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Cocaine Self-Administration Leads to Alterations in Temporal Responses to Cocaine Challenge in Limbic and Motor Circuitry

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Abstract

Chronic use of cocaine is associated with lasting alterations in brain metabolism, circuitry and receptor properties. We used neuroimaging with pharmacologic MRI (phMRI) to assess alterations in response to cocaine (0.5mg/kg) in animals trained to self-administer (SA) cocaine on a fixed-ratio 5 schedule of reinforcement, as well as saline-yoked controls, after 28 days of cocaine abstinence. We fit the cerebral blood volume (CBV) curves for full-width half-maximum (FWHM) as well as peak CBV response. There were significant increases in the FWHM of the response curves in the cocaine-SA animals compared to saline-yoked controls in medial-prefrontal cortex (mPFC) and caudate/putamenm (CPu) and increases in peak CBV in M1 motor cortex, CPu and pedunculopontine tegmental nucleus. Functional connectivity analysis showed increased correlations in the SA rats upon acute cocaine challenge, especially in the S1, mPFC, and thalamus. Since D3 receptors are postulated to increase following chronic cocaine administration we also examined the response to 0.2 mg/kg of the D3 preferring agonist 7-OHDPAT. Cocaine SA animals showed a decreased overall CBV response to this drug, except in the globus pallidus. The hypothalamus showed a negative CBV change in response to cocaine challenge similar to that noted with the D3 agonist and showed a smaller response in the cocaine-SA animals than the controls. Given the good coupling of cerebral hemodynamics with dopamine dynamics previously observed with phMRI, these data suggest that increased persistence of dopamine in prefrontal cortex may be responsible for some of the behavioral alterations observed subsequent to chronic cocaine use.

Keywords

MRI; dopamine neurons; cerebral blood volume; receptors; addiction

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Introduction

Chronic use of cocaine can lead to enduring alterations in brain circuitry including both limbic elements such as the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) as well as alterations in motor circuitry (Schmidt *et al*., 2005; Thomas *et al*., 2008; Koob, 2009). This chronic use can also lead to alterations in cerebral blood flow and metabolism (Volkow *et al*., 2000; Volkow *et al*., 2009) and as well to alterations in receptor densities and/or binding constants. Prior evidence in primates as well as rodents shows that chronic cocaine can lead to decreases in dopamine (DA) D2 receptor binding (Volkow *et al*., 2004; Nader *et al*., 2006) and further, that decreased basal D2 tone in primates (caused for instance by social subordination (Morgan *et al*., 2002; Nader & Czoty, 2005; Nader *et al*., 2008) or in rodents (associated with high impulsivity (Perry *et al*., 2005) can predispose animals to higher cocaine intake. There is also evidence for upregulation of D3 receptors following chronic cocaine intake in rodents (Neisewander *et al*., 2004) or following cocaine overdoses in humans (Staley & Mash, 1996).

Magnetic resonance imaging can provide maps of brain activity across the whole brain associated with stimulation of drugs such as cocaine (Chen *et al*., 1996; Breiter *et al*., 1997; Chen *et al*., 1997; Marota *et al*., 2000; Mandeville *et al*., 2004; Schwarz *et al*., 2004; Choi *et al*., 2006; Luo *et al*., 2009). The response of a given brain region to a drug such as cocaine or amphetamine is associated with both positive and negative hemodynamic changes reflected in either BOLD, cerebral blood flow (CBF) or cerebral blood volume (CBV) measures. We have previously shown for dopaminergic drugs, that decreased CBV is associated with agonism of D2/D3 receptors and that increased CBV is associated with D1/D5 receptor agonism (Chen *et al*., 2005; Choi *et al*., 2006). Therefore the ability to probe D1/D5 and D2/D3 receptor function using MRI provides a useful tool for examining the function of these receptors as opposed to the static picture generated using either Positron emission tomography (PET) or autoradiography studies of ligand binding. This points out one of the fundamental strengths of the MRI experiments that not only does one get a map, but also a time course, and this time course, in the case of drugs such as cocaine or amphetamine, can be associated with the time course of DA in the brain (Chen *et al*., 1997; Chen *et al*., 2005; Choi *et al*., 2006).

In this manuscript we examined the response to both a cocaine challenge as well as a challenge to a D3-preferring agonist 7-hydroxy-NN-di-r-propyl-2-aminotetralin (7-OHDPAT) in animals trained to self-administer (SA) cocaine and saline-yoked controls. In particular we wished to determine what alterations might be observed in the mPFC – limbic circuitry including the nucleus accumbens and pedunculopontine tegmental nucleus (PPTg). We recently showed that the latter region is strongly involved in the limbic circuitry associated with cocaine reinstatement (Schmidt *et al*., 2009). Therefore we postulated that there would be an increased response to cocaine in the limbic circuitry defined by the mPFC – NAc - PPTg in the cocaine SA animals. We further postulated that we would be able to see alterations in the D3 receptor circuitry as a consequence of cocaine self-administration.

Methods

Animals and Housing

Male Sprague Dawley rats (*Rattus norvegicus*) weighing 250-300 g were obtained from Taconic Laboratories (Germantown, N.Y., USA). Animals were single-housed with food and water available *ad libitum*. All animals were housed in a colony maintained on a 12-hr/12-hr light/dark cycle with the lights on at 7:00 a.m. All experimental procedures were performed during the light phase. All experimental protocols were in accordance with the guidelines set forth by the National Institutes of Health and were approved by the Boston

University School of Medicine and Massachusetts General Hospital Institutional Animal Care and Use Committee.

Surgery

Rats were allowed one week to acclimate to their home cages upon arrival. Prior to surgery, the rats were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine (Sigma/RBI, St. Louis, MO). An indwelling catheter (CamCaths; Cambridge, UK) was inserted into the right, external jugular vein and sutured securely in place. The catheter was connected to a mesh backmount, which was implanted subcutaneously above the shoulder blades. In order to prevent infection and to maintain patency, catheters were flushed daily with 0.3 ml of a solution of the antibiotic Timentin (ticarcillin disodium/potassium clavulanate, 0.93 mg/ml) dissolved in heparinized saline. When not in use, the catheters were sealed with plastic obturators.

Cocaine Self-Administration (SA)

After surgery, rats were allowed seven days to recover before behavioral testing commenced. Initially, rats were placed in operant chambers daily and allowed to lever press for intravenous cocaine (0.25 mg cocaine/59 μl saline, infused over a 5 sec period) on a fixed-ratio 1 (FR1) schedule of reinforcement. Each session began with the i.v. administration of 59 μl cocaine (0.25 mg) to fill the catheter. Rats were allowed to self-administer a maximum of 30 injections per 120-minute operant session. Stable responding on the fixed ratio one (FR1) schedule was defined as less than 15% variation in response rates over three consecutive self-administration days. After stable responding was achieved, animals were switched to a fixed-ratio five (FR5) schedule of reinforcement. The maximum number of injections was again limited to 30 per daily self-administration session under the FR5 schedule. For both the FR1 and FR5 schedules, a 20 second time-out period followed each cocaine infusion, during which time active lever responses were tabulated but had no scheduled consequences. The total doses that were self-administered for the rats we scanned were between 6-7 mg/session, for a total of approximately 150mg/rat.

In this experiment, rats were randomly assigned to one of two groups: cocaine-experimental or saline-yoked. Each rat trained to respond for contingent cocaine infusions (cocaine-experimental) was paired with a yoked subject that received infusions of saline. Responses on the inactive lever were not reinforced for the experimental cocaine rat. Lever pressing for the saline-yoked rats had no scheduled consequences, but these animals received the same number and temporal pattern of infusions as self-administered by the paired cocaine-experimental rat. After 21 days of operant training, rats experienced 28 days of forced drug abstinence (spent in home cages), a length of time equivalent to the combined extinction and reinstatement phases of the behavioral studies from the same cohort of animals that were not scanned described in Schmidt et al. (Schmidt *et al*., 2009). Following this period of forced drug abstinence, rats underwent imaging.

