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Imaging cocaine-induced changes in the mesocorticolimbic dopaminergic system of conscious rats

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Abstract

Functional magnetic resonance imaging (fMRI) was used to assess the effects of cocaine on brain activation in fully conscious rats. Methods were developed to image cocaine-induced changes in blood-oxygen-level-dependent (BOLD) signal without the peripheral cardiac and respiratory complications associated with psychostimulant administration. Using spin echo planar imaging (EPI), conscious rats were imaged in a 4.7 T spectrometer prior to and following the intracerebroventricular injection of cocaine (20 μg) in artificial cerebrospinal fluid (10 uL). Within 5 min of injection, there was a significant increase in BOLD signal intensity in the substantia nigra, ventral tegmental area, nucleus accumbens, dorsal striatum and prefrontal cortex, as compared to vehicle controls. Minimal negative BOLD signal changes were observed in response to cocaine and no significant perturbations in normal cardiovascular and respiratory function. These findings demonstrate the technical feasibility of studying psychostimulant-induced brain activity using functional MRI in conscious rats.

Keywords: Psychostimulants; Functional imaging; BOLD technique; Reward system; Nucleus accumbens; Prefrontal cortex; Animal imaging

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1. Introduction

The rewarding and psychomotor properties of cocaine are attributed to changes in neuronal and synaptic activity within mesocortical and mesolimbic systems (Chang et al., 1998; Einhorn et al., 1988). The brain areas involved include the ventral tegmental area and its synaptic targets in the prefrontal cortex and nucleus accumbens. Parallel to these circuits is the nigrostriatal system, comprised of substantia nigra and the dorsal striatum, which mediates the psychomotor activation induced by psychostimulants such as cocaine. Within these brain regions, cocaine blocks synaptic re-uptake of biogenic amines, resulting in enhanced neuronal and synaptic metabolic activity (London et al., 1986; Porrino et al., 1988; Stein and Fuller, 1992, 1993).

The enhanced metabolism accompanying cocaine-induced neuronal and synaptic activity can be assessed with functional magnetic resonance imaging (fMRI). Functional MRI using the blood-oxygenation-level-dependent (BOLD) technique measures brain activity by assessing small magnetic field changes associated with tissue oxygenation state in brain areas that are metabolically active (Ogawa et al., 1990). Neuronal activation elevates the rate of oxygen extraction from local capillary beds (Thompson et al., 2003) and this is immediately followed by enhanced flow of oxygenated blood to the area (Fox and Raichle, 1986; Malonek et al., 1997). Reduced paramagnetic deoxygenated hemoglobin levels within the local vasculature favors a higher T2* relaxation time, resulting in increased MRI signal intensity.

Several human functional imaging experiments have assessed changes in brain activation following intravenous cocaine administration (Breiter et al., 1997; Kaufman et al., 1998b; Li et al., 2000). There are limitations to human imaging experiments, such as the inability to use stronger magnetic field strengths and ultra-fast pulse sequences to improve signal-to-noise and enhance spatiotemporal resolution. This is further compounded by difficulties in including ‘drug-naive’ controls or experimental subjects without a

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history of abuse of multiple addictive substances. Moreover, it is unethical to do prospective studies on the development of drug dependence, withdrawal and craving. This has lead to interest in the use of fMRI in animals. However, recent animal studies have been hampered by the use of general anesthetics needed during imaging sessions to immobilize animals and reduce motion artifact (Luo et al., 2003; Mandeville et al., 2001; Marota et al., 2000). Motor, cardiac and respiratory motion creates image distortions and false positive signal changes mistakenly associated to stimulus presentation (Hajnal et al., 1994). These artifacts may be exacerbated in conscious animals after administration of psychostimulants during imaging. While anesthesia reduces motion, it also diminishes neuronal activity, cerebral metabolism, and cerebral blood flow (Bonvento et al., 1994; Nakao et al., 2001; Ueki et al., 1992) which leads to reduced BOLD signal intensity (Lahti et al., 1999; Peeters et al., 2001; Sicard et al., 2003). The goal of the present study was: (1) to establish methods for imaging fully conscious rats administered cocaine during imaging and (2) to follow cocaine-induced changes in BOLD signal intensity within mesocorticolimbic and nigrostriatal brain regions. Our results show that intracerebroventricular administration of 20 ug of cocaine causes a rapid and robust increase in BOLD signal intensity in brain circuitry involved in cocaine reward.

2. Materials and methods

Male Sprague-Dawley rats weighing 300–350 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 9:00 h) 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).

2.1. Route and dose of cocaine administration

Behavioral, neurochemical and physiological studies were undertaken to select an appropriate dose and route of administration of cocaine for the functional imaging studies. Peripheral cocaine administration increases heart rate, blood pressure and respiratory rate (Tella, 1996), thus complicating BOLD signal interpretation. This effect can be avoided by intracerebroventricular (ICV) administration of a low dose of drug (Jones and Tackett, 1990). Direct administration of cocaine into the lateral ventricles in doses within the range of 25–50 μg and in volumes of 2–10 μl have been shown to be rewarding in rats (Morency and Beninger, 1986). Doses above 50 μg ICV can increase blood pressure, heart rate, and plasma catecholamines (Kiritsy-Roy et al., 1990; Misra et al., 1975).

