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COCAINE-ASSOCIATED ODOR CUE RE-EXPOSURE INCREASES BLOOD OXYGENATION LEVEL DEPENDENT SIGNAL IN MEMORY AND REWARD REGIONS OF THE MATERNAL RAT BRAIN*

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Abstract

BACKGROUND—Cue triggered relapse during the postpartum period can negatively impact maternal care. Given the high reward value of pups in maternal rats, we designed an fMRI experiment to test whether offspring presence reduces the neural response to a cocaine associated olfactory cue.

METHODS—Cocaine conditioned place preference was carried out before pregnancy in the presence of two distinct odors that were paired with cocaine or saline (+Cue and –Cue). The BOLD response to +Cue and –Cue was measured in dams on postpartum days 2–4. Odor cues were delivered to dams in the absence and then the presence of pups.

RESULTS—Our data indicate that several limbic and cognitive regions of the maternal rat brain show a greater BOLD signal response to a +Cue versus –Cue. These include dorsal striatum, prelimbic cortex, parietal cortex, habenula, bed nucleus of stria terminalis, lateral septum and the

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mediodorsal and the anterior thalamic nucleus. Of the aforementioned brain regions, only the parietal cortex of cocaine treated dams showed a significant modulatory effect of pup presence. In this area of the cortex, cocaine exposed maternal rats showed a greater BOLD activation in response to the +Cue in the presence than in the absence of pups.

CONCLUSIONS—Specific regions of the cocaine exposed maternal rat brain are strongly reactive to drug associated cues. The regions implicated in cue reactivity have been previously reported in clinical imaging work, and previous work supports their role in various motivational and cognitive functions.

Keywords

cocaine; olfactory; cue; memory; pups; maternal rat; fMRI

1. INTRODUCTION

The propensity for abstinent cocaine users to relapse upon exposure to drug-associated cues is of great concern. Sensory cues, which in cocaine naïve subjects do not evoke craving or drug seeking, exert a powerful effect upon the behavior of addicts. The continued exposure to cocaine within specific contexts, and in the presence of associative sensory stimuli, may lead to strengthening of drug-cue associative memory. The phenomenon of cue-associated relapse is modeled in various ways within the animal literature. Rats trained to self-administer drugs will reinstate drug seeking after extinction when presented with a drug-associated cue (de Wit and Stewart, 1981; Meil and See, 1996). In conditioned place preference paradigms rats associating particular contexts and sensory cues with the rewarding effects of cocaine administration show a preference for these cues in comparison to neutral or non-rewarding cues (Isaac et al., 1989; Shippenberg and Heidbreder, 1995; Walters et al., 2006).

Relapse vulnerability is especially problematic among females who become mothers. Maternal cocaine use is associated with a variety of behavioral changes for the mother, such as alteration in maternal care potentially leading to abuse or neglect, as well as physical, neurological and behavioral changes in the offspring (Kelley et al., 1991; Peterson et al., 1996; Singer et al., 1992). In rats, pregestational cocaine administration has been shown to alter the quantity and frequency of maternal behaviors (Nephew and Febo, 2010), as well as lower the blood oxygenation level dependent (BOLD) response to suckling stimulation (Febo and Ferris, 2007), which is a natural reward for the maternal rat (Ferris et al., 2005). Cocaine-associated changes in the neurobiology and behavior of the mother directly impact offspring. Subtle changes in maternal behavior have been shown to affect hypothalamic-pituitary-adrenal (HPA) axis reactivity in offspring (Francis et al., 1999; Liu et al., 1997). For example, increased maternal grooming is associated with a decrease in HPA axis activation in response to acute restraint stress in adult offspring (Francis et al., 1999; Liu et al., 1997), as well as social interactions and their interactions with their own offspring (Francis and Meaney, 1999).

During the early postpartum period, pups are a highly rewarding stimulus for the maternal brain. Presentation of pups following maternal separation elicits dopamine (DA) release in the nucleus accumbens (NAc) of postpartum rats (Champagne et al., 2004; Hansen et al., 1993), and maternal rats will lever press for pups (Lee et al., 2000), strongly suggesting that pups are natural positive reinforcers. Furthermore, during the early postpartum period, dams will spend more time in a chamber that is associated with newborn pups than in a chamber associated with cocaine administration (Mattson et al., 2001, 2003). Although pups are highly rewarding and maternal rats show a preference for pup-paired over a cocaine-paired test chamber, it is unknown how the maternal brain will respond to a drug-paired cue if her

pups are not physically present, and whether that responsiveness will be altered or modulated by the presence of pups. Although motherhood may be viewed as involving a natural rewarding bond with offspring, it is still unknown whether offspring are able to alter the responsiveness to cocaine-associated cues in the addicted mothers. We developed a novel neuroimaging model to investigate whether dams who were administered cocaine pre-gestationally will demonstrate altered BOLD functional magnetic resonance imaging (fMRI) responsiveness to a cocaine-associated odor cue during the early postpartum period in the presence of pups compared to when these are absent. Our initial hypothesis was that pups will be able to curtail the neural response to cocaine associated but not saline associated cues, we observed instead that regardless of pup presence cocaine cues still elicit a robust activation across reward and memory regions of the maternal brain.