Imaging studies

The animals were ventilated to maintain pCO2 in a normal physiological range (35-45 mmHg) during the imaging session. Scanning took place under halothane anesthesia (1.2%) and intravenous infusion of pancuronium bromide (2mg/kg/hr) for muscle paralyzation. The tail vein was catheterized for both contrast agent and drug administration. Body temperature was regulated using a circulating warm-water blanket set to 37°C. Arterial blood gas sampling and blood pressure were measured by catheterization of the femoral artery. Blood gases were taken periodically before and during imaging while blood pressure was measured continuously throughout the session. Animals were scanned in a 9.4T magnet (Bruker Instruments, Billerica, MA) using the IRON (Increased Relaxation for Optimized

Neuroimaging) technique to generate maps of relative cerebral blood volume (CBV) (Chen *et al*., 2001). Iron oxide contrast agent was injected through a tail vein to sensitize the images to CBV as described previously (Chen *et al*., 2001; Mandeville *et al*., 2001; Mandeville *et al*., 2004). Briefly, a dextran-coated supramagnetic intravascular contrast agent of monocrystalline iron oxide nanoparticles (MION, 15 mg/kg) was administered after 15 baseline images. Then continuous imaging was performed to attain a post-MION baseline (150 time points) before cocaine administration. The cocaine challenge injection was administered through a tail vein as a bolus (0.5 mg/kg, i.v.). Contiguous coronal slices (180 time points) were obtained for each time point using conventional gradient echo planar images with 16 segments, a field of view (FOV) of 30mm, and 96×96 resolution with 0.75mm slice thickness, and a TR/TE of 625/8ms. Temporal resolution was 10s per time point.

We administered 0.5mg/kg of cocaine since prior studies indicated that this produces a robust response of CBV, and this closely matches the total dose administered for each of the lever presses during the self-administration described above. In order to probe functional alterations in D3 receptors we used the selective D3 agonist 7-OHDPAT that was administered 60 min after cocaine. At this time point cocaine effects have recovered to baseline for CBV and the lifetime for cocaine in the rat brain is about 20 min (Du *et al*., 2009). In macaques and Sprague-Dawley rats, our PET data shows that tracer level doses of cocaine are back to baseline in about 40 min, quite similar to what has been reported in humans (Telang *et al*., 1999). Further studies with much larger doses in mice (20 mg/kg i.p.) show recovery to baseline concentrations by 60 min (Azar *et al*., 1998) similar to that seen in rats by Carmona et al. using 17 mg/kg i.p (Carmona *et al*., 2005). These studies show a t1/2 for clearance, at these high doses, of about 26 min. Our microdialysis studies in rats show that extracellular fluid (ECF) DA levels return to baseline in less than 40 min when using 0.5mg/kg i.v. (Chen *et al*., 2010), this is similar to the DA kinetics reported by others using microdialysis (Othman *et al*., 2007). Therefore the 60 min interval we chose was to allow for clearance of cocaine and DA, but to minimize the time spent under anesthesia. For the 7-OHDPAT the rationale for the dose was to use a dose that evokes strong behavioral effects and also evokes strong alterations in cerebral blood volume, but still shows selectivity for D3 over D2 receptors. We recently published an extensive investigation of the dose-dependence of 7-OHDPAT in naïve rats and that study (Choi *et al*., 2010), consistent with other data from Levant's group (Levant *et al*., 1996), shows that the 0.2mg/kg is still relatively selective for D3 over D2R.

All images were registered onto the same standard brain template for subsequent averaging across animals using an automatic routine developed by Dr. JB Mandeville. Registration between the functional image set and the standard template was performed by adjusting 12 registration variables (3 translations, 3 rotation angles, 3 skew (non-rigid) angles and 3 inflations on the 3 major planes.

The imaging data were analyzed by conversion of changes in signal intensity to CBV values. Images were aligned to a template and regions of interest (ROI) were drawn from a registered atlas (Paxinos & Watson, 1997). The CBV time courses from the ROIs were fit to gamma variate functions using a general linear model and statistical maps were corrected for the total numbers of voxels fit for in the image in the brain only. Maps were constructed for both the self-administering animals as well as the saline-yoked controls of the following: significant changes in CBV, peak CBV values, the full width half maximum (FWHM) of the gamma variate function, and the contrast between all these functions in the self-administering as well as saline animals.

Statistical comparisons of the alterations in mean peak CBV values and FWHM of the curves fit for each of the cocaine SA and saline-yoked animals were compared for each ROI reported were made using a one-way ANOVA.

Seed-based functional connectivity analysis was carried out on the set of CBV study with acute cocaine challenge. CBV temporal profiles from selected seeding areas were used as basis functions (temporally detrained) for correlation analysis through the whole brain on a pixel-by-pixel basis (AFNI 3dDeconvolve). Functional connectivity associated with cocaine challenge was obatined using the contextual dependent approach to deconvolve functional connectivity from the basal resting period. The seeding areas included the following brain areas: CPu (anterial, lateral, and dorsosuperioral portions), M1, S1, S2, mPFC (including cingulate cortex I, cingulate cortex II, prelimbic cortex), thalamus (central portion, and ventroposterior/ventromedial protion), and insular cortex.

Results

CBV response to cocaine challenge

We examined the response to cocaine stimulus (0.5mg/kg, i.v.) in animals trained to self-administer cocaine as well as saline-yoked controls three weeks after their last self-administration session. In both groups, cocaine induces a biphasic signal change with an early negative CBV response followed by a larger positive response. The negative “early-dip” is larger in areas of the brain with the highest expression of DA receptors including nucleus accumbens and caudate/putamen (CPu). Such a map is shown for the cocaine-SA animals and saline-yoked animals in Fig. 1 with the fits to the negative and positive CBV components. Since D2/D3 receptor agonism induces negative CBV changes and D1/D5 receptor agonism induces positive CBV changes (Chen *et al*., 2005; Choi *et al*., 2006) these two components likely represent the response of these two receptor systems. Overall, the pattern of response to the cocaine challenge is reasonably similar between the cocaine SA and saline-yoked controls as shown in the maps in Fig. 1. However, there were significant differences in a number of brain regions. We utilized an ROI-based approach to data analysis comparing the peak CBV changes and alterations in FWHM for the ROIs. The group data are reported in Table 1. Comparisons between the cocaine SA and saline-yoked animals for the brain regions showing significant differences in the two groups are shown in Figs. 2-5. We made maps of significant changes in peak CBV change and FWHM using subtractions between the cocaine SA and saline-yoked animals and also plotted the time course for changes in CBV (for more detail see Methods). There were significant increases in FWHM in dorsolateral (motor) CPu ($F_{1,11} = 7.26$; $P<0.05$) and mPFC ($F_{1,11} = 5.07$; $P<0.05$) (Figs 2 and 3) with trends toward increase FWHM in hippocampus and nucleus accumbens (see Table 1) and increases in peak CBV in dorsolateral CPu ($F_{1,11} = 5.16$; $P<0.05$; Fig. 2), M1 motor cortex ($F_{1,11} = 7.58$; $P<0.05$; Fig. 4), and PPTg ($F_{1,11} = 7.26$; $P<0.05$; Fig. 5). Interestingly, in the hypothalamus cocaine induces a monotonic negative CBV change (Fig. 6). Due to the shape of the curve we did not fit for FWHM but rather integrated the CBV change from either 1-10 min or from 4-10 min. In either case, there was a significant difference between the cocaine SA and saline-yoked controls with the cocaine SA animals having the smaller response (for cocaine SA vs. saline controls respectively the CBV changes were: from 1-10min -4.8±0.8 vs. -9.0 ± 1.5 $p<0.05$ or from 4-10min -5.1±0.8 vs. -9.4±1.8 $p <0.05$). The hypothalamic signal may represent D3 receptor mediated signal change as suggested by our recent data with D3 receptors (Choi *et al*., 2010) as well as the data shown below for challenge with a D3 preferring agonist.

Functional Connectivity in Cocaine SA Animals

In order to assess the brain-wide effects of cocaine self-administration that may not be picked up by simple comparisons of alterations in CBV amplitude we compared the changes in "functional connectivity" (Biswal *et al*., 1995; Li *et al*., 2000; Pawela *et al*., 2008) in three groups of animals: naïve, saline-yoked and cocaine-SA following cocaine challenge. These data analyses were performed using different seed regions and the response during the 16 min during which CBV is elevated following cocaine injection after correction for the effects of the baseline (see Methods for full description of the analysis). These data revealed that at 28 days of abstinence the cocaine group demonstrated increased functional connectivity from almost all seed regions to other brain regions. The effects were particularly strong in numerous cortical areas – especially in S1 as well as mPFC, whether seeded from cortex or from thalamus. Interestingly, functional connectivity to and from the thalamus or mPFC was stronger in the saline-yoked controls than it was in the naïve animals (although weaker than the cocaine SA animals) indicating the effects of the overall protocol on these brain regions. Shown in Fig. 7 are maps demonstrating these effects for representative seed regions in mPFC, thalamus and CPu. Quantitative results are shown in Fig 10.

