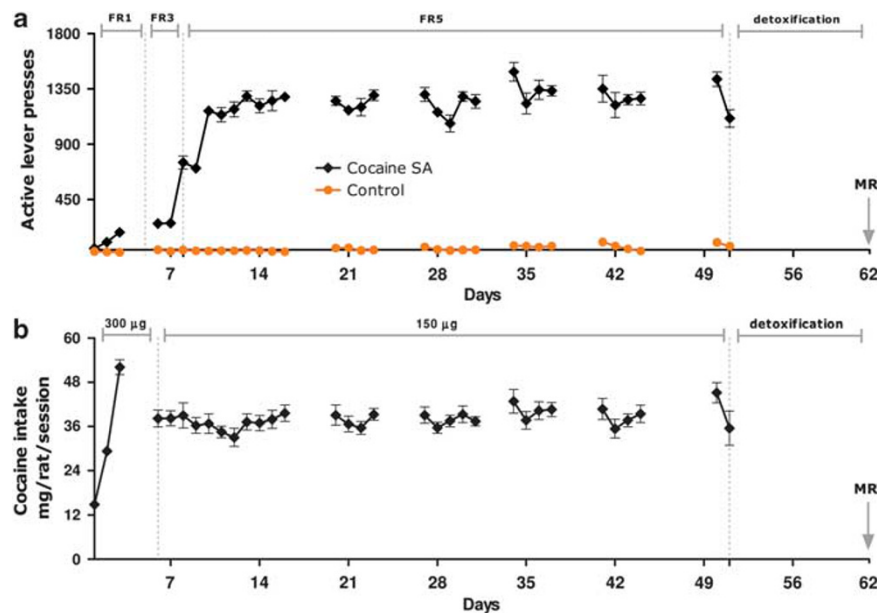


Figure 1 (a) Number of active lever presses recorded in the cocaine SA group ($N = 19$) and control (saline SA, $N = 14$) within the SA sessions. Cocaine SA procedure was initiated under a fixed ratio (FR) 1 schedule of reinforcement. The first three training sessions were terminated after either 50 infusions or 2 h from the start of the session. In the subsequent 30 sessions, the cocaine access time was extended to 12 h and FR gradually increased to 3 (sessions 4–6) and eventually to 5 (remaining 27 sessions). Repeated 48–72 h abstinence periods were introduced on days 16 (session 14, 72 h), 23 (session 18, 72 h), and 31 (session 23, 48 h) to minimize the risk of acute cocaine-induced intoxication. A 10-day detoxification period within the home cage was introduced before the imaging experiment (MRI). (b) Average cocaine intake (mg/rat/session) over the course of the experiment. The dose of cocaine administered per single injection is reported on the top line (300 μ g during the training sessions 1–3, 150 μ g in all remaining sessions, corresponding to 1 and 0.5 mg/kg, respectively).

