Brain Research 1642 (2016) 497–504

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/brainres

Research Report

Cocaine and methamphetamine induce opposing changes in BOLD signal response in rats

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ARTICLE INFO

Article history:
Received 17 December 2015
Received in revised form
7 April 2016
Accepted 16 April 2016
Available online 18 April 2016

Keywords:
Addiction
Blood-oxygenation-level dependent (BOLD) signal
Cocaine
Methamphetamine
Pharmaco-functional MRI

ABSTRACT

Background: Neuroimaging studies in psychostimulant addicts have reported functional neural activity changes in brain regions involved in relapse. However, the difference between the effects of the psychostimulants methamphetamine and cocaine on neuronal activity in a similar setting not been clarified. Since studies in humans are limited by the inability to study the initial impact of psychostimulant drugs, we addressed this issue in a rat model.

Objective: Here, we report methamphetamine and cocaine-induced blood-oxygen-level dependent (BOLD) signal change using functional magnetic resonance imaging (fMRI) in rats receiving drug for the first time during the imaging session.

Methods: Twenty-three male Long Evans rats underwent fMRI imaging and received an intravenous infusion of methamphetamine, cocaine, or saline. Anatomical and pharmacological fMRI (pfMRI) were performed on a 7T BioSpec dedicated research MR scanner under isoflurane gas (1.5–2%). After collecting baseline data for 10 min, rats received drug over the next 10 min for a total 40 min scan time. Data were then preprocessed and statistically analyzed in anatomically defined regions of interest (ROIs) that have been implicated in persistent drug seeking and relapse.

Results: Methamphetamine during the imaging session resulted in a sustained negative BOLD signal change in key regions of the relapse circuit, except for the prefrontal cortex. In contrast, cocaine evoked a positive or unchanged BOLD signal in these same regions. In all of the investigated ROIs, there were no changes in BOLD signal following saline.

Conclusion: Acute methamphetamine and cocaine have distinct patterns of functional activity as measured by pfMRI.

1. Introduction

Methamphetamine (meth) and cocaine are among the most prevalent drugs of abuse worldwide that pose unique challenges for treatment due to their highly addictive nature. As with all abused substances, an incomplete understanding of the drugs' effects on the central nervous system often impedes treatment outcome. Both meth and cocaine users display acute and chronic structural (Dietz et al., 2009; Golden and Russo, 2012), neurochemical (Bennett et al., 1998; Davidson et al., 2005; Ross et al., 2002; Rouge-Pont et al., 2002; Strickland et al., 1998), and metabolic brain changes (Chang et al., 2007). Chronic meth use causes pronounced impairments in memory and attention, time-based prospective memory, reversal learning, and spatial working memory (Kalechstein et al., 2003; Nordahl et al., 2003; Scott et al., 2007). Some studies also suggest that repeated administration of cocaine can result in short- and long-term memory deficits and increased impulsivity (Bashkatova et al., 2005; Muriach et al., 2010; Santucci et al., 2004). As such, it is easy to understand why preventing relapse is a major health need. However, equally relevant is the brain's initial response to psychoactive compounds. In humans, ethical limitations prevent administration of drugs such as meth and cocaine to participants who lack a drug use history. Now, with the advent of new imaging techniques, we can take advantage of animal models to map the functional response to meth and cocaine in the naïve brain.

In humans, pharmaco-functional magnetic resonance imaging (MRI) (Jenkins, 2012; Martin and Sibson, 2008) can identify blood-oxygen-level dependent (BOLD) signal change as a measure of neuronal activity in localized brain regions. This methodology can differentiate responsive and non-responsive brain regions to a

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http://dx.doi.org/10.1016/j.brainres.2016.04.040
0006-8993/

drug and distinguish activation patterns of drug users and non-drug user controls (Zhao et al., 2008). Following an acute cocaine infusion, specific regional increases in BOLD response were reliably measured, despite a 14% decrease in gray matter cerebral blood flow in humans (Gollub et al., 1998). However, some researchers have concluded that the underlying relationships between neuronal activity and drug-induced changes in BOLD signal may be more complex than a direct correspondence (reviewed in Ekstrom (2010)). Changes in BOLD signal depend not only on vasodilation (Chen and Parrish, 2009; Liang et al., 2013) but also vasoconstriction (Kim and Ogawa, 2012). Regardless of the nature of the BOLD signal change, psychostimulant drugs exert both neuronal and vascular changes.

By employing neuroimaging techniques much is known about the different degree of the long-term neuronal changes produced by meth and cocaine abuse (Volkow et al., 2003); however, knowledge about the initial impact of psychostimulants on brain activation is missing. This lack of information is mainly because human studies are limited by the inability to study the initial impact of an acute psychostimulant. Animal studies are without this constraint so use of MRI (a non-invasive measurement method) in animals further enables observations and comparisons of psychostimulant actions and consequences as they occur. Also, comparisons of the drugs transient effects on neuronal and vascular health can be addressed. Important insights gained from these studies include transient differences in neuronal responses and differences in the long term effects of psychostimulants on central nervous system (CNS) function.