CBV response to 7-OHDPAT challenge

We were also interested in probing D3 receptor function as there are suggestions of increased D3 receptor binding in rats abstinent from cocaine for time periods comparable to those of the rats we studied here (Neisewander *et al*., 2004). Therefore, at the end of the cocaine imaging session (sixty min following cocaine injection), we injected a D3 preferring agonist 7-OHDPAT at 0.2mg/kg (i.v.). This drug induces a negative CBV that is larger in the NAc than in the CPu as one would expect from its D3 selectivity (Stanwood *et al*., 2000; Neisewander *et al*., 2004). The overall magnitude of the negative CBV change is larger in the control group than in the SA group comparing either the CBV time course or a map of the integrated CBV change between 10-60 min (Fig. 8). The integral of the CBV change in the NAc (where the D3 receptor density, along with the islets of Calleja is highest in the brain (Bouthenet *et al*., 1991; Sokoloff *et al*., 1992) was -16.4±5.3 in the control group (n=3) and -10.5±3.2 (n=4) in the SA group. There also appears to be a difference between the two groups in the mPFC with the integrated CBV changes being -14.7 ± 6.7 in the controls and -8.7±4.7 in the SA animals. Interestingly, 7-OHDPAT typically leads to a late CBV increase in the globus pallidus in naïve animals ((Choi *et al*., 2006) Fig. 3). As discussed later, this late CBV increase in the internal globus pallidus (GPi) may reflect the result of agonism of D2 receptors in the CPu. The late positive CBV response in the GPi was larger in the cocaine-SA rats than in the saline-yoked controls (Fig. 9).

Discussion

These results indicate a pattern of alterations in limbic and motor circuitry in animals trained to self-administer cocaine. Our data show alterations in brain regions not previously described, such as hypothalamus or little described such as the PPTg. We also found alterations in functional connectivity in cocaine SA rats 28 days abstinent that is dramatically different from both the saline-yoked control group and naïve animals. Our data also show altered time courses for the CBV changes elicited by cocaine in various brain regions that have not previously been described such as hypothalamus. We found that there is a pronounced increase in the response to cocaine, as measured using the amplitude of changes in CBV, in primary motor cortex (M1) and in motor regions of the CPu. Smaller increases were noted in the PPTg. In addition to these increases in magnitude we also noted increases in the full width half-maximum (FWHM) of the CBV time course in the mPFC (but no change in peak CBV) and again in motor CPu. These changes are accompanied by

alterations in functional connectivity in the cocaine SA animals. The alterations show large increases in the functional connectivity overall, with the largest increases occurring to and from cortical areas. These increases are not inconsistent with sensorimotor sensitization noted with cocaine self-administration. Given the ability of cocaine to greatly increase locomotor activity in rodents as well as to produce locomotor sensitization after chronic exposure (Marin *et al*., 2009), it is not surprising that we would see sensitization of sensorimotor cortex and the motor CPu. Given that we only examined rats with saline-yoked controls rather than cocaine-yoked controls (i.e. animals receiving cocaine non-contingently) it is possible that some of the changes we observed in the motor circuitry could be related to the motor conditioning of pushing the bar for the cocaine. We chose not to use a cocaine-yoked control as a substantial body of literature shows that non-contingent cocaine administration is highly stressful (Mutschler & Miczek, 1998; Galici *et al*., 2000; Lecca *et al*., 2007). Locomotor sensitization does occur, however, with non-contingent administration of cocaine (Marin *et al*., 2009).

There is a large body of evidence indicating dysregulation of the prefrontal cortex and its glutamatergic connections to the NAc as a consequence of substance abuse (Thomas *et al*., 2008; Koob, 2009). The frontal cortical-striatal circuitry is responsible for behavioral adaptations that may facilitate craving and the inability to inhibit drug taking (Kalivas, 2009; Koob & Volkow, 2010). A recent review by Koob and Volkow postulates that various elements of the reward/addiction circuitry are modulated at different time points in the addictive process (Koob & Volkow, 2010). These elements include the ventral tegmental area (VTA) and the ventral striatum in the initial intoxication phase, the extended amygdala for the withdrawal negative affect stage and a *widely distributed network* including orbitofrontal cortex, prefrontal cortex, striatum and hippocampus (among others) in the craving/preoccupation phase (Koob & Volkow, 2010). Since our rats were scanned 28 days after cessation of cocaine self-administration, they would presumably fall under the latter stage where much more widespread alterations in frontal, striatal, and hippocampal circuitry are postulated by Koob and Volkow (Koob & Volkow, 2010). It is unclear, however, what form such alterations might take in vivo. Our data show alterations in mPFC (increased FWHM), hippocampus (increased FWHM), and striatum (increased peak CBV and FWHM) and in numerous other brain regions using functional connectivity analysis as noted in Fig 10.

Our data show an increased FWHM in the mPFC in response to cocaine in the SA animals compared to controls. There are two possible explanations for this. The first is that a decrease in basal metabolism/hemodynamics might produce a larger response (Lee *et al*., 2003) as has been observed in fMRI in cases where basal CBF is lowered using something like hypocapnia (Cohen *et al*., 2002). Another possible explanation is that since there is little DAT in the mPFC leading to “volume transmission” of DA (Hitri *et al*., 1994; Haaparanta *et al*., 1996; Hall *et al*., 1999), there could be reduced clearance of DA in the SA animals. This mechanism was tested in both wild-type mice and mice with knockouts of catechol-O-methyltransferase (COMT) where it was found that cocaine induced an increased overflow time for DA without increasing the peak DA amplitude (Yavich *et al*., 2007), corresponding to what we found using phMRI (i.e. increased FWHM but not in peak amplitude). We previously showed that extracellular DA and CBV changes correlate linearly (at moderately high doses) following challenges with either DA releasers (such as amphetamine) or DAT blockers such as cocaine and (–)-2-β-Carbomethoxy-3-β-(4-fluorophenyl)tropane (β-CFT) (Chen *et al*., 1997; Chen *et al*., 2005; Choi *et al*., 2006; Ren *et al*., 2009). These prior studies show that the induced CBV changes following cocaine or amphetamine challenge can be used as a readout for changes in DA concentration. It is also possible that there are alterations in COMT activity in frontal cortex as a consequence of cocaine SA. Genetic polymorphisms of COMT that lead to higher or lower activity (and thereby lead to increased

or decreased DA metabolism) produce correspondingly increased or decreased fMRI response in reward tasks in frontal cortex (Dreher *et al*., 2009).

Human studies show decreased functional connectivity in cocaine abusers compared to controls in either the resting state or in selective tasks using fMRI or following cocaine administration (Li *et al*., 2000; Gu *et al*., 2010; Hanlon *et al*., 2010). Our data show increased functional connectivity in rats. There a several possible interpretations of these differences. First, in humans, there are disparities in total cocaine administered and time of administration compared to our well controlled group. Second, the humans are awake in the scanner and this may lead to profound differences in the connectivities. However, the biggest difference is that in humans and in non-human primates cocaine produces BOLD or CBV decreases whereas in rats it leads to increases in BOLD or CBV (Breiter *et al*., 1997; Gollub *et al*., 1998; Kufahl *et al*., 2005; Mandeville *et al*., 2011).

The mPFC sends excitatory glutamatergic projections to the PPTg (Sesack *et al*., 1989) that in turn sends cholinergic and glutamatergic projections that synapse on DA neurons in the VTA. We recently showed that infusion of the AMPA antagonist CNQX directly into the PPTg attenuated the ability of a priming injection of cocaine to reinstate drug seeking in animals trained with the same self-administration protocol as those used in this study (Schmidt *et al*., 2009). This would accord with increased glutamatergic tone in the PPT that is reflected in increased CBV in response to cocaine and with the increased response we see in mPFC.