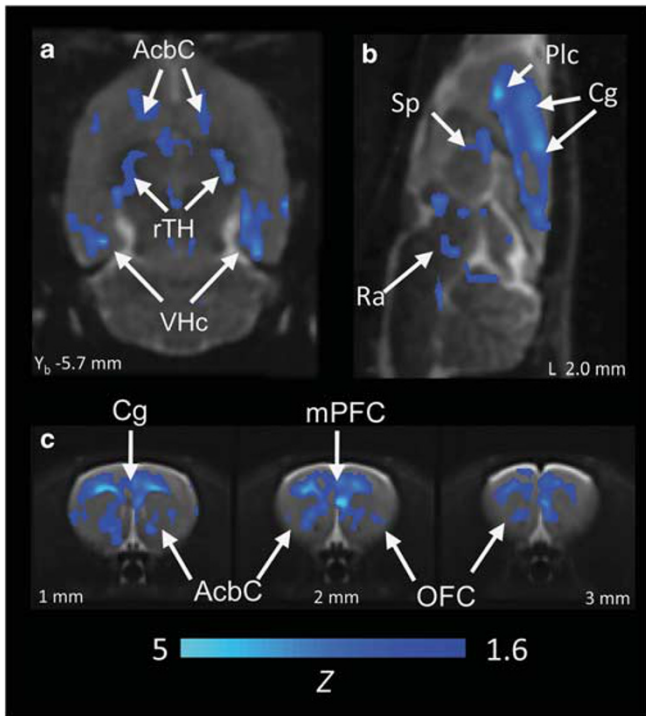


Figure 2 Anatomical distribution of the regions exhibiting significantly lower bCBV in rats chronically self-administering cocaine (cocaine SA; $N=20$) vs control subjects (vehicle SA; $N=14$; $Z>1.6$, cluster correction $p=0.001$) in representative horizontal (a), sagittal (b), and coronal slices (c). The image planes in (a) and (b) refer to two orthogonal sections located at $Y_{\text{bregma}} -5.7$ mm, and lateral (L) 2.0 mm. The rostro-caudal positions of the coronal slice boundaries in (c) are indicated in mm relative to Z_{bregma} . AcbC, core of the nucleus accumbens; Cg, cingulate cortex; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; Plc, prelimbic cortex; Ra, raphe nucleus; rTh, reticular thalamic nucleus; Sp, septum; VHc, ventral hippocampus.

Administration of amphetamine produced transient increases in MABP (Supplementary Figure S6). The effect was not temporally correlated with the functional response, and was well within the blood flow autoregulatory range within which vasopressive responses are homeostatically compensated without producing significant rCBV alterations (Gozzi et al, 2007; Zaharchuk et al, 1999). Arterial blood gases ($p_a\text{CO}_2$ and $p_a\text{O}_2$) were measured before and after the fMRI time series (Supplementary Table S1). No statistically significant difference in mean pre- or post-acquisition $p_a\text{CO}_2$ values between groups was found ($p>0.1$, all groups; one-way ANOVA).

Correlation between Basal and Evoked Activity

In an attempt to establish a correlation between basal and evoked functional activity and to investigate dysregulations in the control of these two states, we measured the correlation between bCBV and amphetamine-induced response in control and animals that self-administered cocaine. No correlation between bCBV and amphetamine-induced rCBV responses was found in either group in any of the regions examined, with the exception of the reticular and posterior-ventral thalamus that revealed, in control subjects, an inverse relationship with amphetamine-induced rCBV in fronto-cortical areas (Supplementary Figures S7