Here, we used pharmacological (p)fMRI (Jenkins, 2012; Martin and Sibson, 2008) to identify BOLD signal changes as a measure of neurovascular response in drug naïve rats with meth and cocaine systemically present during the imaging session. In rats, acute cocaine administration (at doses greater than 0.1 mg/kg i.v.) has previously been shown to increase BOLD signal in brain regions involved in reward processing, executive functions, and emotional regulation (Marota et al., 2000). More specifically, these areas included the striatum, nucleus accumbens (NAC), thalamus, and prefrontal and orbitofrontal cortices (Febo et al., 2004; Marota et al., 2000). An acute amphetamine challenge (3 mg/kg i.v.) given during an fMRI session had a more pronounced effect on BOLD signal and resulted in signal changes in the opposite direction as cocaine in some brain regions. For example, increased BOLD signal change occurred in the olfactory bulb, cingulate cortex, striatum, septum, globus pallidus, thalamus, retrosplenial cortex, hippocampus, lateral entorhinal cortex, inferior colliculus, and pontine reticular nucleus. In contrast, decreased BOLD signal occurred in the frontal, motor, auditory, and parietal cortices (Dixon et al., 2005).

Understanding the differences in regional functional response to psychostimulants is important to elucidate the degree and type of early response the brain makes to these different drugs. The early involvement pattern, along with the knowledge of specific pathways of stimulation may be an early marker for potential long-term neuroplasticity changes or neural damage. However, a controlled pfMRI study with meth and cocaine has yet to reveal the similarities and differences in the acute regional response to these drugs. In this study, we report on meth- and cocaine-induced functional response measured by BOLD signal change in anesthetized rats in brain areas involved in addiction and relapse.

2. Results

2.1. Time course analysis

The time line for the experiment and imaging sessions are depicted in Fig. 1A and B. The first analysis examined the change in BOLD signal from pre-infusion to infusion and post-infusion in anatomically defined regions of interest (ROIs) (Fig. 2). These regions were selected based on their known roles in addiction and relapse. Each ROI was analyzed with a separate mixed analysis of variance (ANOVA) as described in the Methods. Table 1 summarizes the results and presents the data analysis from the mixed ANOVAs and post-hoc tests for each ROI. As shown in Fig. 3, the majority of ROIs showed very little change in BOLD signal in the saline condition, as expected. Some regions showed fairly pronounced decreases in BOLD signal over time in the meth condition and increases in BOLD signal over time in the cocaine condition. According to the mixed ANOVAs, two regions showed a significant Infusion × Time interaction (hippocampus and amygdala) and two regions showed a marginally significant interaction (hypothalamus and NAC; Fig. 3). None of these four regions showed infusion group differences at pre-infusion baseline (according to Tamhane's T2 post-hoc test), but infusion differences emerged at later time points (see Table 1 for statistical statements). Specifically, the amygdala and hypothalamus showed lower BOLD signal for the meth than cocaine condition during infusion and 10 min post-infusion with a marginally lower BOLD signal 20 min post-infusion in the hypothalamus. The hippocampus showed a marginal infusion difference (meth < saline) at both post-infusion time points. Multiple comparisons were not significant in any time window for the NAC. The striatum showed a main effect of infusion, with meth having a lower BOLD signal than cocaine; however, the post-hoc comparison of infusion groups was not significant. The NAC showed a main effect of time, whereby the BOLD signal decreased from pre-infusion to later time points across all three infusion types. There were no significant effects or interactions in the thalamus, prefrontal cortex, or perirhinal cortex.

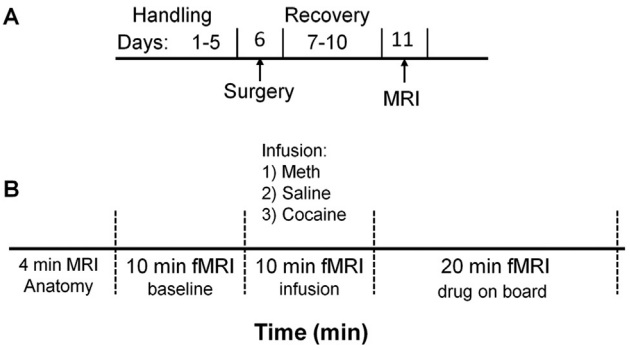

Fig. 1. Time line and study design. (A) Study design. Rats were handled and had a catheter surgically implanted before imaging. Seven days after surgery, rats underwent MR imaging. (B) pfMRI experimental design. The MRI imaging session included anatomical and fMRI scans. The pfMRI component included three modules: pre-infusion, during infusion, and post-infusion.