Interestingly, in the first minute after cocaine infusion there is a transient decrease in CBV that has recently been reported by Luo et al. (Luo *et al*., 2009) and us (Chen *et al*., 2010). Our maps of this early dip show that it is quite close to maps one sees with D2 agonists (Chen *et al*., 2005; Choi *et al*., 2006; Chen *et al*., 2010). We previously showed that D1/D5 agonism using numerous different D1 agonists produces positive CBV changes and D2/D3 agonism produces negative CBV changes and (Choi *et al*., 2006; Chen *et al*., 2010; Chen *et al*., 2010) thus this early dip is consistent with agonism of D2 receptors. Since D2 presynaptic receptors as well as D3 receptors have a much higher affinity for DA than do post-synaptic D2 or D1 receptors (Cooper *et al*., 2003), it is possible these receptors produce an earlier negative response than post-synaptic D1 and D2 receptors.

A previous study found a large decrease in the amplitude of the BOLD response to a cocaine challenge in rats that had been given repeated i.p. injections of cocaine compared to vehicle. Those data were collected in awake rats without control of CO_2. Since CO_2 can dramatically alter the BOLD, CBV or CBF response it is difficult to compare these data. Administration of cocaine can lead to hyperventilation (Sharkey *et al*., 1991) and reduced CBV (see Fig 12.4 in (Chen & Jenkins, 2007)). Since hyperventilation leads to decreased CO_2 it would also lead to decreased BOLD in the cocaine animals (Febo *et al*., 2005). Our data show, however, that there are not huge differences in the amplitude of the response to cocaine in self-administering and saline-yoked controls, but rather more subtle regional differences in peak response and FWHM.

D2 and D3 Receptor Modulation

The effects of cocaine on dopamine receptors have been previously examined using in vivo positron emission tomography scans in rodents and primates including monkeys and humans. These studies show a consistent pattern of decreased D2 binding potential in both animals self-administering cocaine (Volkow *et al*., 1999; Nader & Czoty, 2005; Nader *et al*., 2006), as well as in human cocaine abusers (Martinez *et al*., 2004; Martinez *et al*., 2009; Volkow *et al*., 2009). Not as much work has gone into studying the effects of chronic cocaine administration on D3 receptors. One study of cocaine SA found that 2-8 days after

abstinence that there was no change in D3 binding as measured using autoradiography, but at 30 days of abstinence following cocaine there was an increase in D3 receptor binding (Neisewander *et al*., 2004). Oddly in that paper they used a challenge dose of cocaine 24 hrs before the D3 autoradiography. Dopamine D3 receptor levels were also found to be increased in humans who had died of a cocaine overdose (Staley & Mash, 1996). Other data showed that a single dose of cocaine was enough to increase D3 receptor expression (Le Foll *et al*., 2005). Our data show a decreased response to the D3 preferring agonist 7-OHDPAT in the nucleus accumbens where there is very high D3 expression. We recently demonstrated the in vivo selectivity of 7-OHDPAT at 0.2 mg/kg by showing that the CBV response in NAc is much greater than that in the CPu as opposed to the D2 agonist norpropylapomorphine where the response in NAc and CPu is about equal (Choi *et al*., 2010). It should be noted that the phMRI data is producing a readout on the functional status of the receptors as opposed to autoradiography which looks at a static picture of receptors available for binding. Our data could be explained in two ways. First, Sokoloff's group showed a single cocaine challenge can increase D3 expression at time points greater than about 4 hours after cocaine; at time points less than four hours following cocaine there was a significant decrease in D3R (Le Foll *et al*., 2005). Since we challenged the animals with cocaine 60 min before the D3 agonist it is possible that the decrease in D3R is larger in the cocaine SA animals than in controls. The other possibility is that there is diminished D3 tone overall in the cocaine SA animals, although this explanation would not agree with the D3 findings in either (Neisewander *et al*., 2004) or (Staley & Mash, 1996). Future molecular studies examining the functional status of these receptors should serve to distinguish between these two possibilities.

We previously showed that D2/D3 agonists produce negative CBV changes whereas D1/D5 agonists produce positive CBV changes (Chen *et al*., 2005; Choi *et al*., 2006; Chen *et al*., 2010). Although the primary response to cocaine in both the cocaine SA animal as well as the saline-yoked controls is mostly positive – there are brain regions in which a negative CBV response is found such as the septum or hypothalamus. The response of the hypothalamus to cocaine challenge produced a negative CBV change in both the cocaine SA animals as well as the saline-yoked controls. The time course for this change was quite distinct from that produced in the areas of the brain where mostly positive CBV changes were noted (compare Figs. 1-5 with Fig. 6). Indeed, the time course for the CBV change induced with cocaine in the hypothalamus matches well with the time course produced by administration of the D3 agonist 7-OHDPAT (compare time courses in Fig. 6 with Fig. 9) and both responses are larger in the saline-yoked animals than in the cocaine SA animals. We recently showed that 7-OHDPAT produces negative CBV changes in hypothalamus whereas the D3 selective antagonist PG-01037 produces positive changes in the hypothalamus (Choi *et al*., 2010). Therefore, these data suggest that measurement of cocaine-induced signal changes in hypothalamus may produce a readout on the status of D3 receptors, although this concept requires further proof. In summary, cocaine produces enduring changes in brain circuitry consistent with both motor sensitization (increase response to cocaine challenge in M1 cortex and motor striatum) as well as limbic alterations (altered dynamics in medial prefrontal cortex and decreased response to D3R stimulation in the nucleus accumbens). These data suggest the great potential that in vivo neuroimaging may play in discovering alterations in dopaminergic circuitry in brains subject to chronic intake of psychostimulants.

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Citations

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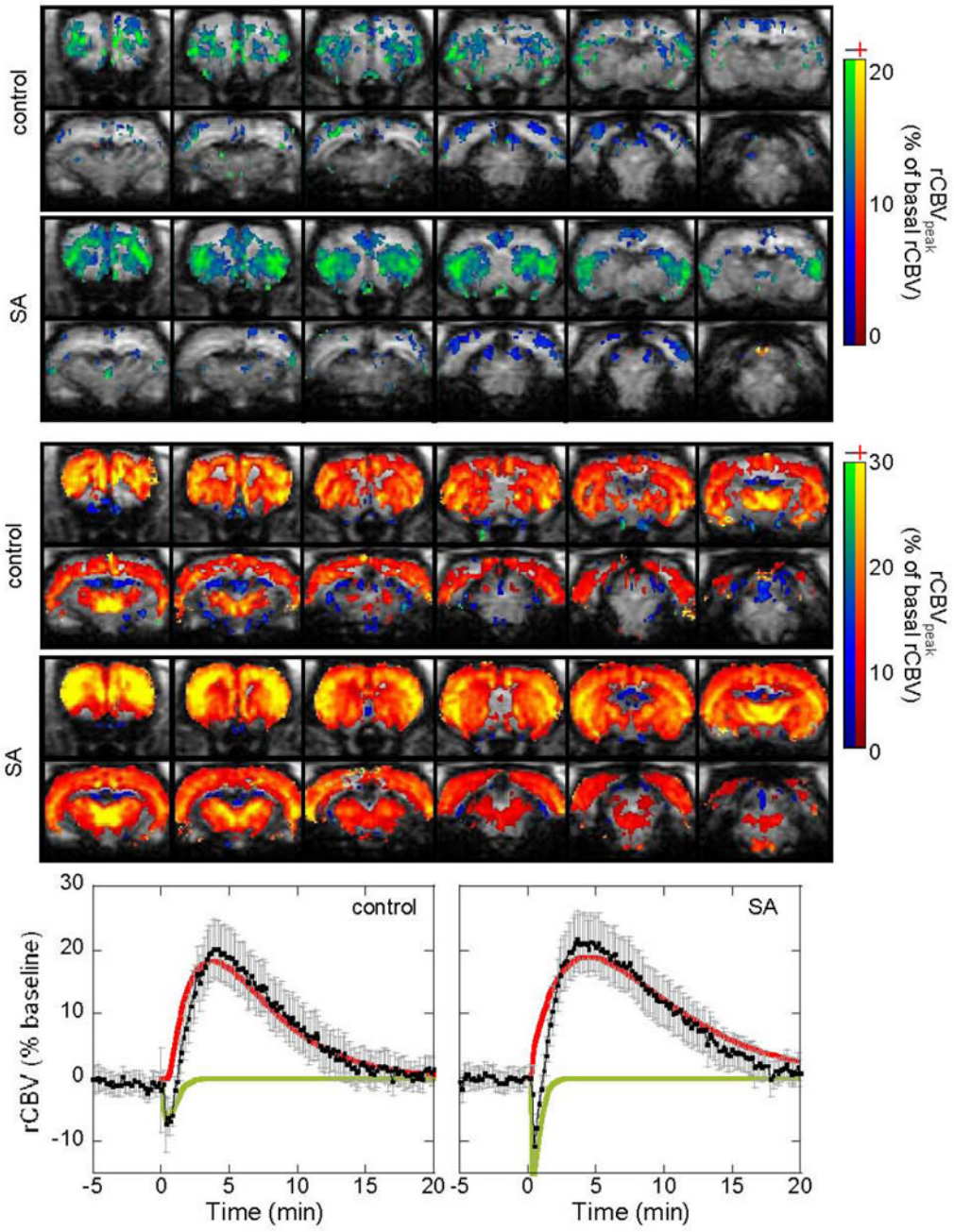

