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BOLD Signal Response to Cocaine Varies with Sexual Receptivity in Female Rats

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Abstract

Blood oxygen level dependent (BOLD) MRI was used to test whether cocaine-stimulated mesolimbic activity varied with sexual receptivity. Rats were randomly screened for lordotic responses and then imaged for their response to centrally administered cocaine. We observed that females expressing no lordosis showed a greater activation of mesolimbic and nigrostriatal structures than lordotic females. Our data suggest that the differential sensitivity to cocaine occurs not only as a result of hormonal changes of the estrous cycle, but also in association with changes in sexual receptivity.

Keywords

lordosis; sexual receptivity; female rat; cocaine; functional MRI; mesolimbic; nigrostriatal; sexual motivation

Introduction

Drug-seeking behavior and sexual motivation are under similar modulatory control by mesolimbic circuitry. Dopamine neurons within the midbrain ventral tegmental area project to forebrain sites such as the medial prefrontal cortex and nucleus accumbens that control reward anticipatory responses and motor aspects of motivated behavior [1]. Ventral tegmental dopamine neurons are also modulated by incoming projections from the medial preoptic area [2], which play a critical role in maternal and sexual behaviors in the female. There is evidence indicating that copulatory performance but not appetitive aspects of sex is influenced by stimulants. Meyerson (1968) reported that amphetamine treatment, which increases extracellular concentrations of dopamine, inhibits hormonally induced copulatory responses in female rats [3]. