The Neural Consequences of Repeated Cocaine Exposure Revealed by Functional MRI in Awake Rats

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The use of functional magnetic resonance imaging (fMRI) in animal models of cocaine addiction is an invaluable tool for investigating the neuroadaptations that lead to this psychiatric disorder. We used blood-oxygen-level-dependent (BOLD) MRI in awake rats to identify the neuronal circuits affected by repeated cocaine administration. Rats were given an injection of cocaine (15 mg/kg, i.p.) or its vehicle for 7 days, abstained from injections for 1 week, and challenged with an intracerebroventricular cocaine injection during functional imaging. Acute cocaine produced robust positive BOLD responses across well-known monoamine-enriched brain regions, such as the prefrontal cortex, nucleus accumbens, dorsal striatum, sensory cortex, hippocampus, thalamus, and midbrain areas. However, repeated cocaine administration resulted in lower BOLD responses in the prefrontal cortex, agranular insular cortex, nucleus accumbens, ventral pallidum, and dorsomedial thalamus, among other brain regions. Reductions in BOLD intensity were not associated with variations in cerebrovascular reactivity between drug naïve rats and those repeatedly exposed to cocaine. Therefore, the lower metabolic activation in response to cocaine could reflect a reduced neuronal and/or synaptic activity upon repeated administration.
Neuropsychopharmacology (2005) **30,** 936–943, advance online publication, 22 December 2004; doi:10.1038/sj.npp.1300653

Keywords: cocaine; drugs of abuse; BOLD imaging; functional MRI; reward circuitry; awake rat; sensitization

INTRODUCTION

The use of animal models of cocaine addiction along with techniques to assess neuronal activity and function has aided the identification of the underlying neuropathology of this brain disorder (Bozarth *et al*, 1980). The behavioral sensitization model assesses the progressive enhancement of locomotor behavior upon repeated cocaine administration in rats (Post and Rose, 1976). The increased locomotor behavior produced by cocaine can persist after prolonged abstinence periods (Pierce and Kalivas, 1997; Robinson and Berridge, 1993; Vanderschuren and Kalivas, 2000). The neuroadaptations that result in behavioral sensitization in rats have been postulated to involve cocaine-induced changes in neuronal activity that lead to psychostimulant-induced psychosis, as well as pathological craving for cocaine in human addicts (Kalivas *et al*, 1998; Post and Rose, 1976; Robinson and Berridge, 1993). The brain circuitry mediating cocaine's initial locomotor and reinfor-

cing action is the mesocorticolimbic dopamine system (Goeders and Smith, 1983; Vanderschuren and Kalivas, 2000). However, this circuit is anatomically and physiologically embedded within other limbic and cortical brain regions (Pierce and Kalivas, 1997). Cocaine-induced alterations within mesocorticolimbic system, such as changes in gene expression (Graybiel *et al*, 1990), synaptic monoamine levels (Kalivas and Duffy, 1993), dendritic spine formation (Robinson and Kolb, 1999), and the biophysical properties of neurons (Ungless *et al*, 2001) could also lead to physiological alterations in other brain areas. Therefore, in order to fully identify, and understand, the functional alterations in the neural substrates targeted by cocaine, noninvasive *in vivo* techniques must be employed.

Cocaine's effects on neuronal activity within the brain can be assessed *in vivo* using the blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) technique. The BOLD fMRI signal is associated with a reduction in paramagnetic deoxyhemoglobin (dHb) concentrations near sites of increased neuronal activity (Ogawa *et al*, 1990). Increased oxygenation of these areas contributes to the elevations in BOLD signal intensity (Thompson *et al*, 2003). An acute cocaine injection increases cerebral metabolic activity (Porrino, 1993) and blood flow (Stein and Fuller, 1992); effects that are likely to contribute to changes in BOLD signal intensity (Febo *et al*, 2004). These alterations could possibly involve effects of

cocaine on synaptic monoamine levels throughout cortical and subcortical structures (Goeders and Smith, 1983; Kalivas and Duffy, 1993). In the present experiment, we used BOLD imaging to assess the effects of cocaine in fully awake rats that were drug naïve or had been previously exposed to the drug.

MATERIALS AND METHODS

Subjects

In total, 24 adult male Sprague–Dawley rats weighing 350–450 g were obtained from Charles River Laboratories (Charles River, MA). Animals were housed in pairs, maintained on 12 : 12 light : dark cycle (lights on at 0900) and provided food and water *ad libitum*. All animals were acquired and cared for in accordance with the guidelines published in the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publications No. 85-23, Revised 1985).

Cocaine Administration

Cocaine hydrochloride was purchased from Sigma-Aldrich (St Louis, MO) and dissolved in sterile saline (0.9%). Rats were given a daily cocaine (15 mg kg^{-1}, intraperitoneal) or saline vehicle (0.1 ml/100 g) injection for 7 consecutive days, and allowed 1 week free of injections before brain imaging experiments (Figure 1a). The i.p. cocaine pretreatment dose and injection regime has been previously reported to produce robust locomotor sensitization in rats that lasts up to 3 weeks of withdrawal (Uslaner *et al*, 2003). Cocaine-induced brain activation was then assessed during functional imaging following 1 week of abstinence from repeated injections. This resulted into three groups: (1) rats that received vehicle injections on all days (vehicle control; $n = 4$); (2) those that were given cocaine only on the day of functional imaging (acute cocaine; $n = 8$); and (3) those that received cocaine injections on all days (repeated cocaine; $n = 8$ per group) (Figure 1a).

Functional Imaging

Studies were performed with a multiconcentric dual-coil, small animal restrainer developed by Insight NeuroImaging Systems, LLC, (Worcester, MA) (Ludwig *et al*, 2004). Prior to imaging studies, animals were acclimated to the restrainer and the imaging protocol as described previously by Febo *et al* (2004). Briefly, animals were lightly anesthetized with 2% isoflurane and secured into the dual-coil restrainer. When fully conscious, the restraining unit was placed into a black opaque tube that served as a 'mock scanner' and a tape-recording of an MRI pulse sequence played for 90 min to simulate the bore of the magnet and an imaging protocol. This procedure was repeated on 3 consecutive days. Experiments were conducted in a Bruker Biospec 4.7-T/40-cm horizontal magnet (Oxford Instrument, Oxford, UK) equipped with a Biospec Bruker console (Bruker, Billerica, MA, USA) and a 20-G/cm magnetic field gradient insert (ID = 12 cm, 120-μs rise time).
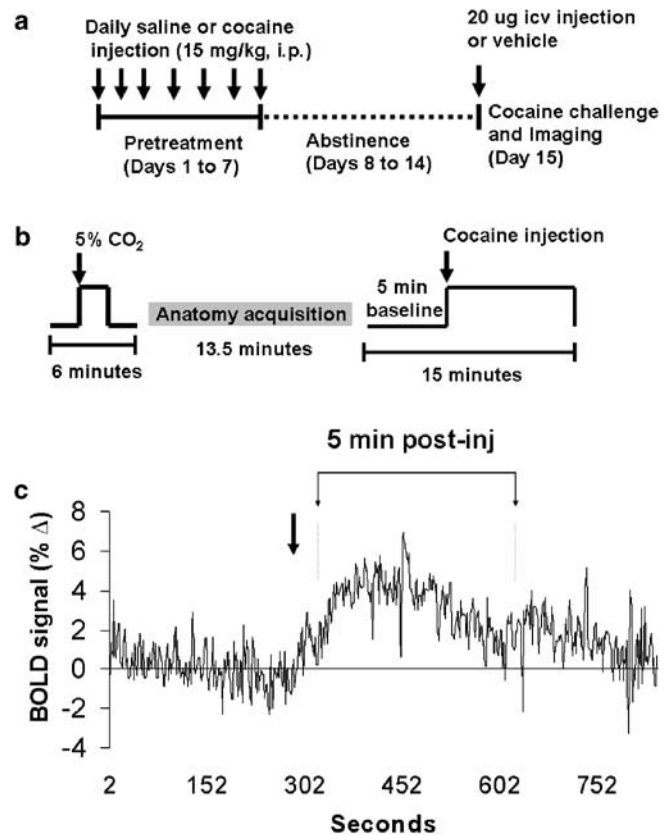

Figure 1 Study design. (a) Cocaine injection dose and injection paradigm used. (b) Functional imaging experimental design. (c) BOLD signal response to cocaine, as reported in Febo *et al* (2004), showing the timepoint at which the peak signal intensity is attained during the timeseries.

Challenge cocaine injections were made via the intracerebroventricular (ICV) route while the animal was inside the magnet. The dose of cocaine used was 20 μg dissolved in 10 μl of artificial cerebrospinal fluid. The injection route and dose have been shown to reduce cardiac and respiratory complications associated with peripheral psychostimulant administration (Febo *et al*, 2004). Immediately prior to imaging, rats were anesthetized under 2% isoflurane, the skull surface was exposed and the landmark suture Bregma located. A 26-gauge cannula of polyethylene tubing (PE-10: inner diameter 0.28 mm, outer diameter 0.61 mm) was implanted into the lateral cerebral ventricle (1 mm caudal to Bregma, 2 mm lateral to the mid-sagittal sinus, and 4 mm ventral to dura) and secured to the skull with surgical glue. Cocaine injections were made via a plastic syringe connected at the end of the tubing. Cannula placement was verified prior to cocaine imaging with a short anatomical MR scan. Only animals with correct placement were included in the study.