Figure 1.

CBV responses to acute cocaine challenge (0.5mg/kg iv) for the cocaine-SA animals and saline-yoked animals with the fits to the negative (green line) and positive CBV components (red line). The upper panel shows brain areas with transient CBV decreases (fit to the green line), the 2nd panel shows brain areas with CBV increases (fit to the red line). Time courses are from the whole CPu.

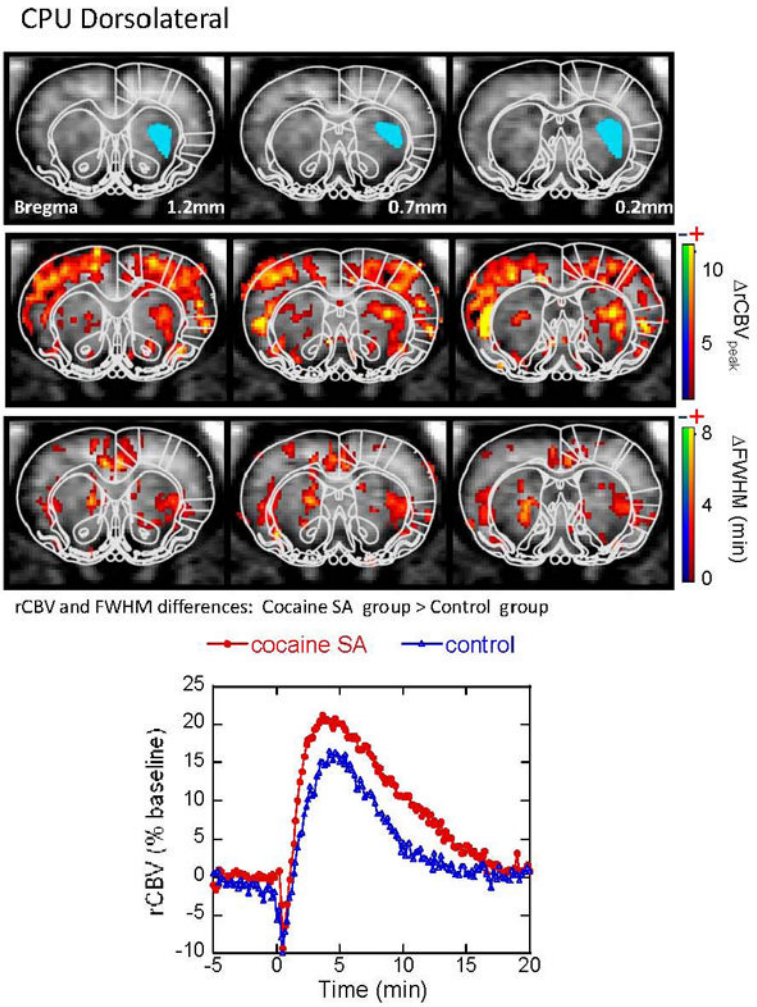

Figure 2.

CBV response to acute cocaine challenge in the dorsolateral CPU. Maps are shown for regions showing significant differences between the cocaine-SA animals and controls in both CBV amplitude and FWHM. Cocaine-SA animals have a greater CBV increass and longer half-life (ie, longer FWHM), compared to the saline-yoked animals. Time courses are taken from ROI (the blue filled areas) in the top panel bilaterally (only one side is shown in blue for clarity).

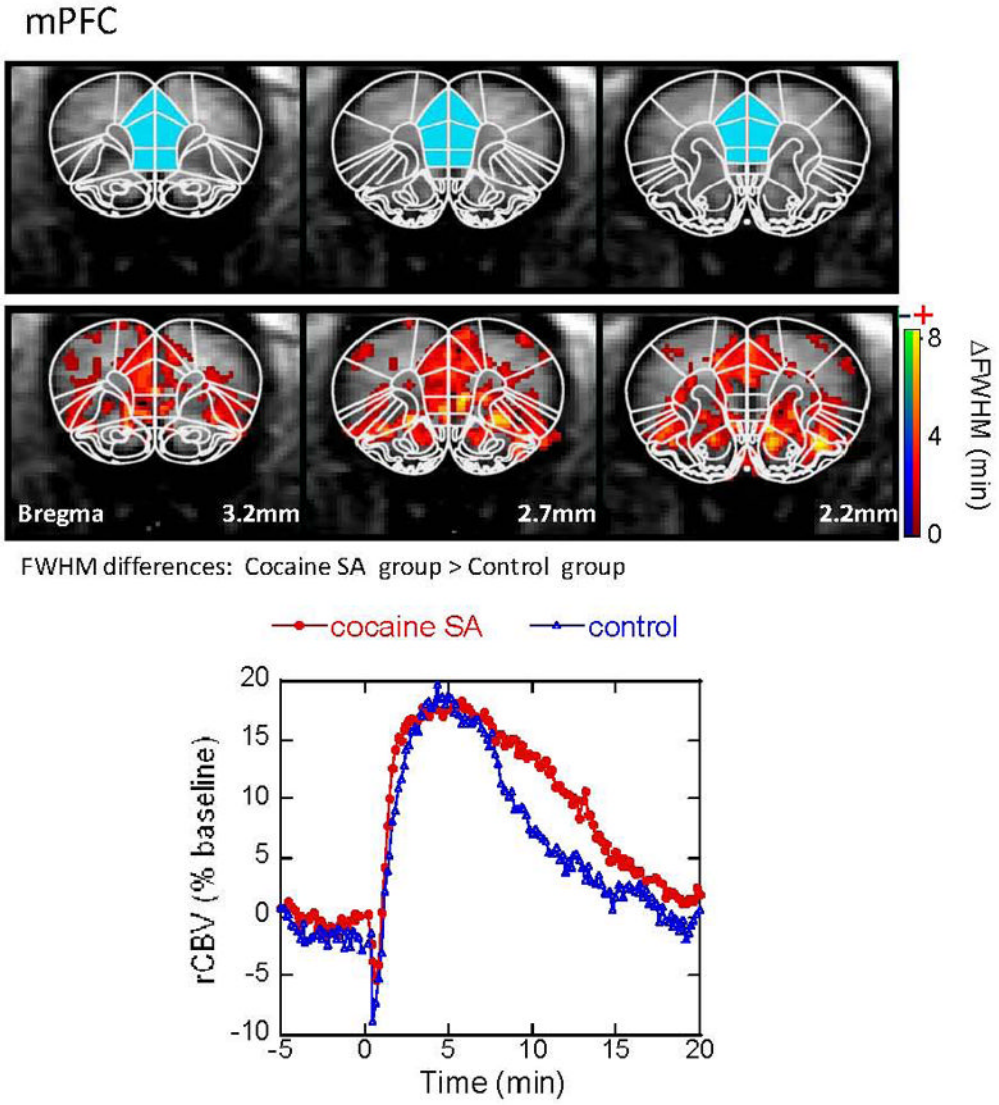

Figure 3.