2. METHODS

2.1 Subjects

Virgin female Long-Evans rats (200–225 g) were purchased from Charles River Laboratories (Wilmington, MA). Females were housed in pairs in a temperature and humidity controlled room and maintained on a 12L: 12D light-dark cycle (lights off at 1900 h). Animals were separated for mating and remained singly housed throughout the remainder of the experiment. Home cages consisted of hanging plastic microisolator cages of standard dimensions with woodchip bedding. Water and Purina rat chow were provided ad libitum. Rats were acquired and cared for in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals (8th Edition, 2011) and adhere to the National Institutes of Health and the American Association for Laboratory Animal Science guidelines. The Institutional Animal Care and Use Committee at Northeastern University approved the protocols used for this study.

2.2 Acclimatization Procedures and Preparations for MR Imaging

All imaging experiments were done in fully awake, unanesthetized primiparous postpartum day 2 to day 4 (P2 to P4) dams. Anesthesia (2–4% isoflurane) was used during rat setup immediately preceding the acclimatization procedures and before MRI data collection, but rats were imaged while fully awake. In order to minimize physiological response and gross motion during magnetic resonance (MR) scanning, all rats were acclimatized to a head restraining unit and magnetic resonance imaging (MRI) sounds prior to drug administration and breeding (King et al., 2005).

2.3 Conditioned Place Preference Procedure

Animals were acclimatized to a three-compartment place preference system (Med Associates, St. Albans, VT). The place preference box contains two test chambers, each 21 cm W × 21 cm H × 28 cm L. One choice chamber has black walls and floor made from 4.8 mm stainless steel rods, placed on 16 mm centers, and the other has white walls and a 1.25 cm × 1.25 cm stainless steel mesh floor. The third neutral chamber measures 21 cm W × 21 cm H × 12 cm L and has grey walls and a smooth polyvinyl chloride (PVC) floor. All three chambers have hinged clear polycarbonate lids. Each female was placed into the center neutral chamber of the three-chambered box, and allowed to freely explore all chambers for one hour. This acclimatization procedure was repeated the following day. One day following acclimatization, each animal was placed into the neutral chamber and again allowed to freely move about the three chambers for 15 minutes. Their preference for either the black or white chamber was recorded. There was no significant preference for either chamber, with every animal spending no more than 470 out of 900 seconds in a particular choice chamber. We determined that approximately half of the animals tested displayed a slight preference for the black chamber, and the remaining animals for the white chamber. For

cocaine administration, these preferences were reversed yielding a biased design, such that cocaine administration took place in the slightly less preferred chamber. Following the ten-day drug-conditioning period, the animals were tested for their chamber preference. The females were once again placed into the center neutral chamber, and allowed to explore for 15 minutes. Their final chamber preference was recorded.

2.4 Cocaine Administration

Following MR restraint acclimatization, and initial place preference determination, drug administration and cue training was carried out. Each animal received two injections daily for ten days, each paired with a scent and contextual cue. Each pair of injections per rat was separated by 5–6 hours. During morning hours between 0700 hrs – 1200 hrs the animals were administered an intraperitoneal (IP) injection of either 15 mg/kg cocaine hydrochloride (Sigma Aldrich, St. Louis, MO) dissolved in 1 ml/kg sterile physiological saline, or 1 ml/kg sterile physiological saline vehicle and returned to their home cage to avoid the development of association between the injection and a particular chamber. After five minutes in the home cage, each animal was confined to the non-preferred chamber of the conditioned place preference box for one hour. Under the floor of the chamber was a lint-free tissue soaked in 15 μ L of peppermint oil (Sigma Aldrich, W284807). In the evening between 1300 hrs – 1800 hrs all animals received an IP injection of sterile physiological saline, and again returned to their home cage. After 5 minutes in the home cage, animals were confined to the preferred chamber for one hour. Under the floor of the preferred chamber was a lint free tissue soaked in 15 μ L of limonene (Sigma Aldrich, 89188). This procedure was repeated daily for ten days. The lengthy 10-day paradigm was used in order to replicate previous findings using 10-day pre-gestational cocaine sensitization (Nephew and Febo, 2010).

2.5 Functional MRI of cocaine associated odor cue in absence and presence of pups

Primiparous rats were imaged for their BOLD response to drug-associated scent cue (+Cue) or saline-associated control scent (–Cue) in the presence and absence of their pups (abbreviated in graphs and text as P and NP, respectively), resulting in 8 MRI scanning groups. Pups were presented using a custom-made plastic presentation platform allowing the maternal rat to visualize, smell and hear the pups throughout the imaging procedure (Nephew et al., 2009). A flexible polypropylene tube was placed into the magnet and secured near the nose of dams to allow for scent presentation during imaging. During the pre-scent baseline period and scent cue presentation, 4–5 pups were placed in the presentation platform along with some home cage bedding. The platform was inserted into the bore of the magnet and placed in front of the restrained dam. The restraint device has an opening in the front, which allows dams to visualize, smell and hear the pups throughout the imaging session. During the baseline, clean air was pumped through the tube to the nose of the maternal rat. For scent presentation scans, 80 repetitions (7.5 s per repetition for a total 600 s) were collected and the scent cue was presented through the tube at image repetition 39–40 (292–300 s). Because of expected differences between saline and cocaine conditioned animals, and the respectively paired odors, the lemon scent (–Cue) always preceded the peppermint (cocaine or +Cue) scent during imaging. However, pup presence during odor cue exposure was fully counterbalanced across all sessions.