Functional images were obtained with a spin echo EPI pulse sequence (64×64 data matrix, TE = 55 ms, TR = 2000 ms). Images were continuously acquired during 15 min that were divided between a 5-min baseline and a 10-min period following ICV cocaine injection (Figure 1b). High-resolution anatomical scans were collected using a fast spin echo pulse sequence (TE = 48 ms, TR = 2500 ms,

FOV = 3 cm, 1.2 mm slice thickness, 256 × 256 data matrix, 16 RareFactor).

In total, 20–25 min prior ICV cocaine injections, drug naïve, and cocaine pretreated rats were exposed to a 2-min 5% CO_2 pulse during a short functional scan (6 min) (Figure 1b). Global cerebral blood flow can be modulated by several physiological parameters; among these is CO_2 (Cohen et al, 2002; Sicard et al, 2003). In general, both cerebral blood flow and BOLD intensity increase in the presence of hypercapnic conditions (Cohen et al, 2002; Sicard et al, 2003). Thus, we used the hypercapnia stimulus as a way of assessing differences in cerebrovascular reactivity between the two treatment groups.

Data Analysis

Selection of regions of interest (ROI) and statistical analysis were performed using the Stimulate software (Strupp, 1996). Movies of functional scans were generated and carefully examined to detect gross movements (ie frequent voxel displacements during time series) and the raw data time series were analyzed for course spikes. Data from two animals in the repeated cocaine group and two in the acute group were discarded due to excess motion artifact. ROI were drawn according to the Atlas of the Rat Brain (Paxinos and Watson, 1997) and are shown in Figure 2a. Before tracing the ROI, functional scans were aligned to a reference anatomical scan using the image analysis software ImageJ (Wayne Rasband, http://rsb.info.nih.gov/ij/; Karl

Schmidt http://www.quickvol.com). Each slice of the functional maps and anatomical images from each subject were manually coregistered to a reference anatomical scan using custom software and the following degrees of freedom: x and y translation, independent x and y scale, and rotation about the z-axis. The original images were then re-sampled without interpolation in the transformation to the atlas coordinate system, and a new set of images was created.

Raw signal intensity values per ROI of each animal were extracted and normalized to their time series baseline (expressed as percent change from baseline). Data for each animal was then averaged during the first 5-min post-cocaine injection in order to make statistical comparisons (Figure 1c). Statistical analysis was performed using a t-test to compare percent changes in BOLD signal intensity and activated pixels between drug naïve and cocaine pretreated rats.

In order to generate positive BOLD activation maps for visualization, the composite functional maps were subjected to a pixel-by-pixel t-test comparing signal intensity during the 5-min preinjection baseline period and 5 min immediately after cocaine injection (Figure 1c). The selected postinjection period corresponds to the timeframe where the peak BOLD response to ICV cocaine occurs (Febo et al, 2004). Pixels whose BOLD percentage change relative to the baseline period was significantly different at a 95% confidence level were overlaid onto the reference anatomical data set.
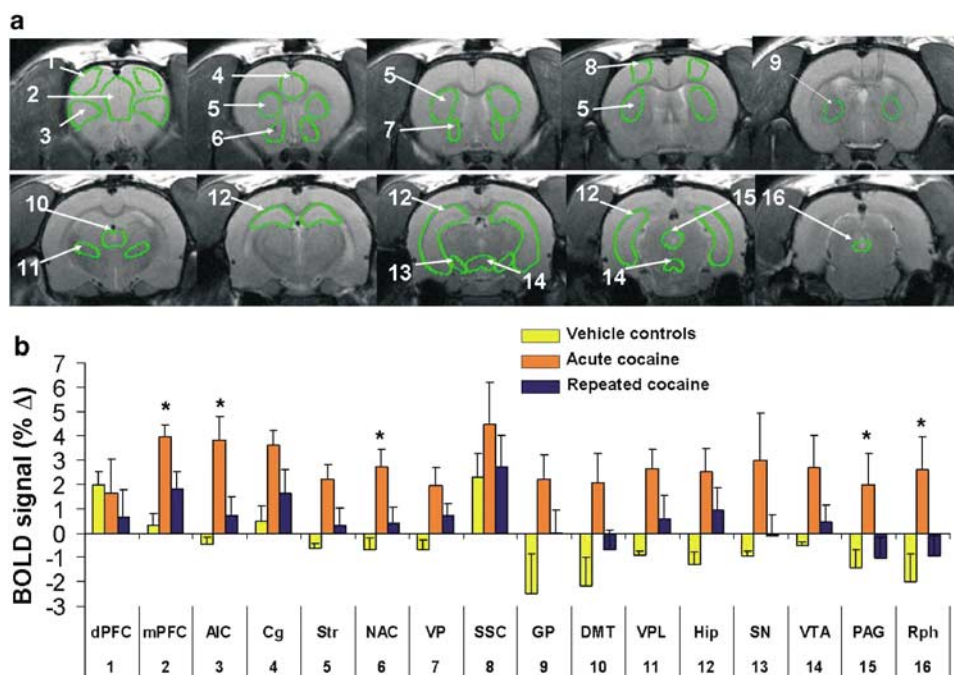

Figure 2 Cocaine-induced BOLD activation in drug naïve and cocaine pretreated rats. (a) Representative high-resolution anatomical images showing regions of interest. Numbers indicate the following regions: (1) dorsal prefrontal cortex (dPFC); (2) medial prefrontal cortex (mPFC); (3) agranular insular cortex (AIC); (4) anterior cingulate cortex (Cg); (5) dorsal striatum (Str); (6) nucleus accumbens (NAC); (7) ventral pallidum (VP); (8) somatosensory cortex (SSC); (9) globus pallidus (GP); (10) dorsomedial thalamus (DMT); (11) ventroposterolateral thalamus (VPL); (12) hippocampus (Hip); (13) substantia nigra (SN); (14) ventral tegmental area (VTA); (15) periaqueductal grey (PAG); (16) raphé (Rph). (b) Average BOLD responses to cocaine in brain areas depicted in (a). Data are averages of the first 5 min after ICV cocaine injections and expressed as mean ± SEM. *denotes p < 0.05 comparing saline vs cocaine pretreated. Numbers along the x-axis indicate the brain areas depicted in (a).

RESULTS

BOLD Response to Acute Cocaine

The spatial pattern of positive BOLD activation following an acute dose of cocaine resembled the distribution of monoamine synaptic terminals (Hokfelt et al, 1974; Thierry et al, 1973) and the pattern of cocaine-induced [^{14}C]2-deoxyglucose labeling in the rat brain (Porrino, 1993). As reported previously by our laboratory, immediate and robust positive BOLD activity was observed in several cortical subregions, such as the medial prefrontal, anterior cingulate, agranular insular, primary motor, and somatosensory cortices (Figures 2 and 3) (Febo et al, 2004). Positive BOLD signal changes were also observed in limbic and extrapyramidal structures (Figures 2 and 3). The temporal activation pattern is consistent with our previous findings (Febo et al, 2004). A peak positive BOLD response of 3.2–3.9% in the nucleus accumbens and prefrontal cortex
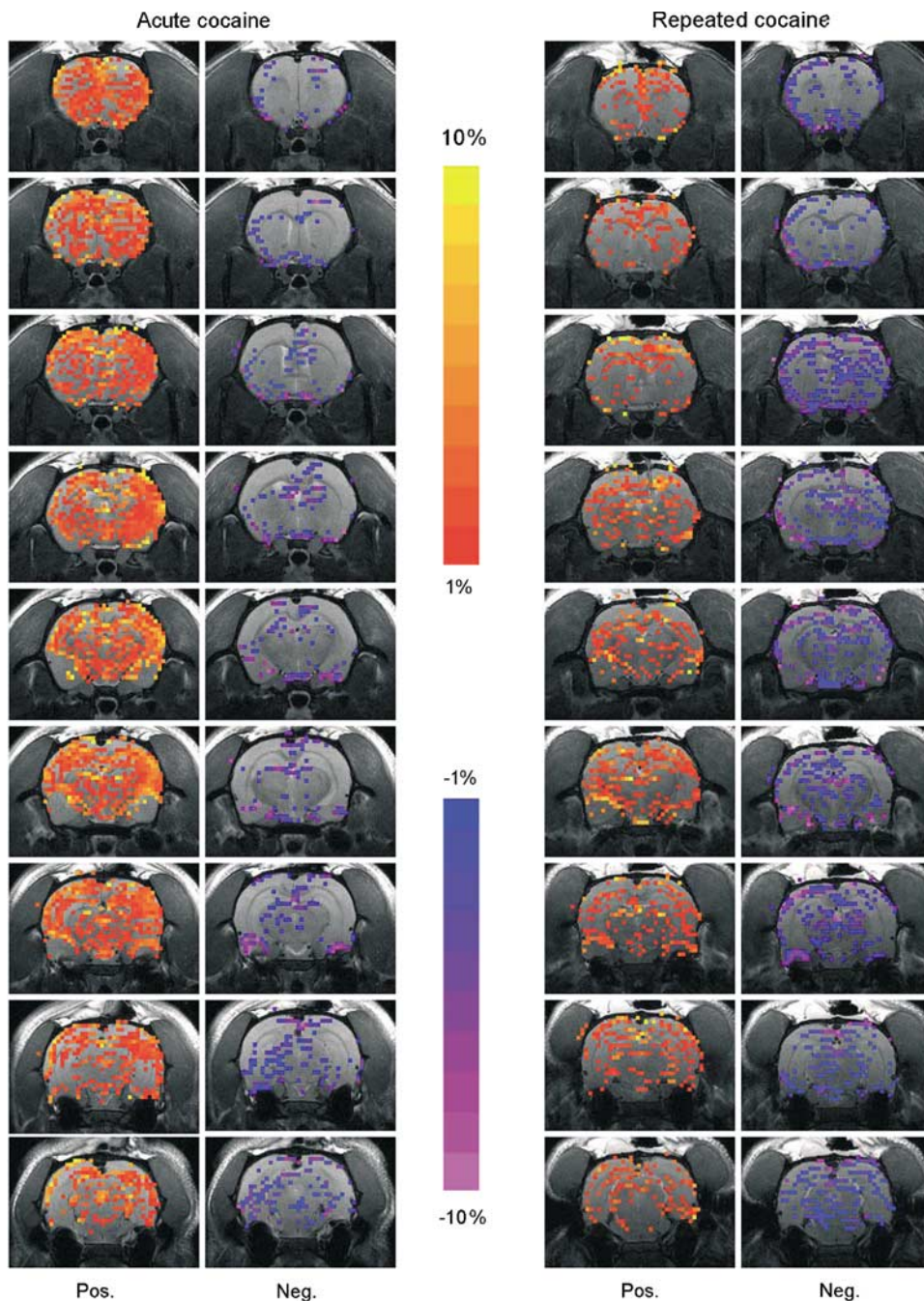