CBV response to acute cocaine challenge in the medial prefrontal cortex (mPFC). Maps are shown for regions showing significant differences between the cocaine-SA animals and controls in FWHM (the changes in CBV amplitude were not significant). Cocaine-SA animals have a larger integrated CBV response due to increased FWHM compared to the saline-yoked animals. Time courses are taken from ROI (the blue filled areas) in the top panel.

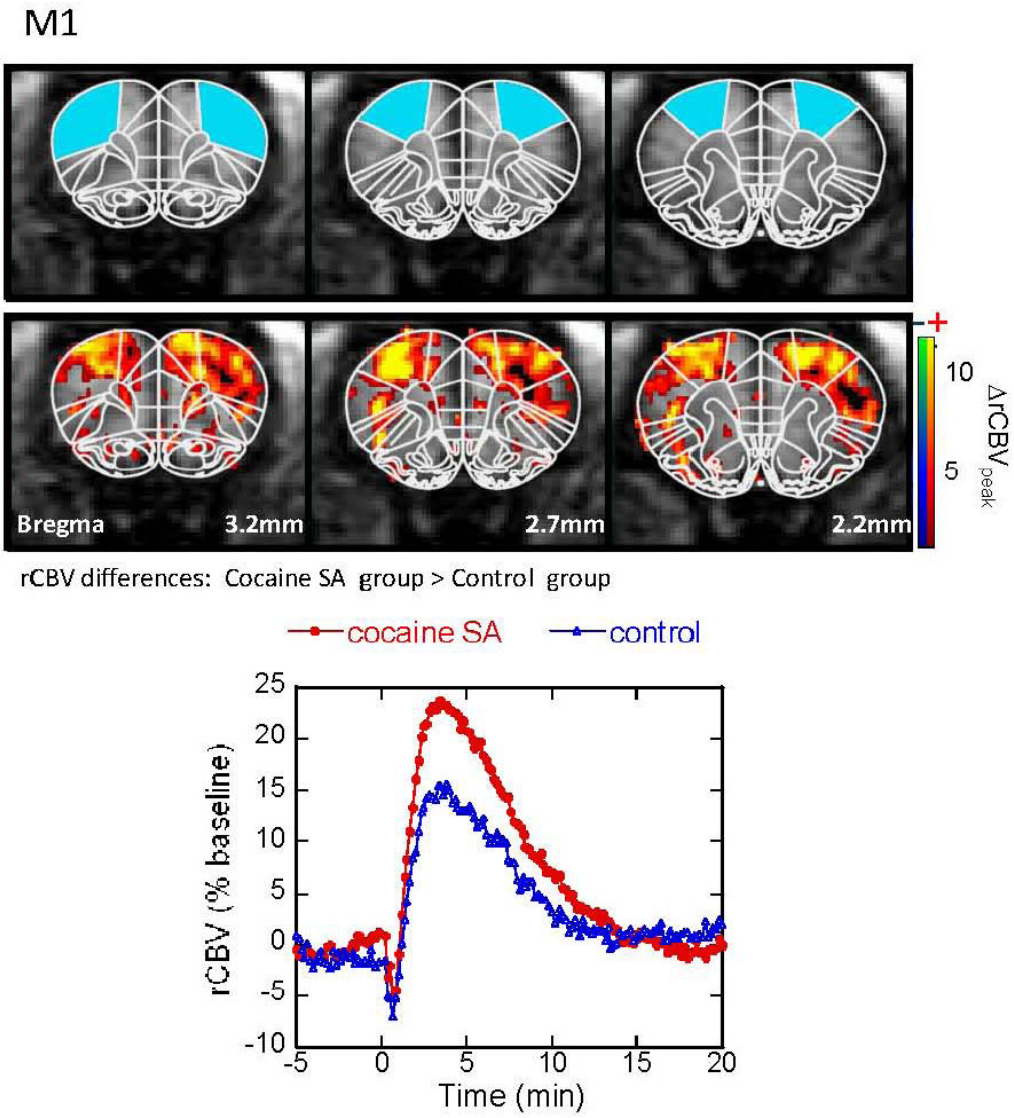

Figure 4.

CBV response to acute cocaine challenge in M1 motor cortex. Maps are shown for regions showing significant differences between the cocaine-SA animals and controls in CBV amplitude. Cocaine-SA animals showed greater CBV increases, compared to the saline-yoked animals (but no change in FWHM). Time courses are taken from ROI (the blue filled areas) in the top panel.

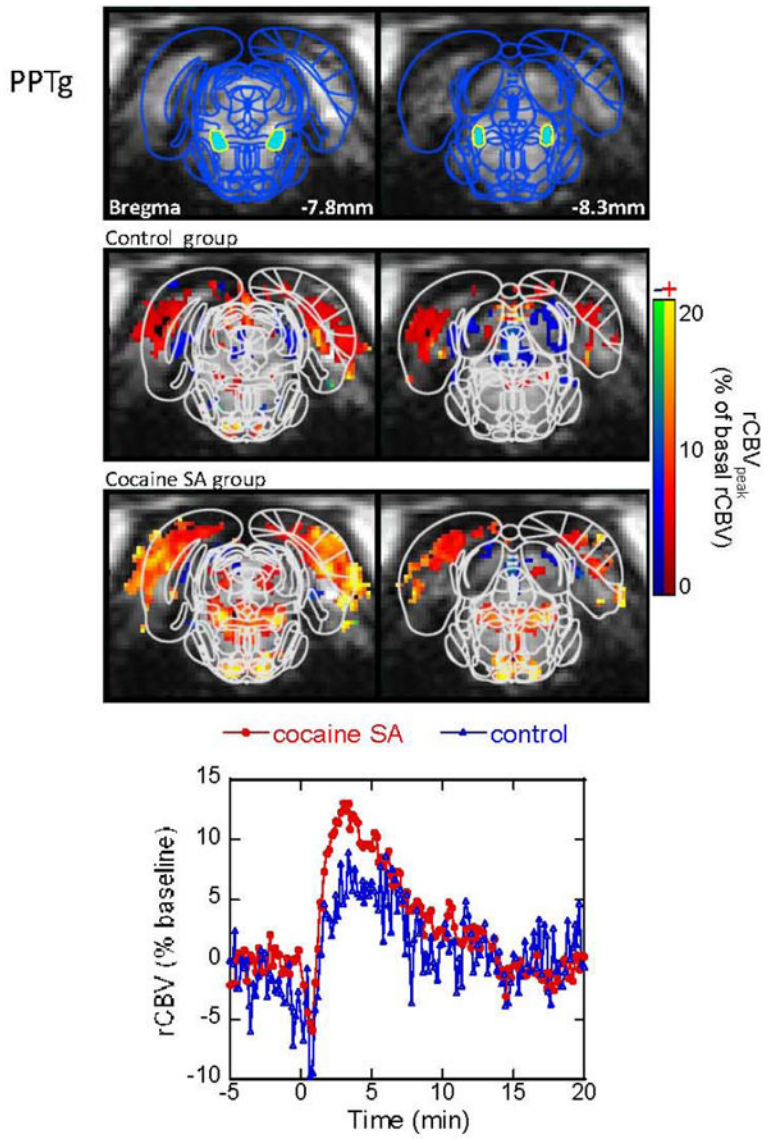

Figure 5.

CBV response to acute cocaine challenge in the PPTg. Maps are shown for significant changes in CBV in both the cocaine-SA animals and saline-yoked controls. Cocaine-SA animals have greater CBV increases, compared to the saline-yoked animals. Time courses are taken from ROI (the blue filled areas) in the top panel.