2.2. Locomotor response to intracerebroventricular cocaine

Twenty-three male rats were anesthetized with ketamine (75 mg/kg, i.p.) and xylazine (7 mg/kg, i.p.), mounted on a stereotaxic apparatus (David Kopf, Tujunga, CA, USA) with the upper incisor bar set 3.5 mm below the interaural line. The skull was exposed and a hole drilled for unilateral placement of a steel guide cannula (dimensions: outer diameter 0.025 in., i.d. 0.013 in.) into a lateral ventricle (Bregma coordinates: AP −1 mm, ML 2 mm, DV 4 mm; Paxinos and Watson, 1997). Behavioral testing commenced 5–7 days following surgery. Horizontal and vertical locomotor activity were measured with an automated animal activity cage system (Versamax^{TM} system, Columbus, OH), as described previously (Febo et al., 2002). On the day of behavioral testing, rats were placed in testing cages and activity measured for 30 min. Rats were then injected with an ICV dose of cocaine (5, 10, or 20 μg) ($n = 5$–6 rats/dose) or drug vehicle (10 μL artificial cerebrospinal fluid, aCSF) ($n = 6$) into the lateral cerebral ventricle using a steel injector inserted into the guide cannula. The injector was connected to a 10 μL Hamilton® syringe by PE-10 tubing. Animals were again placed into the testing cages and behavior measured for an additional 60 min.

2.3. In vivo microdialysis

A group of ten male rats were anesthetized with 2% isoflurane and a guide cannula (CMA/10; CMA, Acton, MA) implanted into the nucleus accumbens (Bregma coordinates: AP 2 mm, ML −1.7 mm, DV 5 mm; Paxinos and Watson, 1997). The cannula was affixed to the skull using dental acrylic cement. A patch of 3 mm^2 skull surface area, caudal and lateral to Bregma was left uncovered for placement of ICV cannula prior to experiments. In vivo microdialysis studies commenced 3–4 days after surgery. On the day of experiment, rats were re-anesthetized with 2% isoflurane and a 26 gauge PE-10 tubing connected to a 20 μL syringe was lowered into a lateral cerebral ventricle. A microdialysis probe (2 mm membrane length, Bioanalytical Systems, IN) connected by inlet tubing to a microinfusion pump (Harvard Apparatus, MA) was inserted into the guide cannula. The animals were then placed into a Plexiglas test chamber (48 cm × 24 cm × 20 cm) and left to recover for 30 min before perfusate sample collection. Three consecutive 15 min baseline samples were collected in microcentrifuge tubes containing 10% perchloric acid at a flow rate of 2 μL/min. Animals then received an ICV injection of aCSF ($n = 4$) or cocaine ($n = 6$), and two additional samples collected. Rats were sacrificed, brains removed and frozen for cryosectioning and verification of cannula placement into the nucleus accumbens (see Fig. 1). Dialysate was analyzed for dopamine metabolite concentrations, homovanillic acid (HVA) and dihydrophenylacetic acid (DOPAC), using high performance liquid chromatography

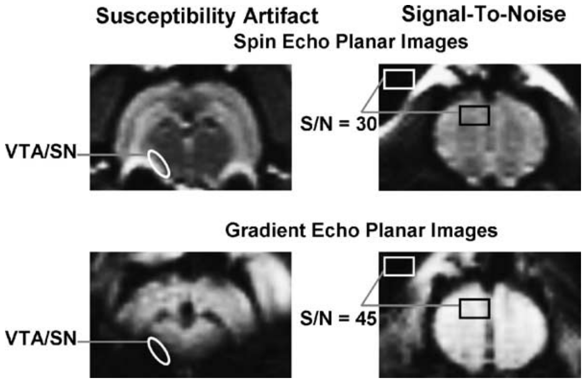

Fig. 1. Comparison between spin echo and gradient echo planar imaging (EPI) pulse sequences. Functional images shown to the left are at the level of the ventral tegmental area and images to the right are at the level of the prefrontal cortex. VTA, ventral tegmental area; SN, substantia nigra; SNR, signal-to-noise.

coupled to an electrochemical detector (Coulochem II, ESA Inc., MA).

2.4. *Cardiovascular and respiratory effects of intracerebroventricular versus intravenous cocaine*

The effect of ICV (20 μg/10 μl) and intravenous (1 mg/kg, 0.2 cc) routes of cocaine administration on cardiovascular and respiratory function were assessed. Animals were tested outside the magnet while set-up in the head and body restrainer used for imaging experiments (described below in Section 2.5). In the ICV group ($n = 6$), rats were anesthetized under 2% isoflurane, the skull surface was expose and the landmark suture Bregma located. A 26-gauge cannula of polyethylene tubing (PE-10: i.d. 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 dura) and secured to the skull with surgical glue. In the intravenous group ($n = 6$), the femoral vein and artery were exposed and cannulated with heparinized PE-50 tubing. The femoral artery tubing was connected to a Statham force transducer for assessing blood pressure and the femoral vein tubing was used for the peripheral administration of cocaine. Animals from both groups were fitted with a tail pulse oximeter (Nonin Medical, Minneapolis, MN) for measuring $p\mathrm{O}_2$, along with a thoracic force transducer for measuring respiratory excursions. A cone was placed over the snout and connected to a capnograph (Surgivet Wisconsin, USA) for measurement of expired CO_2. Continuous recordings of each physiological measure were collected starting 30 min after animals awaked. After a stable baseline period (ca. 10 min) animals were given an injection of cocaine and physiological parameters measured for 30 additional minutes.

