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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 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.

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

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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 19.00 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–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 $W \times 21$ cm $H \times 28$ 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 $W \times 21$ cm $H \times 12$ cm L and has gray walls and a smooth polyvinyl chlorine (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 min. 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 s 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 min. 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 h. During morning hours between 07.00–12.00 h 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 μL of peppermint oil (Sigma–Aldrich, W284807). In the evening between 13.00–18.00 h all animals received an IP injection of sterile physiological saline, and again returned to their home cage. After 5 min 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 μL 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, USA). 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 T_2-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 μm^2 for functional and 117 μm^2 for anatomical scans. A full set of 14 coronal slices across the brain was collected at each effective repetition time every 7.5 s.

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