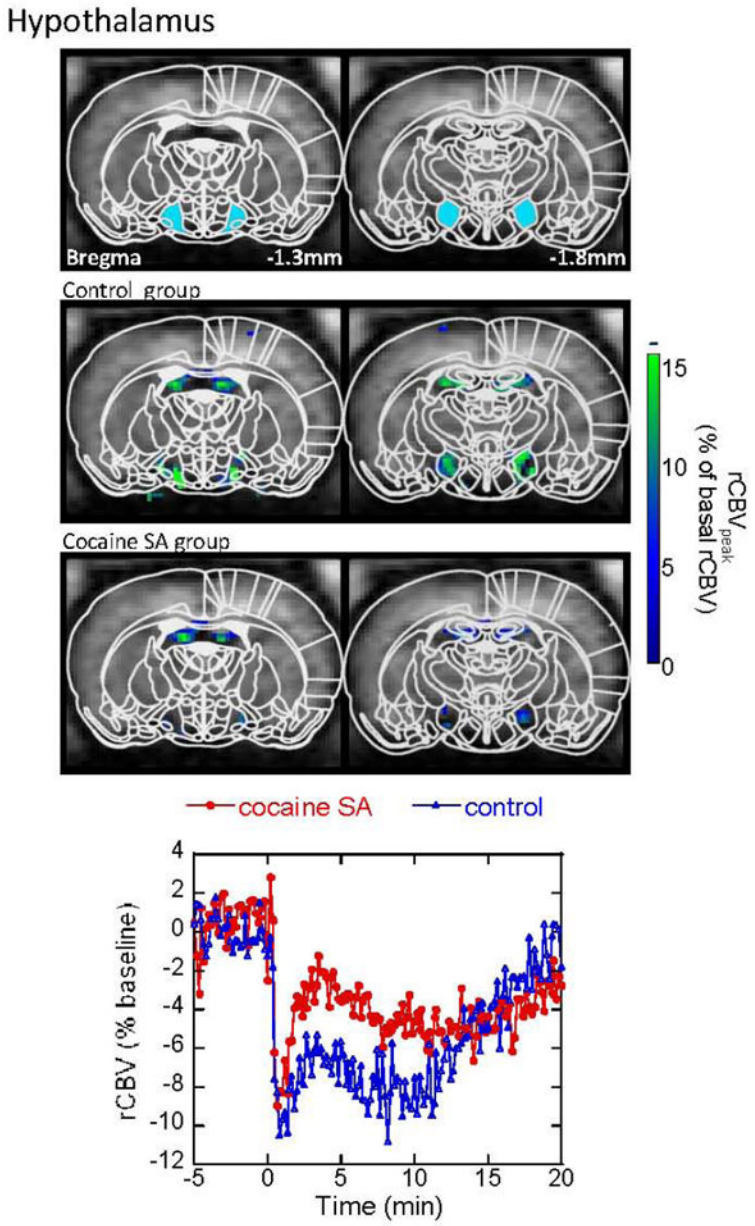

Figure 6.

CBV response to acute cocaine challenge in the hypothalamus. Maps are shown for significant changes in CBV in both the cocaine-SA animals and saline-yoked controls. Note that in this brain regions the changes in CBV induced by cocaine challenge are negative compared to the positive CBV changes observed in the brain regions shown in the prior figures. Cocaine-SA animals have smaller CBV decreases, compared to the saline-yoked animals. Time courses are taken from ROI (the blue filled areas) in the top panel.

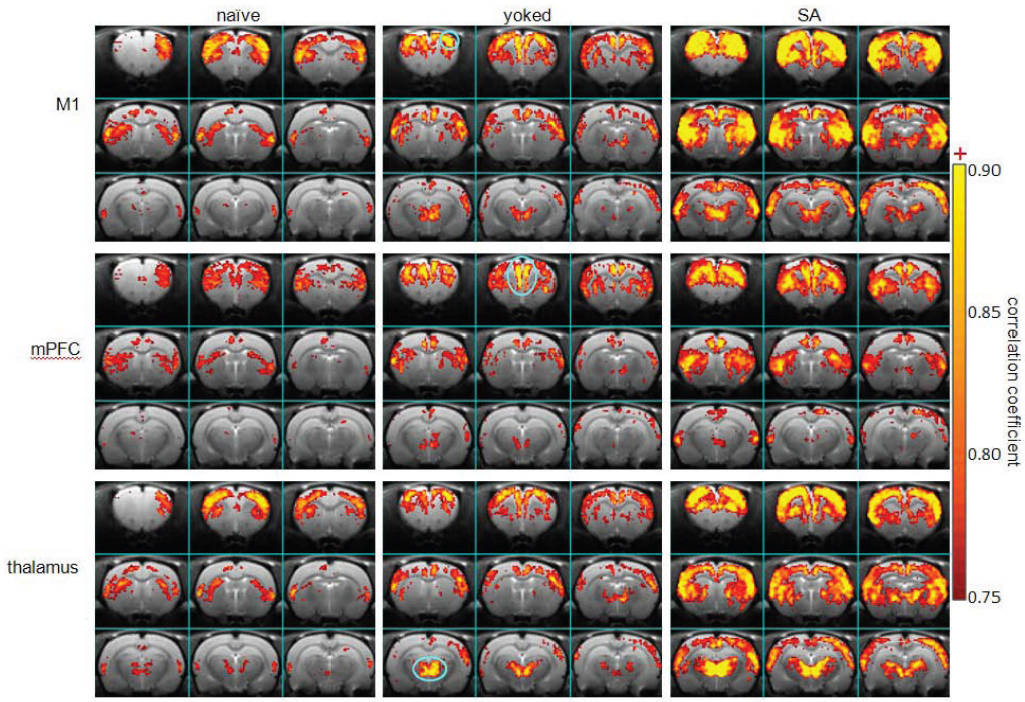

Figure 7.

Maps of functional connectivity from S1, mPFC, and thalamus in responding to acute cocaine challenge (naïve, n=5; yoked control: n=5; cocaine SA: n=7). Functional connectivity from these three seeding areas were slightly increased in the yoked rats and significantly increased in the cocaine SA rats, compared to the naïve rats. Blue lines encircle the ROIs for the seeding areas.

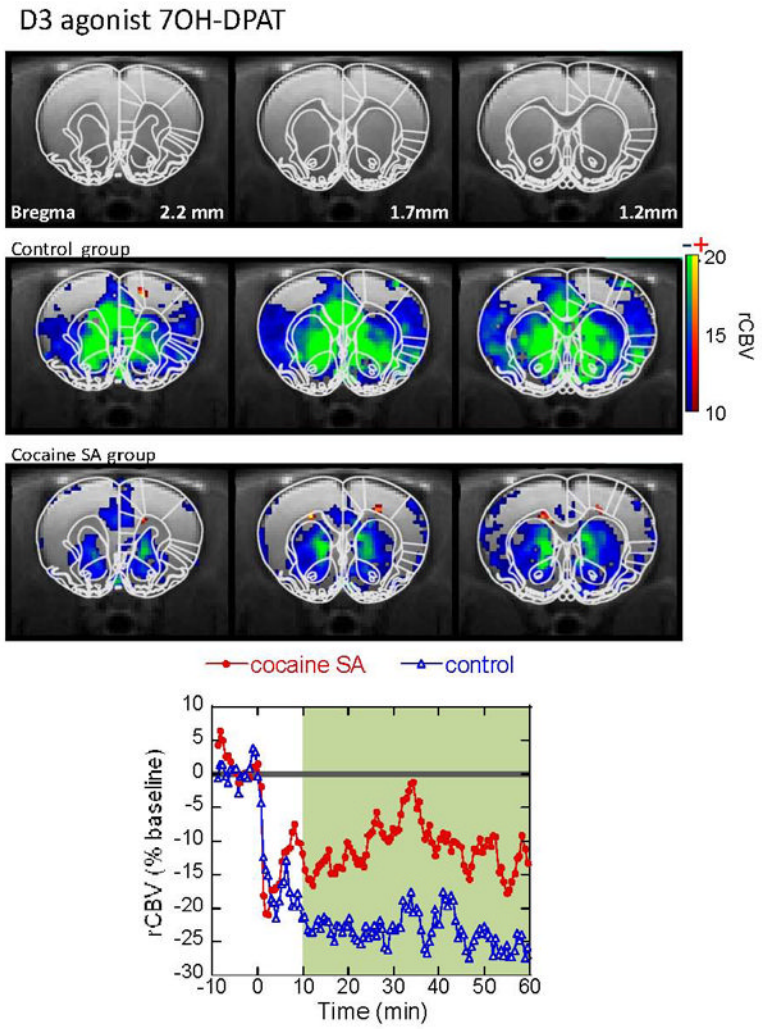

Figure 8.

CBV response to acute challenge with the D3 agonist 7OH-DPAT. Maps are shown for significant changes in CBV in both the cocaine-SA animals and saline-yoked controls. There are significant CBV decreases in the mPFC, medial CPu, and NAc, where D3 receptor density is highest. The degree of CBV decreases to 7OH-DPAT challenge is less prominent in the cocaine-SA animals, compared to the saline yoked ones.

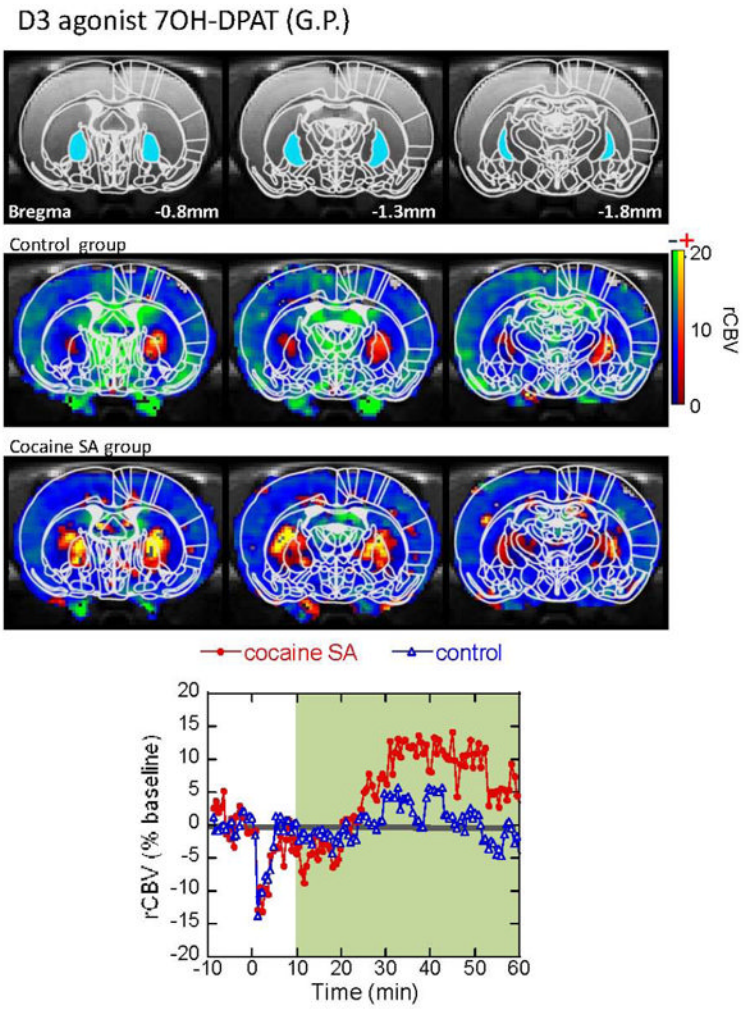

Figure 9.

CBV reseponse to acute challenge with the D3 agonist 7OH-DPAT in the globus pallidus. There is a delayed CBV increase in the globus pallidus in saline-yoked animals, indicating downstream activity of the dopaminergic striato-pallidal neurons. The delayed positive CBV response was greater in the cocaine-SA rats, compared to the saline-yoked rats.

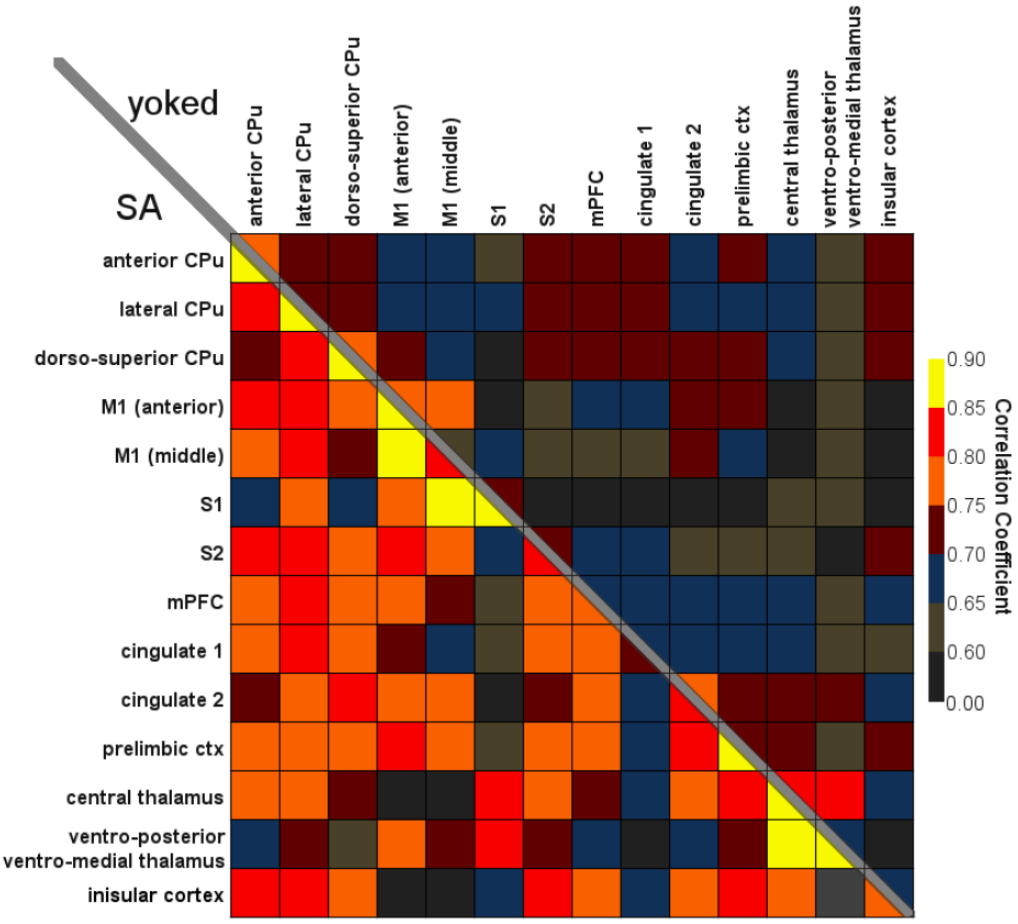

Figure 10.
Cross correlation of functional connectivity upon acute cocaine challenge. Values represent correlation coefficients derived from connectivity analysis made as described in the Methods.

Table 1

Brain Regional Changes in rCBV in Cocaine and Saline Animals After Cocaine Challenge.

Region of Interest	Peak rCBV		FWHM	
	Cocaine SA (n=7)	*Saline (n=5)*	*Cocaine SA (N=7)*	*Saline (n=5)*
CPu	21.1 ± 1.9*	16.7 ± 3.0	8.4 ± 0.7*	5.9 ± 1.1
mPFC	21.5 ± 3.5	22.0 ± 5.0	11.3 ± 1.6*	7.3 ± 1.3
Cing	18.5 ± 3.0	21.1 ± 4.4	9.5 ± 1.6	7.8 ± 1.8
Thal	29.6 ± 4.3	30.0 ± 3.6	7.4 ± 0.6	7.1 ± 0.9
S2	26.6 ± 2.5	24.1 ± 2.5	7.7 ± 0.6	6.2 ±1.0
S1	19.8 ± 3.1	17.7 ± 2.4	6.3 ± 0.9	6.4 ± 0.9
M1	27.7 ± 2.8*	16.6 ± 2.7	6.6 ± 0.6	6.0 ±0.9
NAc	13.1 ± 1.7	16.2 ± 3.8	9.4 ± 1.7	7.5 ±1.7
Hip	13.4 ± 2.3	15.1 ± 2.2	8.6 ± 1.7	6.7 ± 0.3
PPT	12.7 ± 2.1 (n=6)*	8.3 ± 1.5 (n=4)	6.4 ± 0.4	6.3 ± 0.7
VTA	13.1 ± 1.8	12.2 ± 1.0	6.9 ± 0.5	5.5 ± 0.8
HThal	-4.8 ± 0.8*	-9.0 ± 1.5	ND	ND

$p<0.05$ for cocaine SA animals compared to saline-yoked controls. Cocaine was administered at 0.5mg/kg i.v.

ND: not determined; CPu: Caudate putamen; mPFC: medical prefrontal cortex; S1: primary sensory cortex; S2: secondary sensory cortex; M1: primary motor cortex; NAc: nucleus accumbens; PPTg: pedunculopontine tegmental nucleus; VTA: ventral tegmental area