2.5. *Functional imaging methods*

2.5.1. *Small animal restrainer*

Studies were performed with a multi-concentric dual-coil small animal restrainer developed by Insight Neuroimaging Systems, LLC (Worcester, MA), as described elsewhere (Ludwig et al., 2004). Just prior to the imaging session, animals were lightly anesthetized with 2% isoflurane (Surgivet Wisconsin, USA). A topical anesthetic of 2–5% lidocaine gel was applied to the skin and soft tissue around the ear canals and over the bridge of the nose. A plastic semicircular headpiece with blunted ear supports positioned over the ears. The head was placed into a cylindrical head holder with the animal's canines secured over a bite bar and ears positioned using adjustable screws fitted into lateral sleeves. An adjustable surface coil built into the head holder was pressed firmly on the head and locked into place. The body of the animal was placed into a body restrainer. The body restrainer "floats" down the center of the chassis connecting at the front and rear end-plates and buffered by rubber gaskets. The head piece locks into a mounting post on the front of the chassis. This design isolates all of the body movement from the head restrainer and minimizes motion artifact. Once the animal was positioned in the body holder, a volume coil was slid over the head restrainer and locked into position.

2.5.2. *Animal acclimation procedures*

Prior to imaging studies, rats were acclimated to the restrainer and the imaging protocol. Animals were lightly anesthetized with 2% isoflurane, as described above, and secured into the 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. Previous work by King et al. (submitted) show significantly reduced respiratory rate, animal motion and serum corticosterone levels by 3–4 days of acclimation, as compared to the first day.

2.5.3. *Magnetic resonance scanner*

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 (i.d. = 12 cm) capable of a 120 μs rise time. Radiofrequency signals are sent and received with the dual coil electronics built into the animal restrainer (Ludwig et al., 2004). The volume coil for sending RF signal features an eight-element microstrip line configuration that in conjunction with an outer copper shield forms a TEM resonator structure. The arch-shaped geometry of the receiving surface coil provides excellent coverage and high signal-to-noise. To prevent mutual coil interference, the volume and surface coils are actively tuned and detuned.

2.5.4. Pulse sequences parameters

Prior to cocaine imaging experiments, pilot studies were conducted on three male rats using gradient echo planar imaging (EPI) and spin echo EPI pulse sequences to determine which technique is most appropriate for imaging conscious animals for cocaine research. Both pulse sequence techniques were run in the same imaging session acquiring 12 contiguous slices (slice thickness 1.2 mm, field of view 3.0 cm, data matrix 64 × 64) covering the most rostral end of the prefrontal cortex and extending caudally to the mid pons. Spin echo planar images (TR = 2000 ms, TE = 55 ms) and gradient echo EPI (TR = 1000 ms, TE = 25 ms) are shown in Fig. 1. Although gradient EPI produces better signal to noise ratios, there is significant loss of signal in areas of air-tissue interface. This impedes imaging the entire mesocorticolimbic dopaminergic system from VTA to PFC. Spin echo EPI results in slightly lower signal-to-noise without signal loss in caudal brain structures and therefore was used for imaging cocaine-induced neural activity.

2.5.5. Imaging session

On the day of imaging, animals were prepared with ICV PE-10 tubing cannula as described above (Fig. 2A). Cocaine or vehicle injections were made via a 20 μl glass Hamilton syringe connected at the end of the tubing. The length of the cannula tubing was approximately 30 cm. The injection syringe rested just inside the bore of the magnet and could be easily accessed during an imaging session for ICV administration. An additional group of 4 rats received an IV cocaine injection (1 mg/kg, 0.2 ml heparinized saline) through a tail vein. Functional scans of axial 1.2 mm thick sections with a 3 cm field of view (64 × 64 data matrix) were obtained with a spin echo EPI pulse sequence (TE = 55 ms, TR = 2000 ms). Functional images were continuously acquired for 20 min which were divided between a 5 min baseline, and 15 min period following ICV cocaine ($n = 12$) or vehicle ($n = 10$) injection (see Fig. 2B). High resolution anatomical scans were collected using a multi-slice fast spin echo (TE = 48 ms, TR = 2500 ms, FOV = 3 cm, 1.2 mm slice thickness, 256 × 256 data matrix, 16 RareFactor).

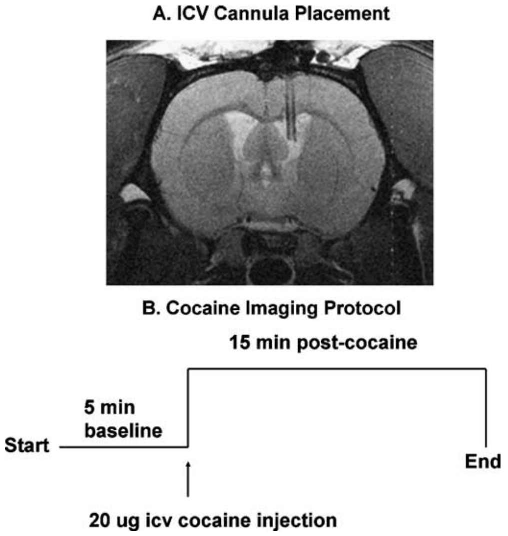

Fig. 2. Intracerebroventricular cannula placement and functional imaging paradigm used in the present study. (A) Prior to imaging sessions a non-magnetic polyethylene cannula was placed into the lateral cerebral ventricle to inject 20 ug of cocaine in 10 ul of artificial cerebrospinal fluid vehicle. (B) A functional imaging session conducted during a 20 min period and cocaine injected 5 min into the scan.

2.5.5.1. Distribution time of an intracerebroventricular injection. One of the limitations to the ICV administration of cocaine is the time-course of drug distribution throughout the brain. Specifically, does the ICV cocaine have access to the mesocorticolimbic areas during the first minutes of imaging acquisition? To address this question, the time of distribution of a contrast agent, gadodiamide (Nycomed Inc., Princeton, NJ), was measured following an ICV injection at a dose of 57.4 μg in a volume of 10 μl of aCSF. This dose of gadodiamide was chosen because it is equimolar to the concentration of the threshold dose of cocaine (20 μg/10 μl) identified in the dose–response studies. Gadodiamide is an uncharged paramagnetic molecule with a formula weight three times that of cocaine. Thus changes in signal intensity in the mesocorticolimbic areas following ICV administration of gadodiamide would suggest that ICV cocaine has the potential to affect these brain areas during the early minutes of image acquisition.

Rats were prepared with an ICV cannula as described above. T1-weighted images were collected using the following parameters: (TR = 235 ms, TE = 6.1 ms, flip angle = 30°, slice thickness = 1.0 mm, number of slices = 18, averages = 1, data matrix = 128 × 128). Forty-two sets of images were acquired for a total acquisition time of 17.5 min. Gadodiamide was injected at repetition number 3, after two baseline images had been acquired. Regions of interest (ROI) were drawn according to the Paxinos and Watson rat brain atlas (1997) and included the same areas of the brain analyzed for the cocaine imaging studies (see Section 2.6 below).

2.6. Data analysis

Selection of ROI and statistical analysis was performed using the Stimulate software (Strupp, 1996). Movies of functional scans for each subject were carefully examined to detect gross movements during imaging sessions. Two out of twelve scans showed high amounts of movements and were excluded from analysis. Regions of interest included the prefrontal cortex, caudate-putamen, nucleus accumbens, substantia nigra and ventral tegmental area. The ROI boundaries were identified based on the Paxinos and Watson rat brain atlas (1997) and analyzed for changes in BOLD signal intensity. BOLD signal intensity values represent averages of individual voxels located within an ROI. The average signal intensity for each ROI was converted to percent change.

In order to generate BOLD activation maps, statistical comparisons were made between the pre-injection baseline period and the post-injection period (Fig. 2B) using a pixel-by-pixel *t*-test analysis. Pixels whose BOLD percentage change relative to the baseline period was significantly different at a 95% confidence level were overlaid onto their respective anatomical data set.

3. Results

3.1. Cocaine-induced behavioral activation

One of the criteria for selecting a dose for imaging studies was the expression of characteristic psychomotor properties of cocaine. Horizontal locomotor and vertical activity were observed to be significantly higher in rats that received a 20 μg ICV cocaine dose when compared to vehicle controls ($F_{1,19} = 5.5$, $P = 0.02$; analysis of variance, $\alpha = 0.05$) or lower doses of cocaine ($F_{1,19} = 9.1$, $P = 0.007$) (Fig. 3A). The 5 μg and 10 μg cocaine doses did not produce any significant increases in horizontal or vertical activity when compared to the vehicle control group. Therefore, the 20 μg dose of cocaine is the threshold dose at which behavioral activation is observed to occur above control levels (Fig. 3A).

3.2. In vivo dopamine metabolism in the nucleus accumbens

A major indicator of cocaine-induced brain activation is an increase in dopamine metabolism in limbic brain areas, such as in the nucleus accumbens. We assessed the effects of 20 ug cocaine on in vivo dopamine metabolism in the nucleus accumbens. Levels of the two main dopamine metabolites, dihydro-phenyl acetic acid (DOPAC) and homovanillic acid (HVA), were significantly increased in the nucleus accumbens following ICV administration of the 20 ug dose of cocaine (Student's *t*-test, $P = 0.01$ for DOPAC and $P = 0.005$ for HVA), but not its vehicle (Fig. 3B).

3.3. Effect of route of administration on the cardio-respiratory physiology

Heart rate, respiratory rate, blood pressure and end tidal CO_2 for each animal was averaged for 5 min prior to injection (baseline period) and at 2, 4 and 10 min following intravenous cocaine, ICV cocaine or vehicle administration. Values were converted to percent change from baseline and statistical analysis made between groups with Friedman's test for repeated measures using the Wilcoxon paired sample test for post hoc comparisons. Percent change data are listed in Table 1. No differences were observed between ICV vehicle versus ICV cocaine injections. Rats given intravenous cocaine showed greater percent changes in heart rate ($\chi^2 = 10.8$, $P < 0.0009$), respiratory rate ($\chi^2 = 3.5$, $P < 0.05$)