Figure 3 Mesocorticolimbic BOLD response to cocaine in drug naïve and cocaine pretreated rats. (a) Activation maps correspond to composite images of rats given acute cocaine ($n = 8$) and repeated cocaine injections ($n = 8$). Colored pixels represent brain areas that showed signal intensity values significantly different from baseline (scale bar hue indicates percent change value). Statistical significance was determined using pixel-by-pixel t-test analysis (Stimulate software, Strupp, 1996) comparing baseline to a cocaine injection period ($\alpha = 0.05$, uncorrected).
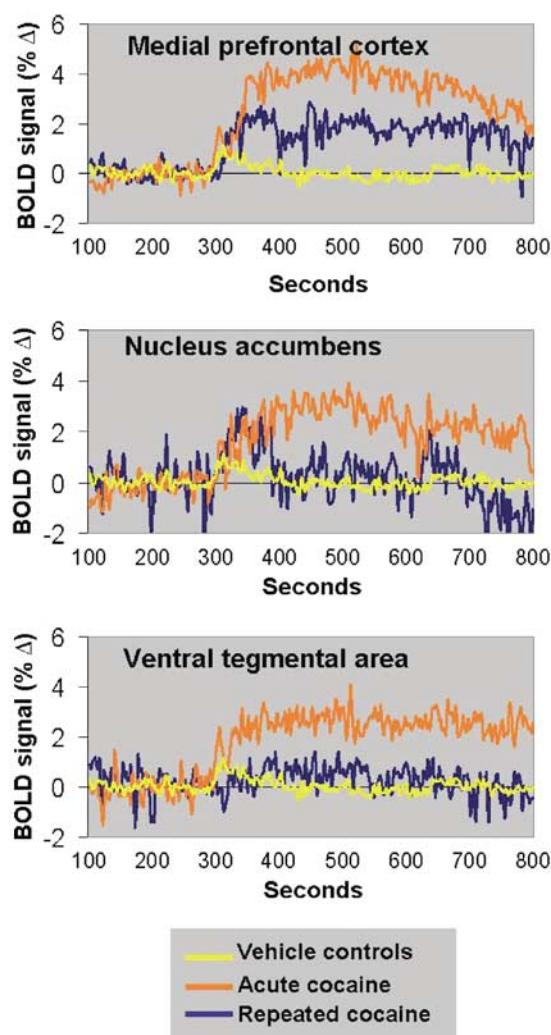
Figure 4 Change in BOLD signal intensity over time following ICV cocaine administration. Data in each treatment group correspond to the average BOLD response across all animals. Arrow indicates the timepoint of cocaine injection. Rats pretreated with cocaine (blue lines) showed lower BOLD responses in the mesocorticolimbic system than rats given an acute cocaine injection (orange lines) or vehicle injections (yellow lines).

and 1.9% in the ventral tegmental area was attained by 100 s following ICV cocaine administration (Figure 4). The amount of negative BOLD pixels in response to acute cocaine was not as dramatic as with positive BOLD pixels. The percentage of negative BOLD pixels sampled per ROI is shown in Figure 5.

BOLD Response to Cocaine Following Repeated Administration

Rats pretreated with cocaine for 7 days showed a lower BOLD response to cocaine challenge than saline pretreated animals (Figure 2). This reached statistical significance for the following brain areas: the agranular insular cortex, nucleus accumbens, ventral pallidum, dorsomedial thalamus, and substantia nigra ($p < 0.05$). Other brain structures showed a nonsignificant tendency towards reduced BOLD activity (Figure 2). These included the medial prefrontal cortex, dorsal striatum, globus pallidus, substantia nigra,

dorsal raphé, and periaqueductal grey ($p < 0.1$). Several other brain areas showed no difference in the BOLD response to cocaine among cocaine and saline pretreated animals (Figure 2). These included the somatosensory cortex, dorsal prefrontal cortex, ventral pallidum, ventroposterolateral thalamus, hippocampus, ventral tegmental area, and the cingulate cortex.

The relative amount of positive and negative BOLD pixels within each region of interest was also quantified (Figure 5). The nucleus accumbens, dorsomedial thalamus, ventral pallidum, dorsal striatum, and the ventroposterolateral thalamus of cocaine pretreated rats showed a lower percentage of positive BOLD pixels than cocaine naïve rats (Figure 5). Cocaine pretreated rats also showed a greater amount of negative BOLD pixels in the cingulate, accumbens, dorsal striatum, raphe, VPL, and globus pallidus (Figure 5).

BOLD Response to 5% CO_2

No significant differences in hypercapnia-induced BOLD signal changes were observed either globally (data not shown) or within specific brain structures of the mesocorticolimbic system (Figure 6). Although naïve rats exhibited a tendency towards greater BOLD signal changes in the prefrontal cortex than cocaine pretreated animals (Figure 6), this effect did not reach statistical significance ($p = 0.08$). Thus, the lower BOLD signal changes in response to cocaine in pretreated animals were not associated with lower cerebrovascular reactivity (Figure 6).

DISCUSSION

The present study supports our previous findings showing cocaine-induced positive BOLD responses across the mesocorticolimbic dopamine system (Febo *et al*, 2004). Monoamine synaptic terminals, which are the primary targets for the actions of cocaine, are extensively distributed throughout the cerebral cortex and subcortical brain structures that showed increased BOLD responses in this study. A major finding of the present study is that 7 days of pretreatment with cocaine significantly reduces the BOLD response to the drug. Although the lower BOLD response to cocaine appeared to be a generalized and nonspecific effect, several brain areas of acutely and repeatedly treated rats did not show differences in BOLD signal intensity (namely, the dorsal prefrontal cortex, cingulate, and somatosensory cortex). In addition, the lower BOLD response was not associated with differences in cerebrovascular reactivity between the two treatment groups, as measured by brief exposure to hypercapnia. Thus, our data support a reduced neuronal response to cocaine instead of a purely cerebrovascular perturbation upon repeated psychostimulant administration. The reduced cocaine-induced BOLD effect, which was observed across several mesocorticolimbic brain areas, could partly comprise the neuroadaptations that underlie cocaine sensitization.

What accounts for the alterations in BOLD signal intensity observed following cocaine administration? Cocaine can have neuronal actions as well as exert vasoactive effects. Studies employing [14C]iodoantipyrene
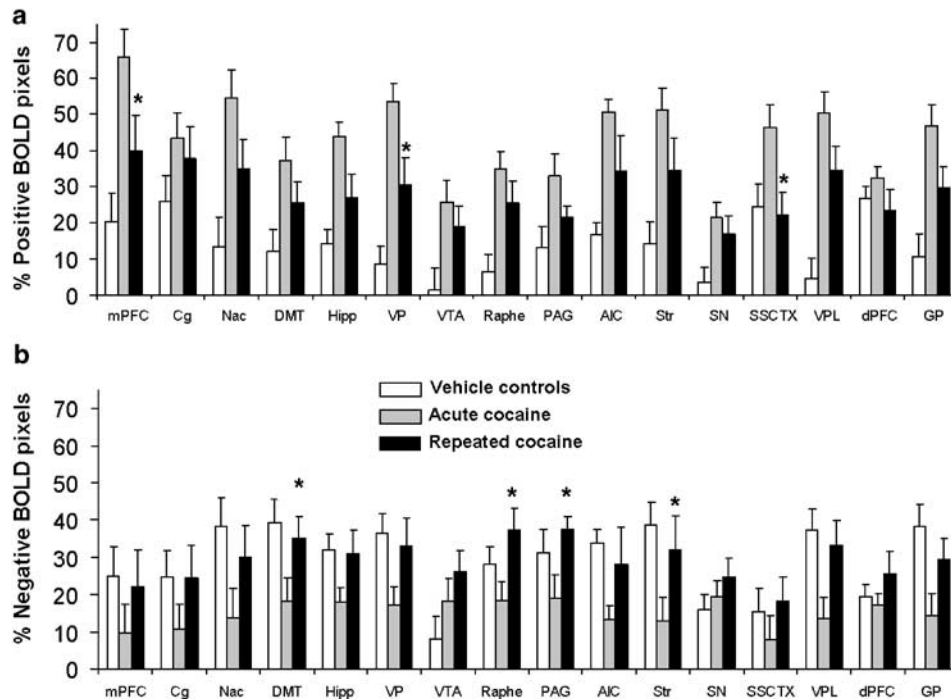
Figure 5 Percent positive and negative BOLD pixels following ICV cocaine injections in drug naïve and cocaine pretreated rats. (a) Positive BOLD pixels. (b) Negative BOLD pixels. Data expressed as mean number of pixels $\pm$ SEM. * denotes $p < 0.05$ comparing saline vs cocaine pretreated. Abbreviations are the same as indicated in Figure 2.

autoradiography have shown cocaine-induced elevations in regions cerebral blood flow (Stein and Fuller, 1992). Increased flow of oxygenated blood to regions of enhanced neuronal activity and metabolism likely contributes to alterations in BOLD observed in this study and Febo et al (2004). One explanation could be that cocaine-induced BOLD signal changes are largely a result of the energetic requirements for the replenishment of neurotransmitter stores and restabilization of membrane ionic gradients (Attwell and Iadecola, 2002) that were perturbed during drug administration. It is possible that several neurotransmitter mechanisms contribute to the BOLD signal changes, since cocaine binds to dopamine, norepinephrine, and serotonin transporters. Elucidation of the neurotransmitter mechanisms involved in generating the BOLD signal in response to cocaine, and other drugs of abuse, will be important for further interpretation of pharmacological MRI data.

We observed that rats given repeated cocaine showed a blunted BOLD response to subsequent exposure. The lower BOLD response to cocaine was observed mainly within brain areas previously postulated to comprise the neural circuitry involved in the induction and expression of behavioral sensitization in rats (Pierce and Kalivas, 1997). Repeated cocaine administration has major effects on synaptic and metabolic activity within these brain areas that could underlie behavioral sensitization to cocaine (Porrino, 1993; Ungless et al, 2001) and could also account for our present findings. The effects of cocaine on local cerebral metabolic rates for glucose ($lCMR_{glu}$) have been extensively studied (Porrino et al, 1988) and appear to depend on the duration of the abstinence period. Hammer

et al (1993) report a decrease in basal cerebral glucose utilization in limbic, extrapyramidal, and cortical sites, 24 h after the last 3-h self-administration session. This was also observed at 6 and 72 h after a 12-h cocaine 'binge' self-administration session (Hammer et al, 1993). Another study found that $lCMR_{glu}$ increases with 8 days of repeated cocaine administration; however, measurements were taken only 45 in after the final injection (Pontieri et al, 1995). Positron emission tomography using [18F]fluorodeoxyglucose (FDG) has shown that metabolic activity is elevated in the frontal cortex of cocaine addicts within 1 week of abstinence, but it decreases with longer periods (Volkow et al, 1991). Thus, with short periods of withdrawal from cocaine administration there is an increase in brain metabolism, whereas reductions may be observed with a longer abstinence. Our present data are consistent with these studies. One explanation for the decreased BOLD response to cocaine found in this study, and the decrease glucose metabolism reported by others (Hammer et al, 1993), could be related to reductions in basal and cocaine-stimulated synaptic monoamine concentrations. This could result from alterations in monoamine uptake transporter function (Kalivas and Duffy, 1993), particularly those involved in regulating dopamine (Imperato et al, 1992) and serotonin concentrations (Parsons et al, 1996). Indeed, reduced dopamine transporter function has been observed following chronic cocaine administration in the rat (Chefer et al, 2003). Alternatively, the reduced BOLD response could in fact reflect differences in basal cerebral blood flow between drug naïve and repeatedly exposed rats. However, our hypercapnia data do not support this effect. More direct measurements of BOLD and CBF, using arterial spin
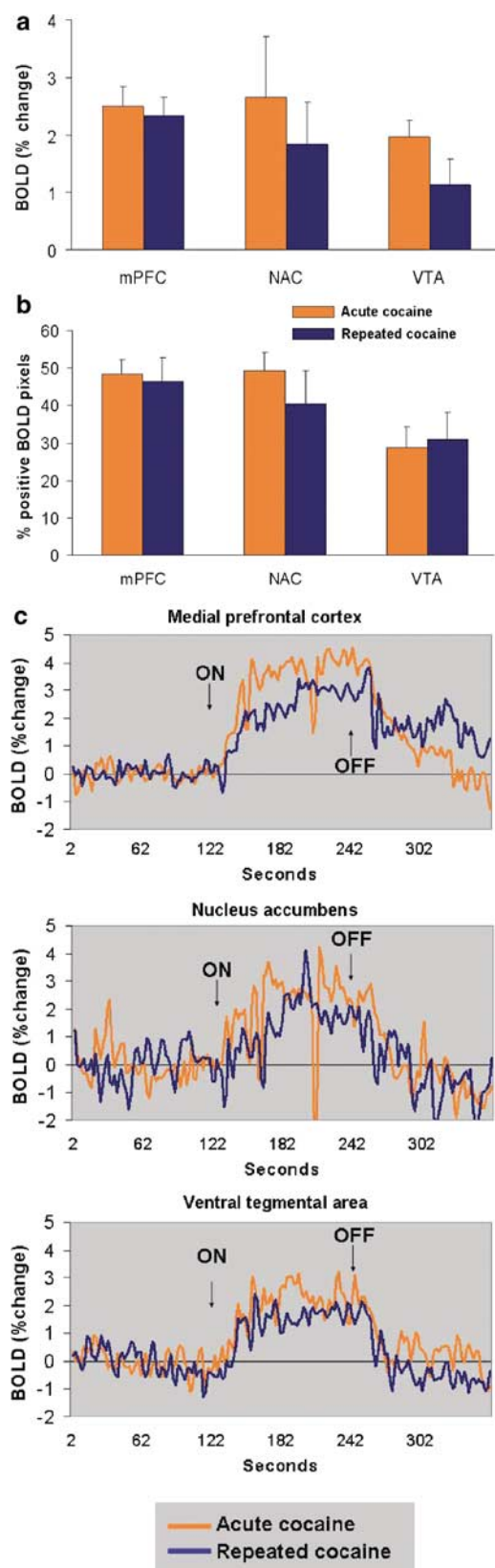
Figure 6 Hypercapnia-induced BOLD response. No differences in hypercapnia-induced BOLD activity were observed in the mesocortico-limbic system of saline and cocaine pretreated animals. Abbreviations: mPFC, medial prefrontal cortex; NAC, nucleus accumbens; VTA, ventral tegmental area.

labeling techniques, will be needed to explore this possibility.

It is important to note that the present experimental design involves the variation of the environment where cocaine was administered and modifications in the route of administration. Both conditions surrounding psychostimulant administration and the route of injection can affect the behavioral response to these drugs (Browman et al, 1998), and can vary the magnitude of neuronal activity (Porrino, 1993). External stimuli, such as a peripheral injections and the cage environment where cocaine pretreatment was given, are not expected to significantly contribute to evoking neuronal activity in the present study. However, the ICV route of cocaine administration activates the mesocorticolimbic dopaminergic system in a reproducible manner. The results of this study likely represent a direct measure of the brain response to the drug itself and not brain activity in response to drug-associated cues.

This study provides the first evidence of a reduced BOLD response to cocaine following repeated exposure to the drug. The lower BOLD activity involved several cortical, limbic, and thalamic circuits that may play a role in the greater behavioral reactivity to cocaine observed with sensitization. Indeed, our present data are consistent with a previous BOLD fMRI study in human cocaine-dependent subjects, both in terms of the neuroanatomical regions activated by cocaine and the temporal BOLD response to the drug (Breiter et al, 1997). However, a significant advance in our study is that we were able to compare cocaine-induced BOLD responses of repeatedly exposed and drug naïve rats. The combined use of BOLD imaging with animal models of drug addiction will be useful in determining the neuronal pathways associated with this mental disease.

ACKNOWLEDGEMENTS

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