2.2. Voxel wise analysis

Voxel wise analysis revealed multi regional involvement in pfMRI changes after drug infusion. In contrast to the time course analysis that analyzed specific regions of interest, this voxel wise analysis illustrates the whole brain effect. Fig. 4 shows the statistical maps comparing drug-induced BOLD signal change (20–30 min) with the baseline phase (pre-infusion). Positive BOLD changes compared to baseline are indicated in blue and negative BOLD signal changes compared to baseline are indicated in red-yellow. When meth was on board relative to saline, negative BOLD signal changes were bilaterally observed in the hippocampus, hypothalamus, striatum, and some areas of cortex ($p < 0.05$ uncorrected). In comparison, when cocaine was on board relative to saline, increased BOLD signal was observed in the thalamus and cortical areas. The contrast between meth and cocaine revealed activation similar to the contrast between meth and

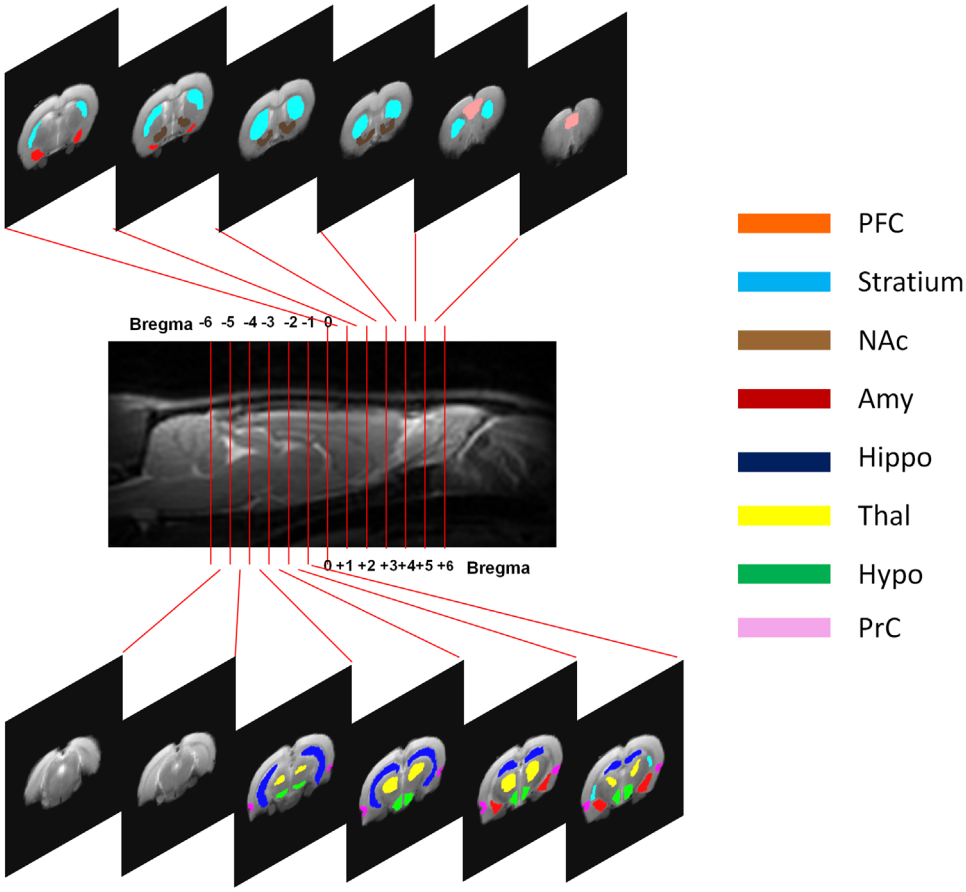

Fig. 2. Representative coronal anatomical images of selected regions of interest (ROI) relative to bregma. Ten slices that included the following ROIs were selected for BOLD analysis: (a) hippocampus, (b) amygdala, (c) hypothalamus, (d) thalamus, (e) nucleus accumbens, (f) striatum, (g) prefrontal cortex, and (h) perirhinal cortex. Note that ROIs span across more than one slice.

Table 1
Results from the mixed ANOVAs and post-hoc tests for each ROI comparing the effect of different infusions at different time intervals post-infusion on BOLD.