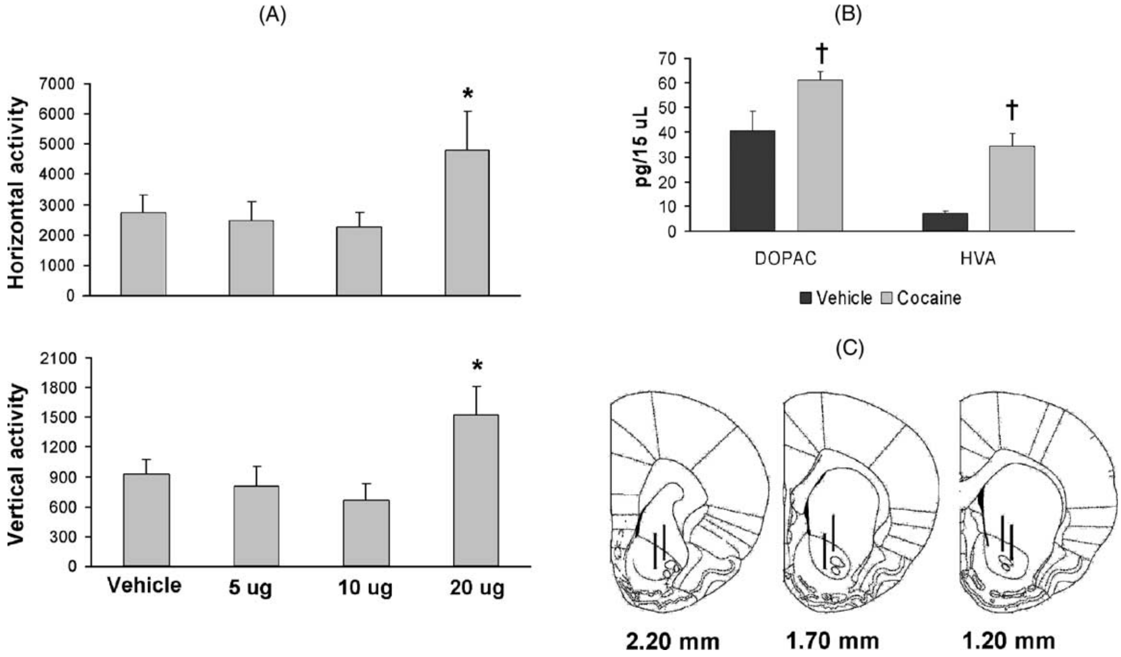

Fig. 3. Effect of intracerebroventricular cocaine administration on behavioral activity and dopamine metabolism in the nucleus accumbens. (A) Automated horizontal and vertical activity counts in response to three ICV cocaine doses. Only rats receiving 20 ug of cocaine showed behavioral activation above vehicle control levels ($^{*}P < 0.05$). (B) The levels of dopamine metabolites, DOPAC and HVA, were increased in the nucleus accumbens following ICV administration of 20 ug cocaine ($^{\dagger}P < 0.01$). (C) Representative coronal sections of the rat brain showing the sites of microdialysis cannula placement into the nucleus accumbens (adapted from Paxinos and Watson rat brain atlas, 1997).

Table 1
Percent change in cardiovascular and respiratory measurements following intracerebroventricular or intravenous administration of cocaine

	ICV vehicle (10 uL)			ICV cocaine (20 ug/10 uL)			IV cocaine (1 mg/kg)		
	2 min	4 min	10 min	2 min	4 min	10 min	2 min	4 min	10 min
Heart rate	−10.9 ± 6.2	−9.3 ± 3.2	−14.3 ± 1.8	−5.9 ± 7.8	−12.9 ± 3.5	−13.3 ± 2.6	−1.3 ± 7.0	7.7 ± 7.0*	10.8 ± 4.5*
Respiratory rate	0.2 ± 0.0	3.4 ± 3.6	0.0 ± 0.1	0.7 ± 1.0	1.4 ± 1.3	3.5 ± 2.9	53.8 ± 20.2†	50.9 ± 20.7†	22.4 ± 18.1
Blood pressure	−0.1 ± 0.3	−0.4 ± 0.3	0.0 ± 0.6	2.1 ± 2.4	2.4 ± 3.8	−0.1 ± 1.4	6.2 ± 1.9	5.8 ± 2.9	5.6 ± 2.8
End tidal CO_2	0.0 ± 0.0	−0.3 ± 0.2	−0.1 ± 0.0	0.5 ± 0.6	0.0 ± 0.6	−0.3 ± 1.4	68.4 ± 67.3	−8.8 ± 14.1	10.4 ± 22.3

(*, †) Statistically significant when compared to ICV cocaine injection (χ^2 values = 3.5–10.8, $P < 0.05$).

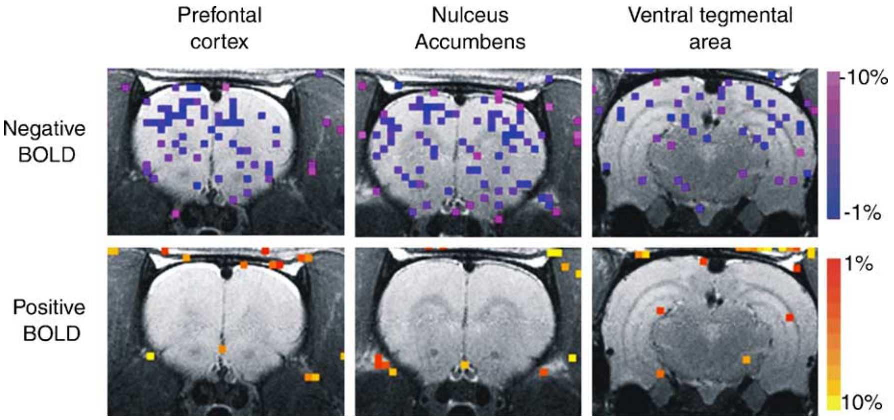

Fig. 4. Activation maps of blood-oxygenation-level-dependent (BOLD) signal change in response to intravenous cocaine administration (1 mg/kg). The colored pixels represent brain tissue volume elements (voxels) that showed signal intensity values significantly different from baseline. Statistical significance was determined with the Stimulate software (Strupp, 1996) using a pixel-by-pixel *t*-test analysis ($P < 0.05$) comparing baseline to a post-cocaine injection period. Pixels from each rat were overlaid on their corresponding high resolution anatomy.