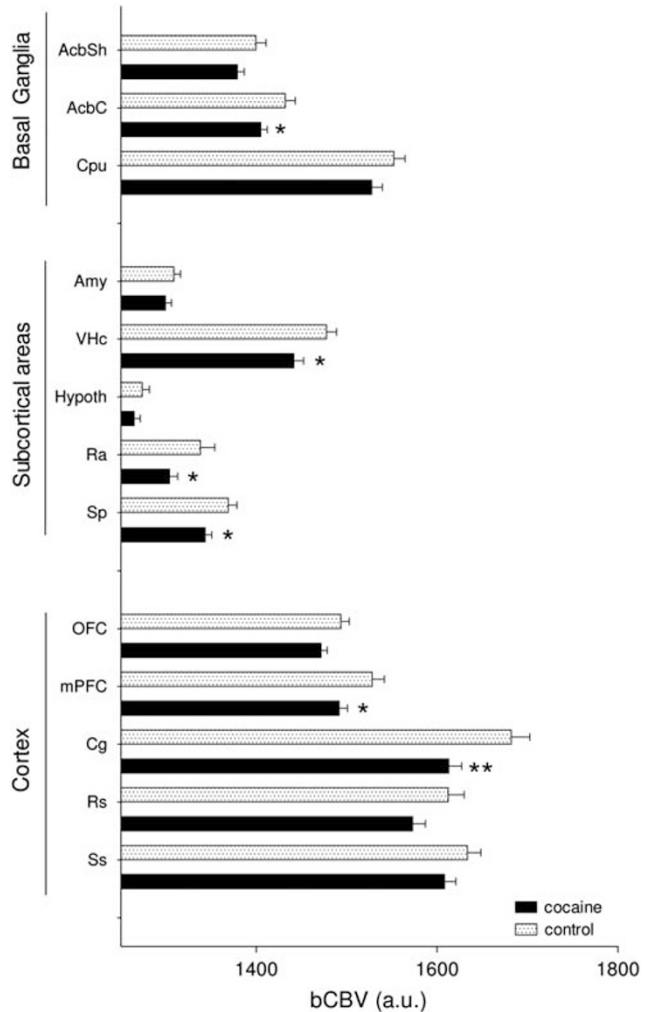


Figure 3 Mean bCBV in representative 3D anatomical volumes (VOIs, Schwarz et al, 2006a) for cocaine SA ($N=20$) and control subjects (saline SA; $N=14$). AcbC, core of the nucleus accumbens; AcbSh, shell of the nucleus accumbens; Amy, amygdala; Cg, cingulate cortex; Cpu, caudate putamen; Hypoth, hypothalamus; mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; Ra, raphe nucleus; Rs, retrosplenial cortex; rTh, reticular thalamic nucleus; Sp, septum; SS, somatosensory cortex; VHc, ventral hippocampus. * $p<0.05$; ** $p>0.01$; one-way ANOVA, followed by Fisher's LSD test.

and S8). No such correlation was present in the cocaine SA group (Supplementary Figure S8).

Histopathology

A histopathological evaluation of brain white and gray matter, glial and interstitial compartments, as well as macro- and micro-vascular, ependymal, and meningeal structures did not highlight any neurocellular, interstitial, or microvascular lesions in either group. Specifically, no signs of cellular pyknosis or atrophy, fiber alteration, necrosis, and interstitial edema were observed in any of the brain regions examined, nor alterations of the microvascular and capillary bed (ie, basal membrane dilation or rupture, hemorrhage, endothelial thickening or wall fibrosis, thrombi or occlusions, and necrosis or vacuolation of endothelial cells).

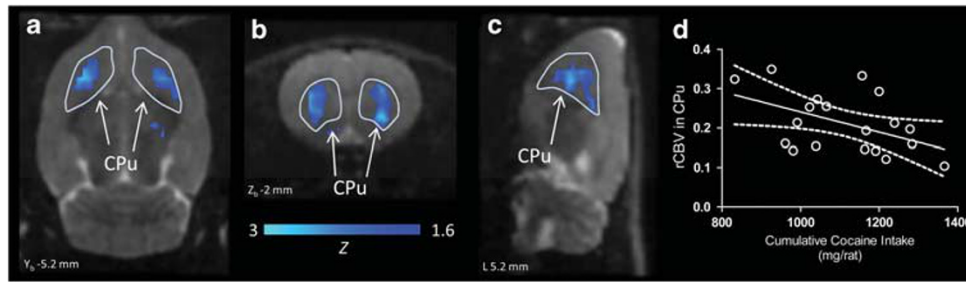


Figure 4 Orthogonal view (a: horizontal, b: coronal, c: sagittal) of the subcortical brain regions exhibiting an attenuated rCBV response to D-amphetamine in rats chronically self-administering cocaine (cocaine SA; $N = 20$) vs control subjects (vehicle SA; $N = 14$; $Z > 1.6$; cluster correction $p = 0.05$). The image planes in (a–c) refer to three orthogonal sections located at $Y_{\text{bregma}} (Y_b) -5.2$ mm, $Z_{\text{bregma}} (Z_b) -1.4$ mm, and lateral (L) 5.2 mm, respectively. (d) Correlation of rCBV response to amphetamine in the striatum and cumulative cocaine intake over 52 days of self-administration ($p = 0.03$, dashed lines represent 95% confidence interval). Cpu, caudate putamen.