2.6 Magnetic Resonance Imaging Scanning Parameters

Experiments were conducted in a 300 MHz Bruker USR 7T/20 cm horizontal magnet (Bruker, Germany) equipped with a Paravision 5.0 console (Bruker, Billerica, MA, U.S.A). Studies were performed with a quad transmit/receive radiofrequency (RF) coil system (InsightMRI, Inc, Shrewsbury, MA). Radiofrequency signals are sent and received with RF electronics built into the animal restrainer (Ludwig et al., 2004). Functional imaging was performed using a multi-segmented T₂-weighted fast spin echo pulse sequence with the

following parameters: repetition time (TR) = 1562 ms, echo time (TE) = 7.5 ms, effective echo time $TE_{eff} = 45$ ms and an echo train length (ETL) = 16. Geometry was setup as follows: 14 slices, field of view of 28 mm, 1.0 mm thick slices with no gaps, data matrix of 64^2 for functional scans and 256^2 for anatomical scans. Thus, the in plane 2-dimensional pixel resolution was $438 \mu m^2$ for functional and $117 \mu m^2$ for anatomical scans. A full set of 14 coronal slices across the brain was collected at each effective repetition time every 7.5 seconds.

Before MR scanning, females were anesthetized with 2–4% isoflurane. A topical anesthetic of 5–10% lidocaine cream was applied to the skin and soft tissue around the ear canals and over the bridge of the nose before the animal was placed inside the quad transmit/receive radiofrequency system under restraint (Ferris et al., 2005). This procedure generally took 5–6 min, after which gaseous anesthesia flow was turned off and the entire unit was placed through the bore of the magnet for imaging. After the entire unit was placed in the magnet, scanning preparations controlled by Paravision 5.0 typically took 15 min and thereafter the entire imaging session including 1 anatomical scan (ca. 6–8 min) and 4 functional scans (ca. 10–12 min each) lasted about 45–60 min. Thus, the entire experiment per animal in an unanesthetized state lasted 60–75 min.

2.7 Statistical Analysis

For conditioned place preference procedures, analysis was conducted as previously described (Isaac et al., 1989; Walters et al., 2006). Briefly, the time each animal spent in the cocaine-associated chamber before drug training was subtracted from the time spent in the cocaine chamber after drug training, and the resulting number was the preference score. A two-way analysis of variance (ANOVA) with odor (lemon vs. peppermint) and drug treatment (saline vs. cocaine) as independent variables was used to test the null hypothesis (no differences between conditions; null rejected at $p < 0.05$ corrected for multiple comparisons).

Full details for the MRI data analysis using in house software has been previously reported (Ferris et al., 2005). The present study includes MRI data for 10 cocaine treated dams and 12 saline controls. Each animal received 4 functional scans, yielding a total of 88 scans. Eight of these scans were dropped from the study because of severe motion artifact. The final number of animals per scan groups are for saline: –Cue/NP $n = 11$, +Cue/NP $n = 10$, –Cue/P $n = 11$, and +Cue/P $n = 12$. For cocaine the number of animals per scanning group are: –Cue/NP $n = 10$, +Cue/NP $n = 9$, –Cue/P $n = 9$, and +Cue/P $n = 8$. Scans were first corrected for motion using FMRIB software library's (FSL's) mcflirt program (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MCFLIRT>). Linear detrending was then used. Quality of motion and drift corrected scans was visually examined and compared with non-corrected scans for any errors in processing. Posthoc review of center-of-mass displacement was also used to judge quality of motion correction.

Region of Interest (ROI) based statistical analysis was done using Medical Image Visualization and Analysis (MIVA) software (Ferris et al., 2005). Each subject was registered to a fully segmented electronic rat brain atlas (Paxinos and Watson, 1997; Swanson, 1999). Statistical t tests were performed on each subject within the original coordinate system. The baseline period used was 38 repetitions immediately preceding stimulus presentation and the stimulation window was 38 repetitions following stimulus presentation. Statistical t tests used a 95% confidence level, two-tailed distribution, and heteroscedastic variance assumptions. In order to provide a conservative estimate of significance, a false-positive detection-controlling algorithm was introduced into the analysis (Genovese et al., 2002). This ensures that the false-positive detection rate is below our confidence level of 5% (Ferris et al., 2005). Statistically significant pixels were assigned

their percentage change values (stimulus mean minus control mean) and exported to Matlab (Natick, MA, USA) for statistical comparisons between groups. A multivariate analysis of variance (*n-anova* program in Matlab) with multiple independent factors (odor x pups x drug) and 4 dependent variables (positive and negative voxels and positive and negative BOLD signal changes) was used. Tukey's least significant difference (LSD) test was used to examine specific differences between the marginal means of the scanning groups and drug treatment conditions ($\alpha = 0.05$; Saville, 1990). A total 54 brain regions were analyzed for the number of significant positive and negative BOLD voxels and percent change in BOLD signal.

3. RESULTS

Figure 1 shows the results for conditioned place preference testing. Cocaine-treated animals spent more time in the cocaine/peppermint-associated side of the conditioned test cage than in the saline/lemon side of the cage (Two ANOVA $F(1,36) = 6.1$, $p = 0.02$; Holm-Sidak posthoc multi-comparison test shows difference at $p < 0.05$ between cocaine –cue and cocaine +cue). Saline animals did not show any preference towards either side of the test cages (Figure 1).

We investigated BOLD signal changes in the maternal rat brain during presentation of an odor that was previously paired with cocaine during conditioned preference testing (+Cue; peppermint). This was compared to another specific associative conditioned stimulus (saline, which appeared to signal the absence of cocaine; –Cue; Lemon), and to saline treated animals presented with the same odors. For all odor exposure conditions we observed increased BOLD in olfactory, limbic and memory formation structures such as the anterior olfactory nucleus (AON), olfactory tubercle, piriform, entorhinal/perirhinal areas, orbital prefrontal cortex, anterior thalamus, amygdala, hippocampus, among others (Figures 2 and 3).

