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Title Page

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 Merlo Pich², Mauro Corsi², Angelo Bifone^{1,2}

¹Center for Nanotechnology Innovation , Istituto Italiano di Tecnologia @NEST, Piazza San Silvestro 12, 56127, Pisa, Italy

² GlaxoSmithKline Medicine Research Centre, Via Fleming 4, Verona, Italy

³GlaxoSmithKline Pathology Department , SA Ware, Hertford, UK

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Corresponding author

Alessandro Gozzi

Istituto Italiano di Tecnologia,

Center for Nanotechnology Innovation, IIT@NEST, ,

Piazza San Silvestro, 12, 56127 PISA

tel: +39 050 509032

alessandro.gozzi@iit.it

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Abstract

Cocaine addiction is often modeled in experimental paradigms where rodents learn to self-administer 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 self-administration 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 ventro-striatal 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 neuro-biological substrate for the behavioral expression of compulsive drug-intake in laboratory animals.

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 fronto-striatal 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 to addictive states (Tomasi et al. 2010; Gu et al. 2010).

Cocaine abuse is often modelled pre-clinically 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 features of drug addiction, including compulsive drug seeking (Vanderschuren and Everitt 2004), uncontrolled drug use (Ahmed and Koob 1998) and increased

motivation to self-administer the drug (Paterson and Markou 2003). These features make these models an experimental tool of excellent face-validity to investigate the neuroplastic events associated to 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 modelled by traditional short-term, limited-access cocaine SA paradigms, where instead “sensitized” (i.e. increased) dopaminergic responses are typically observed (Narendran and Martinez 2008). Moreover, the extent to which these models replicate the multiple neuro-functional 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 hours) 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 analysis 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* histo-

pathological examinations were carried out to assess the potential contribution of direct vascular and neurotoxic effects of prolonged cocaine SA to the imaging findings.

Methods

Experiments were carried out in accordance with 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 self administration

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 Inc., 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 Inc., Plymouth Meeting, PA). Data acquisition and operant-schedule parameters were controlled by a Med-PC software (Med Associates Inc.).

Cocaine SA procedure

Thirty male Lister-Hooded rats (Charles-River, 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 (\pm 10 g).

After their arrival, rats were acclimatized for one 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 self-administration 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 µg 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 hours (6.00 p.m. – 6 a.m.), the unit dose reduced to 0.150 µg/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 (i.e. showed signs of infection) were removed from the study (eleven subjects altogether). Repeated 48-72 hrs abstinence periods were introduced on days 16 (session 14, 72 hrs), 23 (session 18, 72 hrs), 31 (session 23, 48 hrs) 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 due to the necessity to harmonize the timing of MRI scan and self-administration protocol over the relatively large number of subjects employed. A 10-day detoxification period within the home cage was introduced prior to the imaging experiment.

Vehicle SA procedure

A group of fourteen 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 anaesthetized with 3% halothane, tracheotomized and artificially ventilated with a mechanical respirator. A 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 timeseries 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, Ettlingen, Germany) which was used for radiofrequency transmit only. Both coils are standard components provided by the manufacturer.

A T₂-weighted anatomical volume was acquired using the RARE sequence (TR= 5461ms, TE_{eff} = 72 ms, RARE factor 8, FOV 40mm, 256x256 matrix, 20 contiguous 1mm slices) followed by a time series acquisition (TR_{eff} = 2700 ms, TE_{eff} = 111 ms, RARE factor 32, dt=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, 2.67ml/kg of the contrast agent Endorem (Guerbet, 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 (bCBV)

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 basal cerebral blood volume (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 minute 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 multi-level Bayesian inference, with 0.7 mm spatial smoothing, a Z threshold > than 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 minute pre-challenge and 24 minutes post-challenge window. Voxel-wise statistics was carried out using FEAT with 0.7 mm spatial smoothing and using a model function (Figure S1) capturing the temporal profile of amphetamine-induced rCBV response (Schwarz et al. 2006b). Higher-level group comparisons were carried out with multi-level 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, amygdala) was generated using a digital reconstruction of the rat brain atlas (Schwarz et al. 2006a) and used to pre-threshold rCBV time series prior to 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 datasets (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; Schwarz et al. 2007b). A number of representative VOIs were selected based on the results of the inter-group bCBV maps (medial prefrontal, insular, orbitofrontal, somatosensory cortex, caudate putamen, nucleus accumbens, reticular thalamus, 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-meaned bCBV vector across the N subjects in the group from the selected reference structure. 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 minute (4-24 min post injection) 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 minutes infusion of saline. Perfused brains were removed and stored in fixative solution for further 24-72 hours. 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 µm-thin slices, and stained with a combination of Haematoxylin-eosin and Luxol Fast Blue (Scholtz 1977). The slice and brain regions analyzed where 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 self-administration (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 towards an overall increased cocaine intake over time when all homogeneous sessions were compared (sessions 4-31, FR 3-5, binge abstinence intervals 48-72 hrs) (Figure S2).

bCBV

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 that self-administered cocaine showed significantly reduced bCBV in several brain areas compared to control rats (Figure 2 and 3). The effect was prominent in the medial-prefrontal, cingulate, orbitofrontal cortex, septum, ventral hippocampus, in the core region of the nucleus accumbens as well as 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 sub-cortical and cortical areas in both groups of subjects (Figure S3). Rats chronically exposed to cocaine exhibited an attenuated functional response to amphetamine in the striatum compared to control rats (Figure 4, Figure S3). The effect was also apparent in un-detrended rCBV time-profiles (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 (Figure S3 and S5).

Administration of amphetamine produced transient increases in MABP (Figure S6). The effect was not temporally correlated with the functional response, and 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 prior to and after the fMRI timeseries (Table SI). 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 exception of the reticular and posterior-ventral thalamus which revealed, in control subjects, an inverse relationship with amphetamine-induced rCBV in fronto-cortical areas (Figures S7 and S8). No such correlation was present in the cocaine SA group (Figure S8).

Histopathology

A histopathological evaluation of brain white and grey 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, interstitial edema, were observed in any of the brain regions examined., nor alterations of the microvascular and capillary bed (i.e., basal membrane dilation or rupture, hemorrhage, endothelial thickening or wall fibrosis, thrombi or occlusions, necrosis or vacuolation of endothelial cells).

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 play 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 neuro-biological 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 life-span 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 (i.e. ≥ 6 hours) is known to specifically model specific neuro-behavioral 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 to 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 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 towards an increased cocaine intake over consecutive sessions was apparent (Figure S2).

One limitation of the model employed is that it did not include behavioral measurements of drug use despite adverse consequences (e.g. "resistance to punishment" Deroche-Gamonet et al. 2004), a behavioral trait that is considered an essential diagnostic criteria of addiction in humans (V.A. 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 ten-day washout period was introduced prior to the imaging study to rule out acute carry-over 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 neuro-biological phenomena related to acute cocaine abstinence. On the other hand, the observed functional alterations are expected to contain contributions from longer-lasting neuroadaptational processes (i.e. incubation of cocaine craving) which 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 correlate 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 cingulated gyrus, pre-frontal and orbito-frontal cortex, as well as in striatal and hippocampal areas of cocaine SA subjects. The fronto-striatal effect is in excellent agreement with clinical neuroimaging research of cocaine addiction, where reduced frontal and striatal activity have been consistently observed (Strickland et al. 1993; Tumeh et al. 1990; London et al. 1999; Volkow et al. 1992; Volkow et al. 1988) and found to correlate with the cognitive impairments, compulsion

and loss of inhibitory control over drug taking which 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 ventro-striatal 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 over-inhibition 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 this 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 which might exceed the quantity required to produce maximal bCBV alterations.

In attempt to identify an fMRI correlate of the decreased striatal dopaminergic responsivity observed in human PET studies (Volkow et al. 1990; Volkow et al. 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 haemodynamic response produced by amphetamine reflect primarily dopaminergic effects (reviewed in Knutson and Gibbs 2007). For example, amphetamine has been shown to elicit BOLD or rCBV increases in DA-rich ventro-striatal 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. 1999; Chen et al. 1997), 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 towards a reduced-responsivity of ventro-striatal dopaminergic function analogous to what 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 manifestation 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 neuro-adaptational changes induced by cocaine on DA systems, an aspect that does not appear to be adequately modeled by traditional cocaine exposure paradigms, where “sensitized” (i.e. 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, Gozzi A. 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 haemodynamic measures of brain function performed (i.e. 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 in 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 self-administration. 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.