DISCUSSION

The present study documents that chronic, extended-access cocaine SA in the rat produces neuroimaging alterations that closely mimic hallmark imaging findings in human cocaine addicts. Specifically, we observed significantly reduced bCBV, a marker of resting brain function, in regions that have a key contribution in higher cognitive functions and inhibitory control (fronto-cortical areas), craving and anticipation (fronto-hippocampal areas), and reward (mesolimbic areas). Moreover, cocaine SA was associated with reduced striatal reactivity to dopaminergic stimulation, and the presence of putative functional alterations in the inhibitory interplay between reticular thalamus and the activation of fronto-cortical areas. Our results provide neuroimaging evidence of multiple alterations in rat brain function following chronic and voluntary cocaine intake that serve as plausible neurobiological substrate for the behavioral expression of compulsive drug intake in laboratory animals.

Chronic cocaine abuse is often modeled in behavioral paradigms where rodents are trained to voluntarily self-administer the drug. Here, we implemented a prolonged, extended-access cocaine SA protocol with repeated binge-abstinence periods (Parsons *et al.*, 1995; Wilson *et al.*, 1994; Wilson and Kish, 1996) to mimic the characteristics of high-dose, chronic cocaine abuse in humans. Prolonged SA paradigms have been reported to reproduce key clinical features of cocaine addiction including compulsive drug use despite the presence of environmental adversities (Vanderschuren and Everitt, 2004), and high propensity to relapse to drug seeking (Deroche-Gamonet *et al.*, 2004). The chronic protocol employed (covering $\approx 10\%$ of the adult lifespan of a rat, Sharp and La Regina, 1998) permits to mimic patient populations with a significant history (>6 months) of cocaine addiction like those typically enrolled in human neuroimaging studies, thus maximizing the translational relevance of our findings. Moreover, the use of extended access to cocaine (ie, ≥ 6 h) is known to specifically model specific neurobehavioral features of addiction, such as persistent alterations in cognitive functions (Briand *et al.*, 2008; George *et al.*, 2007), increased motivation for cocaine (Paterson and Markou, 2003), and escalation in drug intake (Ahmed and Koob, 1998). Repeated periods of forced abstinence were introduced to reduce the acute toxic effects of the drug and to ensure

sustained motivation to self-administer high doses of cocaine (Roberts *et al.*, 2007). Although the total cocaine intake achieved with the present protocol is higher than that observed with short-access paradigms, the values attained are sufficiently distant from the limit of acute toxicity (Mantsch *et al.*, 2004; Wee *et al.*, 2007), which explains the lack of lethality observed in this study.

Compared with unlimited access protocols, where drug intake exhibits high and low numbers of infusions on alternating days (Wilson *et al.*, 1994), the extended-access protocol used here ensured sustained SA of high doses of cocaine. In contrast to what was reported by other groups (Ahmed and Koob, 1998; Ferrario *et al.*, 2005; Wee *et al.*, 2007), we did not observe unequivocal evidence of dose escalation, although a trend toward an increased cocaine intake over consecutive sessions was apparent (Supplementary Figure S2).

One limitation of the model employed is that it did not include behavioral measurements of drug use despite adverse consequences (eg, ‘resistance to punishment’ Deroche-Gamonet *et al.*, 2004), a behavioral trait that is considered an essential diagnostic criteria of addiction in humans (American Psychiatric Association, 2008). Because this feature is present in ca. 20% of rats exposed to cocaine (Deroche-Gamonet *et al.*, 2004; Ahmed, 2010), the imaging alterations mapped in the present work are likely to include contributions from subsets of subjects exhibiting this behavior. However, whether this trait is characterized by specific functional alterations separate from those highlighted in this study remains to be determined.

A 10-day washout period was introduced before the imaging study to rule out acute carryover effects of cocaine and minimize the potential interference of acute abstinence symptoms on the measures of brain function. Most of the neurochemical and behavioral alterations that can be related to acute withdrawal have an almost immediate onset, peak between 6 and 72 h after termination of drug access, and generally cease within 2–7 days from the last cocaine session (Baumann and Rothman, 1998; Harris and Aston-Jones, 1993; Malin *et al.*, 2000; Mutschler and Miczek, 1998; Markou and Koob, 1992). It is therefore unlikely that the imaging findings contain major perturbations from transient neurobiological phenomena related to acute cocaine abstinence. On the other hand, the observed functional alterations are expected to contain contributions from longer-lasting neuroadaptational processes

(ie, incubation of cocaine craving) that have been shown to build-up after cocaine discontinuation (Lu *et al*, 2004), and that are of translational relevance as they can be related to propensity to relapse.