There were no significant differences between –Cue and +Cue-evoked BOLD signal changes in saline treated rats (Figures 2 and 4). In cocaine-treated dams, we observed two distinctive effects of odor presentation on BOLD signal changes (Figure 3). First, cocaine treated animals showed a blunted or curtailed BOLD signal response to the saline-paired cue (Cue, lemonene scent) compared to saline treated animals. This was observed in a large number of brain regions. The second effect was an enhanced BOLD signal response to the cocaine-paired odor (+Cue, or peppermint scent, compared to –Cue in most cases). This was observed in a smaller number of brain regions. These two general outcomes were observed for both the number of activated voxels (Figures 2–3) and percent BOLD signal changes (Figures 4–5, Table 1). The brain regions are listed in Table 1 and include both subcortical regions involved in motivation, emotion, and motor activity. However, areas involved in sensorimotor processing, memory and emotional memory showed a similar effect (Table 1 and Supplementary Table 2¹). Representative data for several areas of the maternal brain are shown in Figures 4–5. A comparison between Figure 2 and Figure 3 indicates a greater BOLD activation volume with +Cue presentation than with –Cue presentation in cocaine treated animals and saline treated counterparts (in Figure 2). Contrary to this, –Cue exposure elicited a weaker activation in cocaine treated animals (Figure 3) than in saline animals (Figure 2). Therefore, not only does the cocaine-paired odor have a robust effect upon BOLD signal activation in the maternal rat brain but the –Cue also appears to be modified by previous pairings with saline. The latter being a reduced activation compared with control rats. A summary of the specific multiway ANOVA results for percent changes in

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BOLD signal in each of the 54 ROI is provided in Table 1. Voxel activations followed a very similar effect and the statistical results are presented in Supplementary Table 2².

We examined the effect of pup presence on cocaine cue reactivity. Pups were placed in close proximity in front of dams while these were scanned (as in Nephew et al., 2009). There was a significant interaction between pup presence and cue exposure in a subgroup of brain regions with significant responses to the +Cue (Table 1). These were all associated with greater percent changes in BOLD signal. Out of all regions studied, the parietal cortex showed a greater BOLD signal response to +Cue in the presence of pups than in their absence (Tukey's LSD test $p < 0.05$; Table 1). However, no other region studied showed this same effect. In fact, since the BOLD signal changes were close to uniform between the test conditions (of pups versus no pups), the data served as their own positive test/re-test controls within the same imaging session (Figure 4 and Figure 5). Overall, the main observation of the present study is that the cocaine-associated cue enhances neural activity across a number of brain regions, whereas the negative cue (associated only with saline) leads to a weaker response than with the +Cue or in comparison to saline control rats.

4. DISCUSSION

Our results show areas of the maternal rat brain that increase their neural activity in response to a cocaine-associated odor cue. This occurs to a large extent regardless of the presence or absence of pups. Cue-evoked BOLD signal changes were investigated in the absence and the presence of pups. Cocaine-treated dams showed a greater BOLD activation when presented with a cocaine-paired odor than when presented with a saline-paired odor. This was observed across multiple regions including motor, parietal, prelimbic, and retrosplenial cortices, mediodorsal and anterior thalamic nuclei, dorsal hippocampus/dentate gyrus, striatum, amygdala and BNST (Table 1). In addition to the typically identified reward-related functions, many of these regions share functions in learning, memory consolidation, and/or memory recall, which suggests the possibility that the observed differential neural responses may be associated with a cocaine-related memory. The greater activation observed with the drug-related odor cue in cocaine-conditioned rats was not observed in saline treated animals. Importantly, drug treatment ceased before pregnancy and fMRI acquisitions were carried out during the postpartum period, approximately 30 days later. Thus, the present data favor the interpretation that long-term memory and recall mechanisms associated with cocaine's effect might be mediating the observed pattern of BOLD activation.

Previous studies in cocaine and heroin dependent human and animal subjects have provided data on the neural correlates of cue reactivity, and the brain regions involved in craving and addiction. There is a good deal of overlap between these previously identified regions in humans and animals and those identified here in the maternal rat. A subset of the regions identified as responsive to drug cues in the maternal rat include those responsible for memory consolidation, storage, and recall, such as the hippocampus, amygdala, retrosplenium, and anterior nucleus of the thalamus. The hippocampus is thought to be a central player in learning and memory (Burgess et al., 2002) as it is well established that ablation, removal, or inactivation of any number of hippocampus sub regions leads to memory deficits (Cave and Squire, 1992; Eichenbaum, 1997; Kesner et al., 2004; Milner, 1959). It also bears extensive connectivity to a variety of other memory-related regions, including the amygdala, which is known to be involved in emotional memory (Goddard, 1964; McGaugh, 2004; Weiskrantz, 1956) and emotional reactivity (LeDoux, 2000), and the retrosplenium, which is implicated in episodic memory (Shallice et al., 1994; Wagner et al.,

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2005), including emotion-related memories (Maddock, 1999). We also saw differential activation within the anterior nucleus of the thalamus. Lesions to the anterior nucleus of the thalamus cause various memory deficits (Aggleton et al., 1996; Harding et al., 2000; Mitchell and Dalrymple-Alford, 2005; Parker and Gaffan, 1997) including deficits in recognizing a previously learned scent cue (Wolff et al., 2006). This suggests that the above regions may be important in memory of the drug-related cue, which is a crucial component of drug abuse and addiction (Hyman et al., 2006), as well as the subsequent drug experience in these maternal animals.