Disclosure / Conflict of interest

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 three 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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Figure Legends

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 self-administration 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 hours and FR gradually increased to 3 (sessions 4-6) and eventually to 5 (remaining 27 sessions). Repeated 48-72 hrs abstinence periods were introduced on days 16 (session 14, 72 hrs), 23 (session 18, 72 hrs), 31 (session 23, 48 hrs) to minimize the risk of acute cocaine-induced intoxication. A 10-day detoxification period within the home cage was introduced prior to 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 all remaining sessions, corresponding to 1 mg/kg 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 $p = .001$) 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 panels (C) are indicated in mm relative to Z_{bregma} [mPFC: medial pre-frontal cortex; Plc: pre-limbic cortex; Cg:

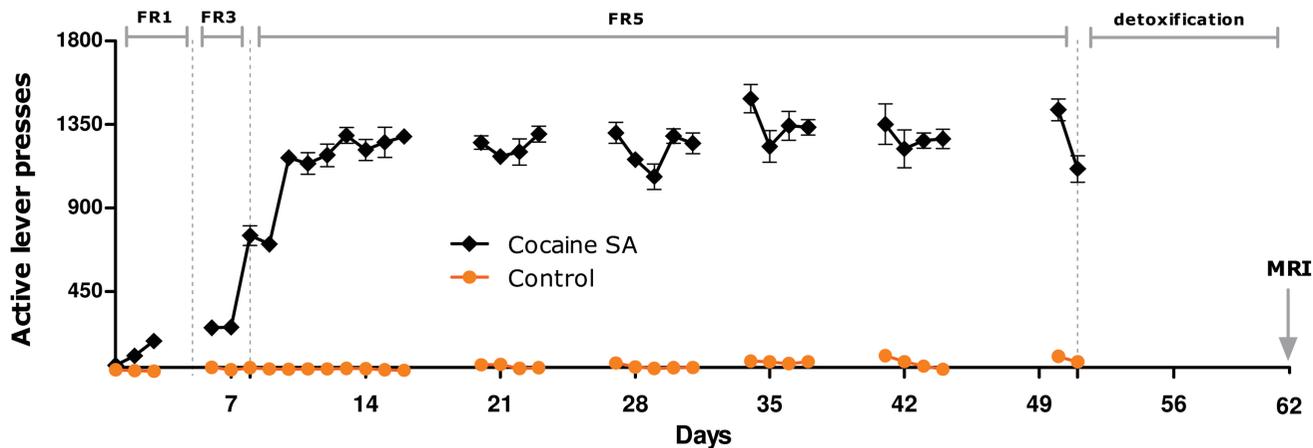
cingulate cortex; Sp; septum; AcbC: core of the nucleus accumbens; Ra: raphe nucleus: rTh; reticular thalamic nucleus; VHc; ventral hippocampus; OFC: orbito-frontal cortex].

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). [Amy: amygdala, mPFC: medial pre-frontal cortex; Cg: cingulate cortex; Sp; septum; AcbC: core of the nucleus accumbens; AcbSh: shell of the nucleus accumbens; Cpu; caudate putamen; Ra: raphe nucleus: rTh; reticular thalamic nucleus; VHc; ventral hippocampus; Hypoth: hypothalamus; OFC: orbito-frontal cortex; Rs: retrosplenial cortex; SS; somatosensory cortex; * $p < 0.05$; ** $p > 0.01$; one-way ANOVA, followed by Fisher LSD test]

Figure 4

Orthogonal view (**A**: horizontal, **B**: coronal, **C**: saggital) 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) and (B) and (C) refer to three orthogonal sections located at $Y_{\text{bregma}} (Y_b) -5.2$ mm, $Z_{\text{bregma}} -1.4$ mm and lateral (L) 5.2 mm, respectively. Cpu: caudate putamen. **D**: correlation rCBV response to amphetamine in the striatum and cumulative cocaine intake over the 52 days of self-administration ($p = 0.03$, dashed lines represent 95% confidence interval).

A**B**