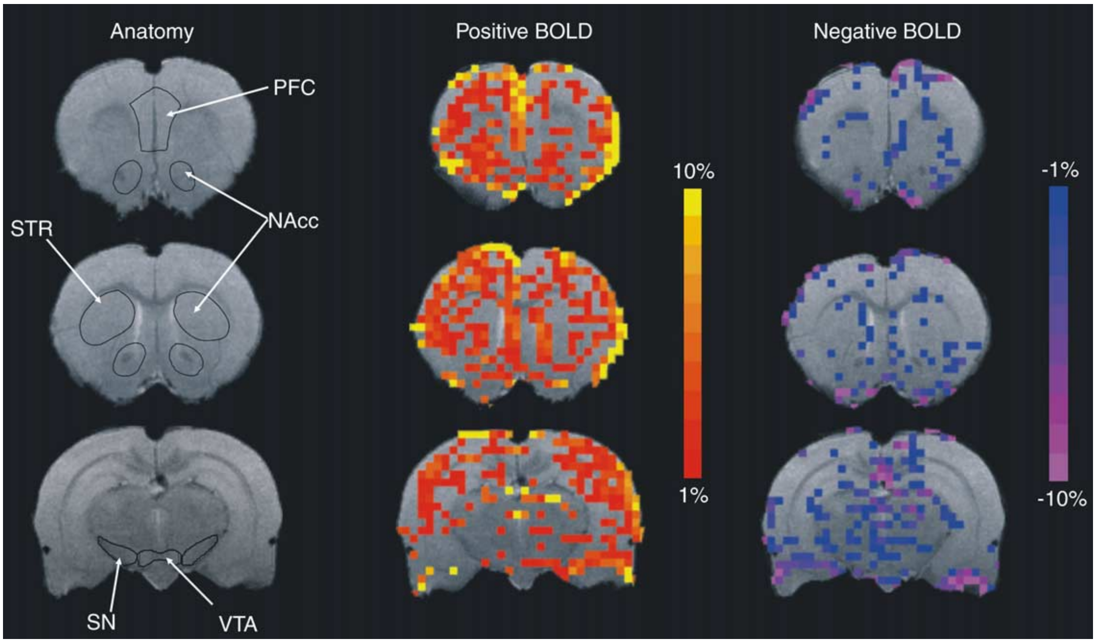

Fig. 5. Activation maps of positive blood-oxygenation-level-dependent (BOLD) signal in response to intracerebroventricular cocaine administration (20 ug/10 uL). The colored pixels represent brain areas that showed signal intensity values significantly different from baseline. Statistical significance was determined with the Stimulate software (Strupp, 1996) using a pixel-by-pixel *t*-test analysis ($P < 0.05$) comparing baseline to a post-cocaine injection period. Activated pixels from each rat were overlaid on their corresponding anatomy. PFC, prefrontal cortex; NAcc, nucleus accumbens; STR, dorsal striatum; VTA, ventral tegmental area; SN, substantia nigra.

Table 2
Percent change in T1-weighted signal intensity following intracerebroventricular injection of gadodiamide (57.4 ug/10 uL)

Region of interest	Time after gadodiamide injection (10 uL)	
	2 min	10 min
Prefrontal cortex	3.9	4.3
Striatum	2.1	6.1
Nucleus accumbens	2.3	2.1
Substantia nigra	1.3	2.2
Ventral tegmental area	5.3	9.0

and blood pressure ($\chi^2 = 8.0$, $P < 0.004$) as compared to rats administered ICV cocaine. Although expired CO_2 was not observed to be significantly different ($\chi^2 = 0.2$, $P < 0.6$), percent change values were largely variable within the intravenous group versus ICV rats.

3.4. Contrast agent study

Intracerebroventricular gadodiamide administration produced T1-weighted signal enhancement within 2 min after injection (see Table 2 for summary). Increases in signal intensity throughout mesocorticolimbic and nigrostriatal areas were observed at 2 min and continued following 10 min after injection of the contrast agent.

3.5. BOLD signal changes in response to intravenous cocaine administration

Fig. 4 shows the results from a representative rat given an intravenous injection of 1 mg/kg cocaine (total $n = 6$). Systemic administration of cocaine consistently produced a global negative BOLD signal change in the brain. No positive BOLD signal activation was observed in any brain area following IV cocaine injection.

3.6. BOLD signal changes in response to intracerebroventricular cocaine administration

Intracerebroventricular administration of 20 μg cocaine produced positive BOLD signal changes in the prefrontal cortex, nucleus accumbens, dorsal striatum, ventral tegmental area and substantia nigra (Figs. 5 and 6). The observed positive BOLD signal changes were greatest in the prefrontal cortex (4%) and nucleus accumbens (3%), two major targets of synaptic inputs from ventral tegmental dopamine neurons. The temporal activation profile, shown in Fig. 5, shows a peak positive BOLD response within the first 5 min after ICV cocaine injection. Following this period the BOLD response declines in the nucleus accumbens and striatum and remains at +2% in the prefrontal cortex and midbrain areas. The negative BOLD response to cocaine was sparse throughout mesocorticolimbic areas and preferentially localized to dorsal thalamic areas (Fig. 5). Administration of artificial CSF did not produce positive BOLD signal changes in the mesocorticolimbic areas analyzed. Vehicle injections did result in negative BOLD signal changes (approximately −1 to −2%), mainly in midbrain and striatal brain regions (Fig. 6).