Functional MRI studies of humans have also demonstrated the role of memory in cue reactivity. Garavan et al. (Garavan et al., 2000) reported cue-induced BOLD fMRI activation in cocaine dependent subjects viewing a video showing crack cocaine use, and compared this to a nature or a sex film. They reported significant increases in BOLD activation in various cortical and subcortical regions that subserve functions associated with emotional memory recall such as the medial and dorsolateral prefrontal cortex, anterior and posterior cingulate, inferior parietal lobule, insula, temporal pole, and anterior nucleus of the thalamus (Garavan et al., 2000). Kosten et al. (2006) correlated treatment effectiveness scores (which provide an indication of relapse) and cocaine cue-evoked BOLD signal changes. The areas that showed increased activation in individuals with lower treatment effectiveness scores included sensory and motor areas of the cortex, the posterior cingulate (retrosplenium) and superior temporal gyrus (Kosten et al., 2006). Furthermore, a positron emission tomography (PET) study using a similar experimental design as ours reported that cocaine-related cues increased regional cerebral metabolic rates for glucose (rCMRG) to a greater extent than neutral cues in cocaine dependent subjects but not in control non-dependent subjects in dorsolateral and medial orbital prefrontal cortical areas, temporal and parietal cortices, and peristriate areas of the visual cortex (Grant et al., 1996). The overlap between the regions identified in the human imaging studies and those we have identified in maternal rats indicates the likely similarity in processing of drug cues between maternal and non-maternal mammals. The primary systems that appear to be recruited in response to drug cues in both the maternal rat as well as human male indicate an important role of learning and memory systems in cue-induced drug seeking and taking.

In addition to learning and memory related areas, and the limbic and motor associated areas discussed above, the data presented here also revealed an increased response of the dorsal striatum of cocaine treated dams in response to the +Cue compared to -Cue. The dorsal striatum has previously received attention for its role in cue reactivity and craving, and spans the learning and memory as well as limbic and motor circuits described above. Volkow and colleagues (2006) showed a significant increase in cue induced dorsal striatal DA release using PET in cocaine dependent subjects, and in rats, See et al. (2007) reported an attenuation of cocaine seeking behavior following inactivation of dorsal lateral striatum and testing for reinstatement of cocaine self-administration. However, further work demonstrated that localized inactivation of the same region interferes with cocaine- but not cue-induced reinstatement, although a trend was cited (Gabriele and See, 2011). Together these data demonstrate a great overlap in regions typically responsive to drug associated cues in the non-maternal animal with those identified here in the maternal rat. These overlapping circuits suggest that the cue is indeed eliciting some aspect of drug responsiveness in the maternal brain, although the behavioral outcomes of such activity are as of yet unexplored.

While similar studies in humans reinforce the data we collected from maternal rats, Johnson et al. (2013) recently carried out an experiment in male rats, which examined the BOLD fMRI response to a cocaine-associated odor versus an unpaired odor cue (Johnson et al., 2013), and provided an alternate view of cue reactivity. The study was supported by

intracranial self-stimulation data showing that exposure to a cocaine-related odor cue causes reductions in brain reward threshold in male rats (Johnson et al., 2013). The fMRI results showed a significantly greater BOLD activation in the agranular insular cortex. However, none of the other regions reported in the present study were observed to be significantly different between the +Cue and –Cue. One possible explanation for the different findings could be that of sex differences in reactivity to drug-related cues (Russo et al., 2003b). Johnson et al. (2013) carried out their study in male rats, whereas maternal rats were used here. Differences in neuroendocrine status and stress reactivity between male and early postpartum maternal rats may account for the differential patterns of BOLD activity observed between the studies (Li et al., 2005; Russo et al., 2003a), and explanation that is supported by human fMRI work investigating sex differences in cue evoked BOLD signal responses and their correlation with craving and stress reactivity (Potenza et al., 2012). However, differences in the behavioral paradigm preceding the imaging of the respective cue fMRI studies could be important as well. Johnson et al (2013) passively exposed male rats to cocaine and peppermint odor across 10 days and on alternating days with saline alone in the absence of a specifically vehicle-paired scent. Here, we used a behavioral paradigm that measures the animals' conditioned association between a side of a cage and an odor paired with cocaine's rewarding effect; and, the other cage side was paired with a different odor and saline. Therefore, the present study design may have allowed a somewhat better specificity in determining odor-evoked BOLD activation across the brain.

An intriguing result is the suppressed neural response to the –Cue in cocaine exposed rats. This was not anticipated but could signify an important underlying mechanisms deserving further investigation. Compared with saline treated animals, cocaine treated rats showed a significantly curtailed BOLD response to the lemon odor, which was paired with saline injections. One explanation is that the regions showing this suppressed response to the –Cue odor might be responding to the signaling of the absence of cocaine. On the other hand, one might expect that cocaine addiction might entail significant responding to rewarding cues while suppressing stimuli that are not expected to have any motivational salience.