MRI measures of bCBV allow high-resolution mapping of resting brain function that tightly correlates with regional energy metabolism and cerebral blood flow (Gaisler-Salomon *et al*, 2009; Hyder *et al*, 2001; Gonzalez *et al*, 1995). Our data showed the presence of reduced bCBV in the cingulate gyrus, prefrontal cortex, orbitofrontal cortex, as well as in striatal and hippocampal areas of cocaine SA subjects. The frontostriatal effect is in excellent agreement with clinical neuroimaging research of cocaine addiction, where reduced frontal and striatal activities have been consistently observed (Strickland *et al*, 1993; Tumeo *et al*, 1990; London *et al*, 1999; Volkow *et al*, 1992, 1988) and found to correlate with the cognitive impairments, compulsion, and loss of inhibitory control over drug taking that may lead to relapse (Goldstein *et al*, 2010; Kalivas *et al*, 2005; Kalivas, 2004; Hong *et al*, 2010; Strickland *et al*, 1993). Importantly, cognitive deficits have been observed in rats allowed extended (but not limited) access to cocaine (Briand *et al*, 2008; George *et al*, 2007), a phenomenon that involved working memory and sustained attention tasks (two prefrontal cortex-dependent tasks) as well as object recognition measures (a hippocampus-dependent task). The involvement of hippocampal systems is also consistent with the role played by this brain structure in contextual conditioning and memory, two functions that are altered by cocaine use and are believed to play a role in cue-elicited craving (reviewed by Koob and Volkow, 2010). Likewise, the reduced bCBV in the nucleus accumbens was not unexpected, given the established interconnection between fronto-cortical activity and ventrostriatal DA cell firing and release (Kalivas *et al*, 2005; Peoples *et al*, 2007). In keeping with this, recent PET imaging studies showed lower levels of endogenous DA in cocaine addicts relative to comparison subjects (Martinez *et al*, 2009) and primate research revealed reduced glucose utilization in the striatal areas upon chronic cocaine use, a feature that became more pronounced with increased cocaine exposure (Porrino *et al*, 2007).

Focal bCBV reductions were also observed in reticular thalamic and raphe nuclei. The former finding is consistent with human neuroimaging studies showing altered GABAergic neurotransmission in the thalamus of abstinent cocaine abusers (Volkow *et al*, 1998) and recent electrophysiological evidence of a state of protracted overinhibition of reticular thalamic areas following binge administration of cocaine (Urbano *et al*, 2009). Interestingly, as serotonin exerts a direct excitatory action on GABAergic neurons in the reticular thalamus (McCormick and Wang, 1991), the reduced activity of these nuclei and that observed in regions of the raphe may be functionally interrelated and part of a single defective circuit.

No correlation between total cocaine intake and bCBV was found in any of the VOIs examined. The lack of correlation could reflect different individual susceptibility to the effect of the drug, or could be related to the high amount of cocaine self-administered that might exceed the quantity required to produce maximal bCBV alterations.

In an attempt to identify an fMRI correlate of the decreased striatal dopaminergic responsivity observed in human PET studies (Volkow *et al*, 1990, 1993; Martinez *et al*, 2004), we also mapped the functional response elicited by the DA-releaser amphetamine using a pHMRI protocol (Schwarz *et al*, 2004; Bifone and Gozzi, 2010). Several pHMRI studies have provided compelling evidence that the striatal hemodynamic response produced by amphetamine reflects primarily the dopaminergic effects (reviewed in Knutson and Gibbs, 2007). For example, amphetamine has been shown to elicit BOLD or rCBV increases in DA-rich ventrostriatal areas that are linearly correlated to synaptic DA concentrations (Dixon *et al*, 2005; Ren *et al*, 2009; Choi *et al*, 2006; Schwarz *et al*, 2007b; Preece *et al*, 2007). Moreover, amphetamine-induced rCBV responses are abolished in the DA denervated areas (Chen *et al*, 1997, 1999), an effect that can be later restored after fetal or stem cell transplantation (Bjorklund *et al*, 2002; Chen *et al*, 1999). Thus, the sum of these data indicates that amphetamine-induced rCBV responses can be reliably used as a marker of striatal DA neurotransmission. Within this framework, the presence of an attenuated striatal rCBV response to amphetamine in the cocaine SA group points toward a reduced responsivity of ventrostriatal dopaminergic function analogous to what was observed in PET studies in humans (Narendran and Martinez, 2008). This finding provides for the first time a plausible preclinical neuroimaging correlate of one of the most replicated clinical manifestations of cocaine addiction, which is believed to play a key contribution to the 'hypohedonia' and amotivation reported by drug-addicted subjects during protracted withdrawal (Volkow *et al*, 1997). This result documents a potentially important correspondence between clinical and preclinical neuroadaptational changes induced by cocaine on DA systems, an aspect that does not appear to be adequately modeled by traditional cocaine exposure paradigms, where 'sensitized' (ie, increased) dopaminergic responses are typically observed (reviewed by Narendran and Martinez, 2008). As similarly attenuated striatal responses were not observed in rodent neuroimaging studies using short-term (5 days) drug administration protocols (Febo *et al*, 2005; Reese *et al*, 2004; and A Gozzi, unpublished results), our data suggest that, for this characteristic to be modeled in rodents, prolonged and extended access to high doses of cocaine may be required. Importantly, no appreciable microscopic lesions in the vascular, neurocellular, and interstitial compartments of brains exposed to cocaine were observed. This result is important, as it permits to rule out a potential contribution of abnormal cerebrovascular processes on the hemodynamic measures of brain function performed (ie, bCBV and rCBV).

Correlation analysis between resting and amphetamine-evoked (rCBV) responses revealed an inverse relationship between bCBV in reticular thalamic areas and amphetamine-induced frontal activation in control subjects, but not in cocaine group. Previous studies have demonstrated that inhibition of reticular thalamic activity can enhance fronto-cortical dopaminergic neurotransmission (Jones *et al*, 1988), a finding consistent with the functional connectivity of these regions (Paxinos, 2008) and the high GABAergic density of reticular thalamic nucleus

(Paxinos, 2008). As prefrontal projections to the thalamic reticular nucleus play a unique circuit for attentional mechanisms (Zikopoulos and Barbas, 2006), we hypothesize that the loss of correlation between basal and evoked function observed in the cocaine SA group may be related to the attentional deficits observed in rats allowed extended access to cocaine (Briand et al, 2008; George et al, 2007). A putative role for thalamo-frontal dysfunctions in cocaine addiction is supported by recent neuroimaging studies showing altered thalamo-cortical connectivity in cocaine abusers under resting conditions (Gu et al, 2010) and when performing a cognitive task (Tomasi et al, 2007). However, as correlation measurements do not reflect causal association, further research is warranted to elucidate the exact nature of this finding.

In summary, we provide evidence of altered brain function in rats that underwent prolonged and extended-access cocaine SA. Consistent with clinical neuroimaging findings, cocaine-exposed animals revealed reduced basal brain function in fronto-cortical and thalamic areas, and attenuated responsivity in striatal regions upon challenge with the DA-releaser amphetamine, an effect that was significantly correlated with the total cocaine intake. The consistency of these findings with neuroimaging measures in cocaine-addicted patients supports the use of prolonged and extended-access SA paradigms in the rat to investigate the neuroadaptations underlying cocaine addiction.

ACKNOWLEDGEMENTS

We thank Valerio Crestan and Giuliano Turrini for their excellent technical support to the pHMRI measures, and Pamela Rodegher from Histolab, Verona, Italy, for the histological preparations.

DISCLOSURE

All the authors are employees of GlaxoSmithKline. The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)