ROI were also analyzed in terms of percent pixels showing positive BOLD changes. Cocaine administration increased the percentage of positive pixels in all major areas of the mesocorticolimbic dopaminergic system (Table 3). This reached statistical significance ($P < 0.05$) in the prefrontal cortex, nucleus accumbens, dorsal striatum, and ventral tegmental area.

Table 3
Percentage of pixels activated within regions of interest following intracerebroventricular administration of cocaine or vehicle

Region of Interest	Positive BOLD	
	Vehicle	Cocaine
Prefrontal cortex	9.9 ± 3.4	48.5 ± 5.4*
Nucleus accumbens	6.3 ± 2.0	35.9 ± 6.3*
Striatum	4.8 ± 1.6	26.1 ± 6.4*
Ventral tegmental area	5.2 ± 1.7	14.9 ± 1.8*
Substantia nigra	3.5 ± 1.3	6.3 ± 1.9

* Statistically significance determined with analysis of variance, $F_{1,16} = 8.2–32.5$, $P < 0.05$, post hoc analysis using Tukey's HSD test showed $P < 0.0005$.

4. Discussion

The acute behavioral effects of cocaine are associated with activation of dopaminergic pathways in the mesocorticolimbic system. In the present study, ICV cocaine caused a significant activation in all of the major components of this system most notably the VTA, nucleus accumbens, and prefrontal cortex. These results using BOLD fMRI corroborate findings from previous animal studies. Metabolic mapping with radiolabeled deoxyglucose showed cocaine-induced, site specific glucose utilization in the multiple areas of the brain (London et al., 1986; Porrino et al., 1988). With a temporal resolution of minutes and spatial resolution of light microscopy, Stein and Fuller (1992, 1993) mapped the entire brain for cocaine-induced changes in cerebral blood flow using [14C]iodoantipyrene. These radio labeled metabolism and blood flow studies are excellent for following the acute effects of cocaine on brain activity but unfortunately require sacrifice of the animal. On the other hand, non-invasive fMRI allows for repeated studies on the same animal enabling researchers to follow developmental changes in the brain's responsiveness to cocaine that occurs with sensitization and addiction.

There are many methodological issues concerning imaging cocaine-induced brain activation in conscious animals. The most significant of which is the control of cardiovascular and respiratory variables that affect cerebral blood flow and alter BOLD signal changes independent of cocaine's direct psychostimulant effect on the brain. The signal

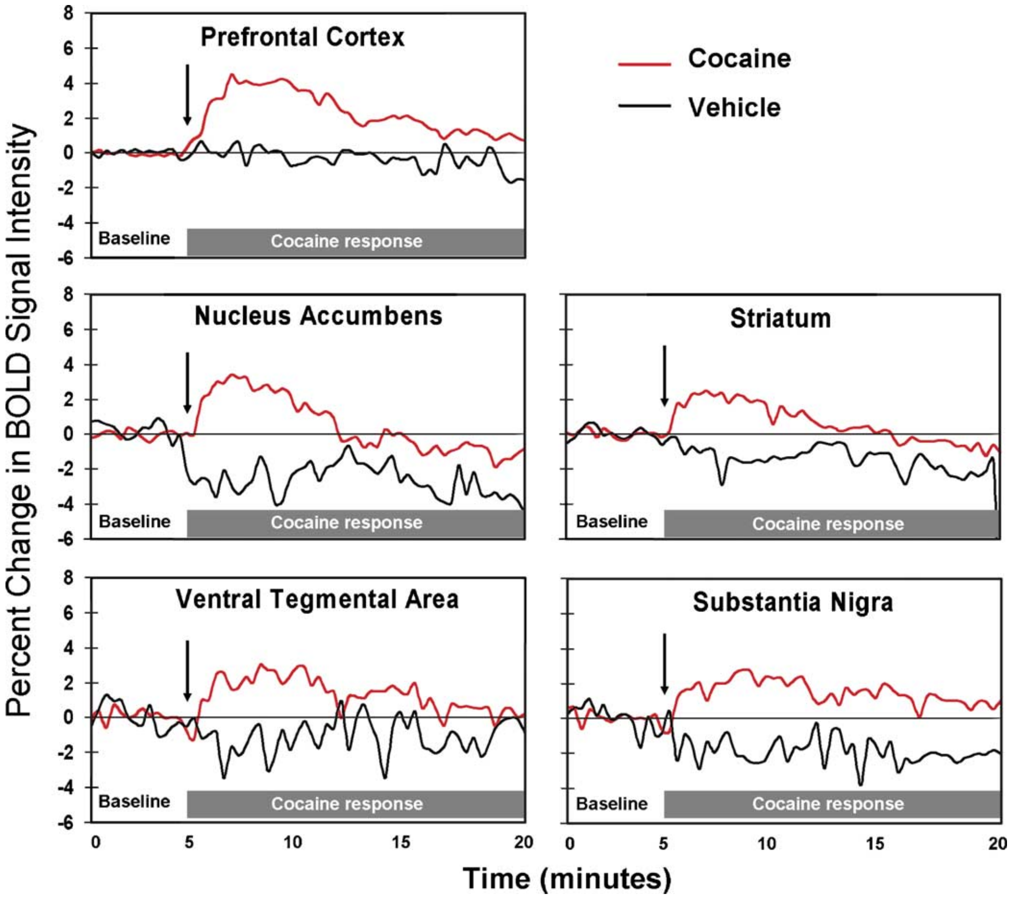

Fig. 6. Percent change in BOLD signal intensity over time in mesocorticolimbic and nigrostriatal brain areas. Data represent average signal intensity from voxels within the regions of interest of each rat. Percent change in signal intensity from baseline was calculated for each subject. Red lines represent cocaine injected rats and the black lines show data for vehicle injected animals. Arrows indicate the time of injection during the 20 min imaging session.