In addition to identifying the regions of the maternal brain that respond with increased BOLD activation to a previously cocaine-paired cue, a major goal of the study was to examine the effect of pup presence (proximity to the dams during scanning) on this response. The conceptualization of the study attempted to mimic in part aspects of the re-exposure of the mother to drug related cues during early newborn care. We hypothesized that if a cocaine-associated cue strongly activates regions of the reward system of cocaine-exposed dams, pup proximity would blunt this effect. This would be primarily associated with the rewarding and motivational drive that occurs in dams while in the presence of their pups (Numan and Numan, 1995). In maternal rats it has been demonstrated that in the early postpartum period they prefer pups over cocaine (Mattson et al., 2001), which has been associated with c-fos labeling in the medial prefrontal cortex and nucleus accumbens (Mattson and Morrell, 2005), and dams will train to lever press to gain access to pups, which provides evidence that pups are reinforcing stimuli (Lee et al. 1999; 2000). Based on this data, we expected interactions between cocaine cue exposure and pup presence, primarily involving regions of the reward system; however, this was not the case. The only brain region to show a significant effect for pup presence was the parietal cortex (Fig. 4 and 5). The role of the parietal cortex in cocaine addiction is not new. Cocaine users show significant hypoactivity in the parietal cortical neural response to affective stimuli (Asensio et al., 2010). This region also shows less activity in working memory tasks in cocaine dependent subjects (Bustamante et al., 2011) and is also associated with the development of maternal plasticity in multiparous rats (Fleming and Korsmit, 1996). Thus one might construe a novel role for this region in cocaine cue reactivity that is modulated by the presence of pups in maternal rats. This deserves further investigation.

The lack of broad evidence that the presence of pups modulates the reward related drug cue reactivity of the dam is not entirely unprecedented, as it is known that postpartum rats will show high and sustained levels of intravenous self-administration, even in the presence of pups (Hecht et al., 1999). These data, combined with those we present here, suggest that although the maternal animal does display an altered response to the drug cue in the presence of her offspring, that this alteration may not be sufficient to ameliorate drug cue memory and drug seeking. However, although the reward system response to drug cues seems unaffected by pup presence, at this stage it is unclear how the blunted neural response to the cues in the motor and somatosensory cortices and lateral septum may affect drug seeking, or conversely, maternal care.

There are several caveats to the study. Scent cues were not fully counterbalanced, and every animal in the cocaine group received cocaine in the morning paired with peppermint, and saline in the evening paired with limonene, while no animals received cocaine paired with limonene, or vice versa, so it is difficult to generalize the findings to odor cues other than peppermint. Furthermore, peppermint was always presented first in the imaging session, followed by limonene. However, it is unlikely that there is any effect of this method, due to the absence of a large population of BOLD signal differences between the saline treated animals presented with peppermint and the saline treated animals presented with limonene. Another important aspect of the study is the motivational value of the odors themselves, which might determine the outcome of the BOLD responses to the odors regardless of the cocaine pairing. Peppermint odor appears to be naturally avoided by young rat pups, as measured by time spent in proximity to or away from odor-saturated pine shavings (Brown and Willner, 1983). Rats spend more time away from the peppermint compared to control pine shaving scent and more time near maternal-related odors (Brown and Willner, 1983). However, this has been tested at 500 μ L, which is 100 times greater in concentration than the amount used in the present work (5 μ L). Moreover, our previous collaborative work shows that cocaine-associated peppermint odor reduces brain reward thresholds (indicating an increased rewarding effect of peppermint) (Johnson et al., 2013). Saline controls showed no differential BOLD responses between the two odors, this suggests that inherent motivational differences elicited by the peppermint and lemonene are not a significant factor in modulating the BOLD responses to cocaine-paired cues. If this were the case, however, it brings up an interesting, and perhaps novel strategy to investigate drug cues and stress interactions using fMRI in rodents. Pairing with cocaine could possibly heighten stress reactivity to the peppermint odor, and if this is the case then one additional effect of cocaine/odor pairing is a greater stress-related activation than in saline treated animals. Although this warrants further refinement of the experimental design in future experiments, it also brings up potential directions to pursue in subsequent experiments seeking to investigate the interactions between cocaine cues and stress (Potenza et al., 2012).

Animals in this study were administered cocaine passively, as opposed to self-administration, and there is some evidence that passive cocaine administration as compared with active cocaine seeking may produce slightly altered response patterns in the brain (Stefanski et al., 2007). Finally, it is important to note that our design did not account for whether or not cue exposure impacts neural responses to pups. In order to address this, the study design would need to examine the neural responses to pups, for instance, suckling stimulation as in Ferris and colleagues' study (2005) before and during re-exposure to a cocaine-associated cue (Ferris et al. 2005).

Our present data collectively suggest that the maternal response to a drug cue is remarkably similar to that of a non-maternal animal. It is likely that the drug cue is eliciting many of the mechanisms responsible for cue-induced drug seeking and relapse within the maternal brain, in a similar manner to the non-maternal animal. It is also likely that interactions with stress

reactivity to the drug cue or conditions of drug exposure may impact the final outcome, which is a heightened response to the drug-associated cue, and lowered response to a cue signaling the absence of cocaine. As a consequence this highlights the importance of studying relapse during the postpartum period with well-defined behavioral models and neuroimaging techniques. Cocaine abuse before and during pregnancy may impact the maternal brain and cause significant neurochemical and physiological alterations underlying modified motivational and reward systems (Elliott et al., 2001; Johns et al., 2004, 1998, 1997). This could impact important developmental stages for the mother as for the offspring during pregnancy, parturition, lactation, and postnatal care. An attempt to model these changes in the rat, particularly the *in vivo* neurobiological changes that may occur in the maternal brain is of major importance in the field of addiction research and merits further investigation. How the observed pattern of cue reactivity, combined with activity of the distinct subset of regions identified here regardless of the absence and the presence of the offspring will affect maternal care and subsequent drug seeking behaviors is yet to be explored in great detail.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Conditioned place preference score in saline and cocaine treated female rats tested before pregnancy. Data are shown as mean time (in seconds) spent in cocaine-associated side (patterned bars) compared with the saline side of the test cage (black bars). All data are mean $\pm$ standard error. *Significantly different from time spent in -Cue side (two way ANOVA, $p < 0.05$).