Region of Interest	Infusion × Time interaction	Main effect time	Main effect infusion	Tamhane's T2 test for multiple comparisons			
				Pre	Infusion	Post10	Post20
Hippocampus (Fig. 3a)	$F(6,38)=2.5$, $p<0.041$	NS	$F(2,20)=3.4$, $p<0.053$	NS	NS	(M < S)	(M < S)
Amygdala (Fig. 3b)	$F(6,38)=2.9$, $p<0.021$	NS	NS	NS	(M < C)	(M < C)	NS
Hypothalamus (Fig. 3c)	$F(6,38)=2.1$, $p<.0751$	NS	$F(2,20)=4.4$, $p<0.027$	NS	(M < C)	(M < C)	(M < C)
Thalamus (Fig. 3d)	NS	NS	NS	NS	NS	NS	NS
NAC (Fig. 3e)	$F(6,38)=2.2$, $p<0.0631$	$F(3,18)=3.7$, $p<0.032$	NS	NS	NS	NS	NS
Striatum (Fig. 3f)	NS	NS	$F(2,20)=3.6$, $p<0.048$	NS	NS	NS	NS
PFC (Fig. 3g)	NS	NS	NS	NS	NS	NS	NS
PRH (Fig. 3h)	NS	NS	NS	NS	NS	NS	NS

NAC=nucleus accumbens, PFC=prefrontal cortex, PRH=perirhinal cortex, M=methamphetamine, C=cocaine, S=saline, NS=not significant.

saline; however, in this case the activation difference was more pronounced. Fig. 4 indicates that, in general, meth induced a widespread decrease in BOLD signal, while cocaine induced an increase in BOLD signal in fewer regions after infusion.

3. Discussion

Here we compared BOLD signal change in response to an acute infusion of cocaine, meth, and saline using pfMRI in rats. A major advantage of the current study was the evaluation of differences in signal intensity over time using a treatment protocol relevant to drug self-administration studies. Furthermore, we characterized differences between drugs within the same exact protocol based on separate previous reports of amphetamine induced negative BOLD change (Preece et al., 2007) and positive BOLD changes with cocaine (Febo et al., 2005). Here, we demonstrated that acute administration of meth or cocaine had opposing BOLD signal changes using pfMRI. Specifically, our time course analyses showed that initial meth exposure during the imaging session caused a sustained negative BOLD signal change in regions implicated in persistent drug seeking and relapse. In contrast, initial cocaine exposure resulted in no difference in BOLD signal in several of the same ROIs. The voxel wise comparison corroborated the time course analysis, showing opposing signal changes when cocaine and meth were compared to saline. Taken together, these results confirm that different psychostimulants can produce quite different effects on patterns of brain activity.

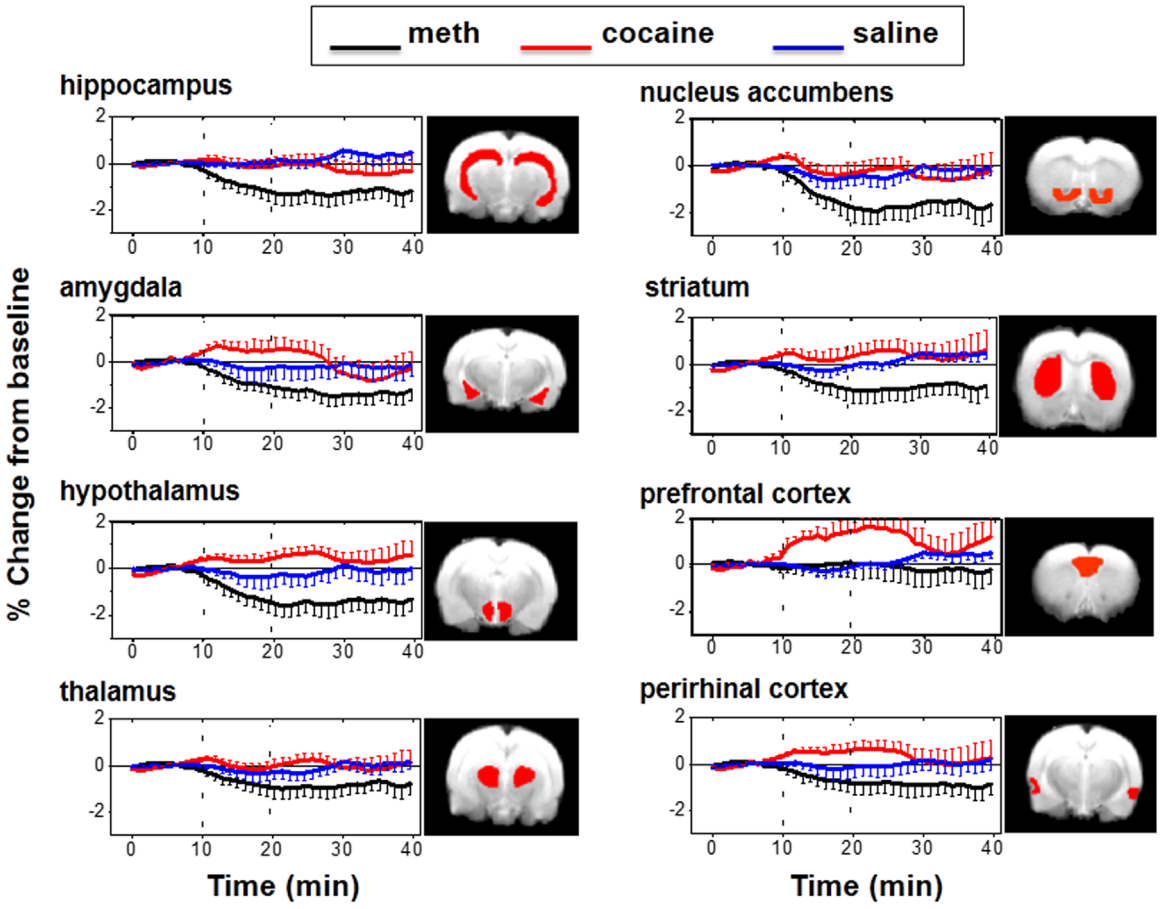

Fig. 3. ROI analysis of pfMRI time series. Functional activation as measured by percentage change in BOLD signal intensity over time following an i.v. infusion of meth ($n=8$), cocaine ($n=7$), or saline ($n=8$). Average activation is shown on the left of each panel with corresponding ROI on the right. The first 10 min of pfMRI was used as the baseline. The infusions started 10 min into the scan session over a 10 min period. Percent signal change from the baseline is represented as mean ± SEM. The results of the statistical analysis is shown in Table 1.

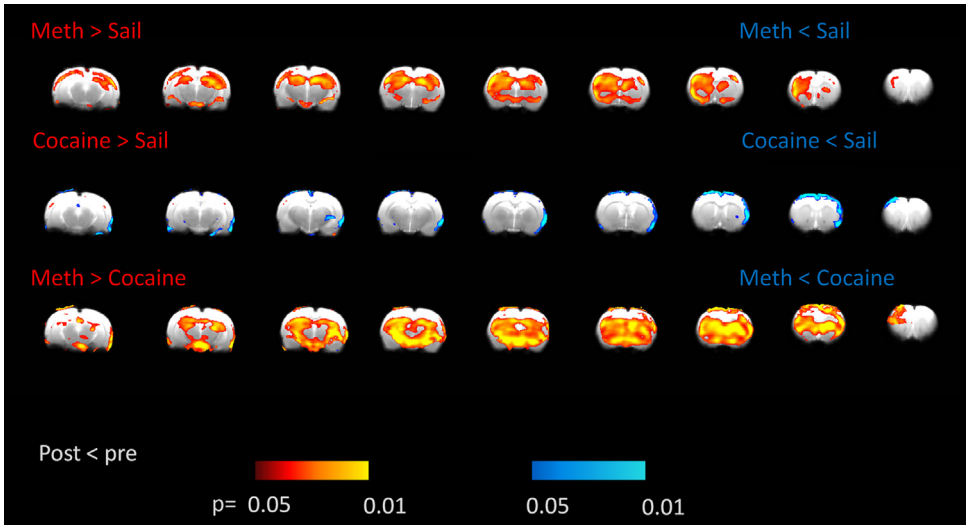

Fig. 4. Voxel wise statistical analysis of pfMRI data. pfMRI activation (Z statistical maps) produced by drug (meth or cocaine) administration vs. vehicle (saline) with significantly activated voxels ($P<0.05$ uncorrected) overlaid on anatomical images. All rats (meth ($n=8$), cocaine ($n=7$), and saline ($n=8$)) were imaged under anesthesia induced by 2% isoflurane. For each contrast, hot color represents voxels with probability that post-injection (20–30′) BOLD is lower than pre-injection (0–10′) BOLD signal, for the comparison between drug indicated on top of each row. The cold color represents voxels with probability that post-injection (20–30′) BOLD is lower than pre-injection (0–10′) BOLD signal for the opposite comparison. The top row compares the meth with the saline group, Meth > saline pixels are shown in red and Meth < saline pixels are in blue. The middle row compares cocaine with saline, cocaine > saline pixels are shown in red and cocaine < saline pixels are shown in blue. The bottom row compares meth with cocaine, Meth > Cocaine pixels are shown in red and Cocaine < Meth are shown in blue.

We observed a significant positive BOLD signal after cocaine in the prefrontal cortex relative to meth, but not saline. In the other ROIs, cocaine failed to induce a significant signal change relative to saline treated rats. This finding is not congruent with a previous study (Febo et al., 2005) that reported a peak in signal intensity immediately after cocaine treatment. The most likely explanations

for this discrepancy are administration protocol and route of cocaine delivery used between reports. Febo et al. (2005) infused the drug via the intracerebroventricular route (20 μg) after a 5 min habituation period in the magnet and only recorded for the ensuing 10 min. We infused cocaine intravenously over a 10 min period (1.2 mg total) following a 10 min habituation while the subject was inside the magnet. We designed our protocol to correspond with the amount of drug a rat typically infuses during the first 10 min of operant self-administration procedures (Reichel et al., 2011, 2012a, 2012b). Regardless, cocaine resulted in an overall positive (albeit not significant) or no signal change in the ROIs investigated. A more interesting and striking difference occurred between cocaine and meth. In all subcortical nuclei, meth and cocaine had opposing signal changes, in that meth caused a pronounced negative signal change in multiple brain areas. In a previous study, amphetamine (3 mg/kg i.v.) evoked a negative BOLD signal change with the greatest effects observed in NAC (Preece et al., 2007). A negative cerebral blood volume change was also observed in the hypothalamus of rats that went through cocaine self-administering in response to cocaine challenge during the scan session (Chen et al., 2011). Interestingly, acute cocaine administration in human cocaine-dependent subjects also generated negative BOLD signal in the amygdala (Breiter et al., 1997; Kufahl et al., 2005) and NAC, subcallosal cingulate, ventral tegmental area, right parahippocampal gyrus, and right posterior orbital gyrus (Kufahl et al., 2005). A possible explanation for disagreement between our data and human cocaine data is that the repeated exposure to cocaine alters the polarity of BOLD signal in various ROIs as seen in cocaine-dependent subjects. However, to the best of our knowledge, there is no data on human naïve subjects to directly compare with our data.

The interpretation of a negative BOLD signal change in fMRI studies is currently debated (Kim and Ogawa, 2012). Depending on the interplay between hemodynamics and metabolism, negative BOLD can result from decreased neuronal activity (Shmuel et al., 2006), changes in cerebral blood volume or cerebral blood flow (Harel et al., 2002; Polesskaya et al., 2011), or increased neuronal activity (Schridde et al., 2008), or even neuronal damage (Gold et al., 2009). Schridde et al. (2008) found one explanation for a sustained negative BOLD signal in their seizure model was due to disproportionately high neuronal activity and cerebral metabolic rate of oxygen (CMRO2), with an under-compensating cerebral blood flow (CBF)/cerebral blood volume (CBV). Combined these findings lead to accumulation of deoxygenated Hemoglobin (dHb) and the ensuing negative BOLD change even with increased neuronal activity. Likewise, the negative BOLD response to meth we observed could involve a much more potent level of neuronal activity relative to cocaine. Although speculative, this response may perhaps be a signature of early neurotoxicity of this drug (Gold et al., 2009).

While the explanation above is certainly plausible, the more parsimonious explanation meth induced negative signal change is that changes in cerebral blood volume or blood flow resulted from sustained vasoconstriction following meth and/or physiological factors via other organ systems (e.g., respiration). For example, meth causes significant physiological effects on the cardiovascular system including increased heart rate, respiration rate, and blood pressure (Barr et al., 2006), all of which can potentially interfere with interpretation of a BOLD signal due to changes in cerebral blood flow and volume (Schmidt et al., 2006). In our fMRI data analysis, however, the negative BOLD signal change persisted even when respiration was included as a covariate in ROI analysis. We therefore suspect that the negative BOLD signal change is related to changes in blood flow or volume resulting from vasoconstriction.

Cocaine and meth induce cerebral vasoconstriction (Kaufman et al., 1998a, 1998b, 2001; Kiyatkin et al., 2007; Kousik et al., 2011). Notably, the time course of vasoconstriction differs between the drugs as cocaine produces more time limited vasoconstriction than meth. For example, in rats vasoconstriction lasted less than 5–6 min after a single cocaine exposure of 1 mg/kg i.v. (Du et al., 2006), while vasoconstriction lasted about 30 min in mice after a single meth exposure (5 mg/kg i.p.) (Polesskaya et al., 2011). Also, vasoconstriction following meth exposure may exacerbate meth-induced brain hyperthermia, indicating altered neuronal activity (Kiyatkin and Sharma, 2009).

Despite many similar behavioral and physiological effects, meth and cocaine significantly differ in their mechanisms of action and central effects. Meth has a clearance half-life of approximately 10 h in humans, resulting in sustained stimulation (Cruickshank and Dyer, 2009), while the 1 h half-life of cocaine in humans results in more transient stimulation (Verstraete, 2004). Meth has a longer half-life (53–66 min) in rats (Riviere et al., 2000) than cocaine (8.2 min) (Tsibulsky and Norman, 1999). Both drugs cause changes in brain regional glucose metabolism, an indicator of regional cerebral metabolic activity (for a review on brain regional change see Aron and Paulus (2007)). Cocaine causes changes in glucose utilization in different anatomical regions. Cocaine abusers have decreased glucose metabolism in cortical and subcortical portion of limbic system (Lyons et al., 1996), with particular effects in neocortical areas, basal ganglia, thalamus, and midbrain (London et al., 1990; Lyons et al., 1996; Zocchi et al., 2001). A high dose of acute cocaine increases the rate of glucose metabolism in the subthalamic nucleus and substantia nigra. Most of these brain metabolic changes from acute cocaine result from dopamine transporter blockade (Thanos et al., 2008). Non-dopamine related drug actions also affect glucose influx (Wakabayashi and Kiyatkin, 2015). During meth intoxication, whole brain metabolism was higher compared to controls, while metabolism in several different anatomical regions specifically in striatal and thalamic areas (Volkow et al., 2001), and in anterior cingulate and insula (London et al., 2004) were reduced. Taken together, these data indicate meth and cocaine exposure provoke somewhat distinct regionally specific cerebral metabolic activity that results in regionally specific BOLD activation.