detected from BOLD- or T2*-weighted functional imaging is dependent on changes in neurovascular coupling, particularly, between the oxygen demand of neuronal activity and cerebrovascular adjustments to meet these demands. Increased neuronal activity transiently elevates the levels of paramagnetic deoxyhemoglobin which reduces BOLD signal intensity (reduced T2* relaxation time). The site of neural activity is 'flooded' with oxygenated blood which results in enhancement of BOLD signal intensity (due to increased T2*-relaxation time). Intravenous cocaine causes cerebral vasoconstriction (Kaufman et al., 1998b) and reduces cerebral blood volume by as much as 15–20% in human volunteers (Kaufman et al., 1998a). Thus, cocaine can alter the neurovascular coupling that gives rise to BOLD signal changes through direct effects on systemic cardio-respiratory parameters, in addition to its effects on neuronal activity.

In these studies on rats, we minimized the cardio-respiratory effects of cocaine by direct ICV injection of drug. Key to this route of administration was the dose–response data identifying 20 μg of cocaine as the threshold dose that elicited stereotypic behavior and release of dopamine in the nucleus accumbens without significantly disturbing cardio-respiratory function. However, there was a general nonspecific decrease in heart rate of about 10% following ICV injection of cocaine or artificial CSF vehicle in a volume of 10 μl. The mechanism of this response is unknown but may be due to the volume injection and subtle changes in CSF pressure. Perhaps this decrease in heart rate is responsible for the nonspecific 1–2% change in negative BOLD signal characteristic of the vehicle injection. This vehicle effect would be expected to diminish the cocaine-induced increase in BOLD signal activity. Nonetheless, a significant increase in BOLD signal intensity was noted in all of the mesocorticolimbic areas analyzed following ICV cocaine.

As expected, the intravenous injection of cocaine caused perturbations in all cardio-respiratory measures. Heart rate increased by 10% over baseline while the rate of respiration exceeded 50% of baseline. Blood pressure rose by a modest but significant 5–6%. As compared to ICV administered cocaine, expired CO_2 levels fluctuated unpredictably with intravenous injection with changes ranging from a negative 8% to a positive 68%. Over the 10 min data acquisition period following intravenous cocaine administration there was a progressive and robust appearance of negative BOLD signal over most of the brain. This generalized negative BOLD response probably reflects a decrease in cerebral blood volume caused by cerebral vasoconstriction in the presence of enhanced metabolic activity.

In a recent study, Luo et al. (2003) controlled for peripheral cardiovascular effects of cocaine in an animal study with a derivative drug, cocaine methiodide, which unlike native cocaine does not cross the blood brain barrier. Both cocaine and cocaine methiodide caused a significant increase in blood pressure following intravenous administration. However, only cocaine activated components of the mesocorticolimbic system, particularly the prefrontal cortex, thus supporting the use of the BOLD technique to study the neurobiology of cocaine despite cardiovascular alterations. However, the pattern of negative BOLD activity showed pronounced changes in the ventral surface of the brain including such areas as the piriform cortex, olfactory tubercles, ventral pallidum, shell of the accumbens, amygdala and temporal cortex. The aforementioned metabolic mapping and blood flow studies in animals (London et al., 1986; Porrino et al., 1988; Stein and Fuller, 1992, 1993) reported cocaine activation of these sites. The most likely explanation for these disparate results is the use of anesthesia. To eliminate motion artifact during BOLD imaging, Luo et al. (2003) anesthetized animals with urethane and induced paralysis with galamine.

In the present studies, we demonstrated that it is technically feasible to use BOLD imaging to study the acute effects of cocaine in fully conscious, non-paralyzed rats. Major areas of the mesocorticolimbic system, e.g. VTA, nucleus accumbens, and prefrontal cortex all showed significant increases in BOLD signal within minutes after ICV cocaine administration. One technical concern about the ICV route of administration was the timely distribution of cocaine to the different areas of the mesocorticolimbic system. ICV administration of a contrast agent in a volume of 10 μl showed that it is possible for cocaine to cross the ependymal lining of the ventricular system within the first 1–2 min of injection to directly affect the relevant brain circuits mediating its psychostimulant actions. Nonetheless, it is not certain whether the increase in BOLD activation in VTA, nucleus accumbens and prefrontal cortex was due to a direct action of cocaine on these sites or indirectly through other neural pathways.

Our results indicate that ICV administration of cocaine is suitable for examining the direct effect of this psychostimulant on brain activity using the BOLD technique in fully conscious animals. It is understood, that the peripheral autonomic activation, which coincides with the acute cocaine 'high' experienced by humans (Smith et al., 2001), is not mimicked with the ICV route of administration. Nevertheless, the present imaging methods can be used to design experiments to determine differences in cocaine-induced brain activation between naive and cocaine pre-exposed animals. For example, animals can be sensitized to cocaine by the normal route of intravenous or intraperitoneal administration. Cocaine can then be given ICV to these sensitized animal and drug naïve controls to assess changes in BOLD activity in the mesocorticolimbic system. The spatial and temporal resolution of fMRI using spin echo EPI pulse sequence allows undoubtedly generate a wealth of information on the fundamental neural adaptations occurring in the addicted brain.

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