Figure 2.

Composite positive BOLD signal maps of saline treated dams. Data are shown for animals presented with a lemon odor (–Cue) and a peppermint odor (+Cue), in the absence and presence of pups (NP and P, respectively). Both lemon and peppermint odors were paired with saline during conditioning trials. Imaging groups and number of animals per group are shown above and below the atlas maps. Voxels on the 2D atlases showing red-to-yellow color gradation represents localized changes in BOLD signal between 1 to 10% (scale bar at bottom) corresponding to a minimum threshold p value of 0.05, FDR corrected.

Abbreviations (from top to bottom): ACg, anterior cingulate cortex; PrL, Prelimbic area; AON, anterior olfactory nucleus; MCx, motor cortex; IL, infralimbic area; OTb, olfactory tubercle; INS, insular cortex; NAc, nucleus accumbens; dSTR, dorsal striatum; VPal, ventral

pallidum; SSCx, somatosensory cortex; LSpt, lateral septum; GPal, globus pallidus; BNST, bed nucleus of stria terminalis; AThal, anterior thalamic nucleus; ParCx, parietal cortex; PVN, hypothalamic paraventricular nucleus; PirCx, piriform cortex; BAmy, basal amygdala; dHC, dorsal hippocampus; AudCx, auditory cortex; EntCx, entorhinal cortex; CAmy, cortical amygdala.

Figure 3.

Composite positive BOLD signal maps of cocaine treated dams. Data are shown for animals presented with a saline-paired lemon odor (–Cue) and a cocaine-associated peppermint odor (+Cue), in the absence and presence of pups (NP and P, respectively). Imaging groups and number of animals per group are shown above and below the atlas maps. Voxels on the 2D atlases showing red-to-yellow color gradation represents localized changes in BOLD signal between 1 to 10% (scale bar at bottom) corresponding to a minimum threshold p value of 0.05, FDR corrected. Abbreviations are as in Figure 2.

Figure 4.

BOLD signal response to odor cues paired with saline or cocaine (–Cue and +Cue, respectively). The same odors were presented to saline treated animals (left, black bars) and cocaine treated animals (patterned color bars to the right). Data are shown as mean $\pm$ standard error. * indicates significantly different from Cocaine –Cue/NP condition; **significantly different from Cocaine –Cue/P condition; *** significantly different from all conditions (saline or cocaine) except Cocaine +Cue/P or Cocaine +Cue; θ significantly different from Cocaine +Cue/NP (Tukey's least significant difference posthoc test $p < 0.05$).

Figure 5.

BOLD signal changes in response to odor cues paired with saline or cocaine (–Cue and +Cue, respectively). Data from cocaine treated rats presented with –Cue and +Cue as percent signal changes over time. Arrow indicates the time of odor presentation. Data are shown as mean $\pm$ standard error.

Table 1

Effect of pup presence on maternal brain BOLD signal changes in response to a saline vs. a cocaine paired odor cue. Presented are the results of a full factorial multi-way analysis of variance and posthoc test (α value < 0.05).

Region of Interest	Multiway ANOVA: Main Effects and Interactions (F statistic, p value) ^a	Posthoc Test (Group codes) ^b
Anterior amygdala **	Main effect odor ($F_{1,72} = 8.6$, $p = 0.004$)	6 vs 8; 5 vs. 7
Nucleus accumbens (core)	n.s.	n.s.
Nucleus accumbens (shell)	n.s.	n.s.
Agranular insula	n.s.	n.s.
Anterior olfactory nucleus **	Main effect drug ($F_{1,72} = 10.4$, $p = 0.002$)	2 vs. 6; 4 vs. 8
Anterior thalamus **	1 Main effect odor ($F_{1,72} = 6.8$, $p = 0.01$)	8 vs. 1,2,3,5,6; 7 vs. 5
	2 Drug x odor interaction ($F_{1,72} = 4.1$, $p = 0.04$)	
Auditory cortex	n.s.	n.s.
Basal amygdala	n.s.	n.s.
BNST **	1 Main effect odor ($F_{1,72} = 6.2$, $p = 0.01$)	6 vs 8; 5 vs. 7
	2 Main effect drug ($F_{1,72} = 8.2$, $p = 0.005$)	
CA1 dorsal	n.s.	n.s.
CA1 ventral **	Main effect pups ($F_{1,72} = 5.0$, $p = 0.02$)	n.s.
CA2 dorsal **, †	Drug x odor interaction ($F_{1,72} = 5.2$, $p = 0.02$)	5 vs. 7
CA2 ventral	n.s.	n.s.
CA3 dorsal **	1 Main effect odor ($F_{1,72} = 5.6$, $p = 0.02$)	8 vs 4; 7 vs 3
	2 Drug x odor interaction ($F_{1,72} = 4.1$, $p = 0.046$)	
CA3 ventral	n.s.	n.s.
Central amygdala **	Main effect odor ($F_{1,72} = 4.8$, $p = 0.03$)	8 vs 6
Cortical amygdala **	Main effect odor ($F_{1,72} = 6.2$, $p = 0.02$)	n.s.
Dentate gyrus **	Main effect odor ($F_{1,72} = 5.7$, $p = 0.02$)	7 vs 5
Striatum (DL) **	1 Main effect odor ($F_{1,72} = 7.3$, $p = 0.008$)	6 vs 8; 5 vs. 7
	2 Drug x odor interaction ($F_{1,72} = 4.5$, $p = 0.04$)	
Striatum (DM) **	1 Main effect odor ($F_{1,72} = 5.7$, $p = 0.02$)	8 vs 6,4; 5 vs. 7
	2 Drug x odor interaction ($F_{1,72} = 5.9$, $p = 0.01$)	
Striatum (VL) **	Drug x odor interaction ($F_{1,72} = 9.3$, $p = 0.003$)	8 vs 6,4; 5 vs. 7
Striatum (VM) **	1 Main effect odor ($F_{1,72} = 6.9$, $p = 0.01$)	6 vs 8; 5 vs. 7
	2 Drug x odor interaction ($F_{1,72} = 10.3$, $p = 0.002$)	
Entorhinal cortex	n.s.	n.s.
Globus pallidus **	Main effect odor ($F_{1,72} = 4.2$, $p = 0.04$)	n.s.
Habenula **	Drug x odor interaction ($F_{1,72} = 7.6$, $p = 0.008$)	6 vs 8; 5 vs. 7