One potential explanation for the difference between meth and cocaine evoked BOLD signal could be the result of neurovascular decoupling effect of anesthesia used in this study. Isoflurane may have notable effects on CBF and cerebrovascular coupling relative to the awake condition, thus affecting the BOLD response (Sicard et al., 2003). Inhaled 3% isoflurane for one hour decreased the concentration of dopamine (DA) in striatum of male rats as shown with in vivo microdialysis (Adachi et al., 2005). Further, isoflurane anesthesia enhanced the direct inhibitory effects of cocaine on dopamine transporters and had an indirect effect on dopamine D2 receptor subtypes in male rhesus monkeys as shown with positron emission tomography (PET) in combination with microdialysis (Tsukada et al., 1999).

The importance of a change in the polarity of a BOLD signal between meth and cocaine merits further investigation in relation to the physiological and neurovascular effects of the drugs. Future studies may seek to determine how the mechanisms of meth differ substantially from cocaine (e.g., serotonin and norepinephrine reuptake blockade or changes in the intraneuronal vesicular monoamine transporter (VMAT)) in generating different BOLD responses.

4. Experimental procedure

4.1. Subjects

Twenty-three male Long Evans rats (Charles-River) weighing

250–300 g upon delivery in a temperature- and humidity-controlled vivarium on a reversed 12:12 light-dark cycle. Rats received *ad libitum* food and water (Harlan, Indianapolis, IN, USA). Procedures were conducted in accordance with the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, 1996) and approved by the IACUC of the Medical University of South Carolina. At the end of the experiment, rats were euthanized according to standard approved protocol.

4.2. Surgery

Five days before the imaging session, rats were implanted with a jugular cannula for drug infusion. Anesthesia consisted of intraperitoneal (i.p.) injections of ketamine (66 mg/kg; Vedco Inc, St Joseph, MO, USA), xylazine (1.3 mg/kg; Lloyd Laboratories, Shenandoah, IA, USA), and Equithesin (0.5 ml/kg; sodium pentobarbital 4 mg/kg, chloral hydrate 17 mg/kg, and 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol, 10% ethanol solution). Ketorolac (2.0 mg/kg, i.p.; Sigma, St. Louis, MO, USA) was given just prior to surgery as an analgesic. One end of a silastic catheter was inserted 33 mm into the external right jugular and secured with 4.0 silk sutures. The other end ran subcutaneously and exited from a small incision just below the scapula. This end attached to an infusion harness (Instech Solomon, Plymouth Meeting, PA, USA) that provided access to an external port for i.v. drug delivery. An antibiotic solution of cefazolin (10 mg/0.1 ml; Schein Pharmaceuticals, Florham Park, NJ, USA) was given post-surgery and during recovery along with 0.1 ml 70 U/ml heparinized saline (Elkins-Sinn, Cherry Hill, NJ, USA).

4.3. Neuroimaging

The time line for the experiment and imaging sessions is depicted in Fig. 1A and B. Imaging was performed on a 7T BioSpec research dedicated MR scanner (Bruker Biospin, Ettlingen, Germany), equipped with 500 mT/m (rise time 80–120 μs) gradient set (for performing high resolution small animal imaging) and a small bore linear RF coil (ID 119 mm) as the RF transmitter and a four channel surface array coil as the RF receiver. Rats were anesthetized using isoflurane gas (induction dosage 2–3%, maintenance dosage 1.5–2%), at 1 L/min N2O/O2 (70/30) flow under spontaneous respiration, during the duration of the study. Real time monitoring of physiological parameters (heart rate, respiratory rate, and body temperature) were collected during the imaging session for signs of distress and for fMRI data analysis by using SAII monitoring instrument (Small Animal Instruments Inc., Stony Brook, NY). Inside the magnet body, temperature was maintained with controlled warm airflow. The time line for the imaging sessions is depicted in Fig. 1B. First, anatomical images were collected to establish regions of interest for the fMRI experiment with the following parameters: T2 weighted-2D Rapid Acquisition with Relaxation Enhancement (RARE), TR/TE 4000/65 ms, field of view (FOV) 3.2 cm × 3.2 cm, slice thickness 1 mm, slice gap 0.1 mm, number of slices 16, matrix 256 × 256, number of averages 5, receiver bandwidth 100, kHz. Base line data for fMRI were collected for 10 min before drug administration. These functional images were obtained with a multislice spin-echo, echo planar imaging (EPI) sequence (Keilholz et al., 2004) using the following optimized parameters: TR/TE = 2000/16.5 ms, BW = 250 KHz, FOV = 3.2 × 3.2 cm, Matrix size = 80 × 64, number of repetition = 1200, slice thickness 1 mm, slice gap 0.1 mm, number of slices 16. The geometrical parameters were optimized for a high signal to noise ratio (SNR) and minimum susceptibility distortion. Following base line data collection, rats were administered meth, cocaine, or saline during the ensuing 10 min period, and the scan commenced for an additional 20 min resulting in a 40 min scan session. Specifically, rats received meth (0.02 mg/50 μl bolus, total 0.12 mg), cocaine (0.20 mg/50 μl bolus, total 1.2 mg) or saline (50 μl bolus) delivered in a total of six infusions of over a 10 min period. The selected cocaine and meth doses approximate the amount that a rat self-administers during the first 10 min of a self-administration session in our laboratory (Reichel et al., 2011, 2012a, 2012b).

4.4. Data processing and analysis

4.4.1. Regions of interest

We created a custom rat brain template based on all rats included in the analysis by aligning each rat's high resolution T1 image to a randomly selected T1 image from one rat, which served as the standard. Each rat brain was aligned to the standard brain and all normalized brains were averaged to generate the final template (Tang et al., 2010). ROIs were manually traced on the template using a rat atlas (Paxinos and Watson, 2008) as a guide.

4.4.2. Image processing

For pfMRI data, preprocessing and data analysis were carried out using FSL software (version 4.1.7, FMRIB Software Library, Oxford Center for Functional Magnetic Resonance Imaging of the Brain, Oxford University, Oxford, U.K.). For each rat, functional runs were motion corrected and spatially smoothed with an isotropic Gaussian filter kernel with full width at half maximum (FWHM) size of 1.1 mm. The 4D data set was also normalized by a single scaling factor to make the average brain signal intensity equal to 10,000. Preprocessed images were then registered via the corresponding high-resolution anatomical scans to the study-specific rat brain template. Within each ROI, the time series of signal intensity values were extracted, with the six head motion parameters and measured respiratory waveforms removed from the BOLD time series (via regression of the signal versus time). The residual time series were then averaged to create 1-min intervals. The signal intensity from the time series for each rat and each ROI were then converted to percent signal change using the first time point's intensity value as a baseline. Each time series was then down sampled to create four 10-min time windows (0–10, 10–20, 21–30, 31–40 min), which were then submitted to statistical analyses.

ROI data were analyzed with separate mixed ANOVA's for each ROI using IBM Statistics (Chicago, IL). The between groups variable was drug infusion (meth, cocaine, and saline) and the repeated factor was the time course within a scanning session (four 10-min time bins: pre-infusion, infusion, post-infusion – first 10 min and last 10 min). Results from the multivariate tests are reported because sphericity assumptions did not hold (see Hertzog and Rovine (1985)). When Drug Infusion × Time interactions emerged, we used simple effects analysis (Keppel and Zedeck, 1989) to determine at which time point infusion effects emerged. Tamhane's T2 post-hoc comparisons were used to determine which of the three infusion conditions were different at each time point. Tamhane's T2 test is a conservative pair-wise comparison based on a t-test that is used when variances are unequal across groups.

In addition to the ROI analysis, voxel-wise statistical analyses were performed to reveal any other brain regions that could be sensitive to cocaine or meth effects. At the individual rat level, four events were defined using FSL's double gamma hemodynamic response function and a temporal derivative: baseline (0–10 min), infusion (10–20 min), 20–30 min, and 30–40 min, using the general linear model (FEAT, version 5.98). The main contrast of interest was between baseline and 20–30 min post-infusion, as this represents the immediate neural response to the drug relative to the non-drug state. Individual-subject contrasts of Pre > Post-infusion were then submitted to group-level analyses.

The average group activation for each drug type (meth, cocaine, and saline) was calculated using FSL's mixed-effect analysis (FLAME stage 1) with a threshold of $z=1.7$ and corrected cluster $p=0.05$. Activation maps were generated to show the significant effect of meth and cocaine on whole brain (multi regional involvement in fMRI changes after drug infusion). These changes are represented by (1) the probability of observing post-injection (20–30 min) BOLD being higher than pre-injection (0–10 min) BOLD signal for cocaine, and (2) the probability of observing post-injection BOLD being lower than pre-injection BOLD signal for meth.

Disclosure/conflict of interests

No conflict of interest is reported.

Acknowledgment

This research was supported by NIDA Grants DA024401 (JEJ), HD055885 (CMR), DA034727 (CMR, ST), and NIH Grant C06 RR015455. The authors thank Jennifer Hergatt and Shannon M. Ghee for technical assistance.

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