Region of Interest	Multiway ANOVA: Main Effects and Interactions (F statistic, p value) ^a	Posthoc Test (Group codes) ^b
Infralimbic area	n.s.	n.s.
Insular cortex	n.s.	n.s.
Lateral amygdala **	Drug x odor interaction ($F_{1,72} = 4.5$, $p = 0.03$)	n.s.
Lateral hypothalamus **	Drug x pups x odor interaction ($F_{1,72} = 4.1$, $p = 0.047$)	7 vs 5,3
Lateral septum **, †	Main effect odor ($F_{1,72} = 6.8$, $p = 0.01$)	7 vs 5
Mediodorsal thalamus **	1 Main effect odor ($F_{1,72} = 6.5$, $p = 0.01$)	8 vs 1–6; 7 vs 1–6
	2 Drug x odor interaction ($F_{1,72} = 8.2$, $p = 0.005$)	
Medial amygdala	n.s.	n.s.
Motor cortex, primary **, †	Drug x odor interaction ($F_{1,72} = 6.5$, $p = 0.01$)	7 vs 5,3
Motor cortex, secondary **, †	Drug x odor interaction ($F_{1,72} = 6.3$, $p = 0.01$)	7 vs 5,3
Medial septum **	Main effect odor ($F_{1,72} = 5.5$, $p = 0.02$)	8 vs 6
Orbital area	n.s.	n.s.
Olfactory tubercles **	Drug x pups x odor interaction ($F_{1,72} = 4.1$, $p = 0.046$)	7 vs 3
Posterior amygdala **	Main effect drug ($F_{1,72} = 4.9$, $p = 0.03$)	n.s.
Piriform cortex	n.s.	n.s.
Prelimbic area **	1 Main effect odor ($F_{1,72} = 9.3$, $p = 0.003$)	6 vs 8; 5 vs. 7
	2 Drug x odor interaction ($F_{1,72} = 5.8$, $p = 0.02$)	
Parietal cortex **	1 Main effect odor ($F_{1,72} = 5.4$, $p = 0.02$)	8 vs. 1,2,3,4,5,6,7
	2 Main effect drug ($F_{1,72} = 9.6$, $p = 0.002$)	
	3 Drug x odor interaction ($F_{1,72} = 8.5$, $p = 0.005$)	
PVN	n.s.	n.s.
Nucleus reuniens	n.s.	n.s.
Retrosplenium **	Main effect odor ($F_{1,72} = 6$, $p = 0.02$)	8 vs 6
SN, compacta **	Drug x pups x odor interaction ($F_{1,72} = 5.6$, $p = 0.02$)	n.s.
SN, reticulata	n.s.	n.s.
Somatosensory cortex, primary **	Drug x odor interaction ($F_{1,72} = 5.4$, $p = 0.02$)	n.s.
Somatosensory cortex, secondary	n.s.	n.s.
Subthalamic nucleus **	Drug x odor interaction ($F_{1,72} = 4.4$, $p = 0.04$)	n.s.
Subiculum **	Main effect pups ($F_{1,72} = 4.8$, $p = 0.03$)	n.s.
Temporal cortex	n.s.	n.s.
Ventral pallidum	n.s.	n.s.
Ventral tegmental area	n.s.	n.s.

^a Multi-way ANOVA used with independent variables of drug x odor x pups (saline vs. cocaine; –cue vs. +cue; no pups vs. pup presence, respectively). Null hypothesis evaluated for each factor and interactions.

** Significant difference (null hypothesis rejected).

† denotes effect of pup presence. A posthoc least significant difference test used to examine specific differences.

^b Group codes in columns for the posthoc test are **1** = Sal/–Cue/NP, **2** = Sal/–Cue/P, **3** = Sal/+Cue/NP, **4** = Sal/+Cue/P, **5** = Coc/–Cue/NP, **6** = Coc/–Cue/P, **7** = Coc/+Cue/NP, **8** = Coc/+Cue/P (NP, no pups and P, pups present).

^c *Abbreviations:* n.s., not significant; SN, substantia nigra; PVN, paraventricular nucleus; BNST, bed nucleus of stria terminalis; DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial.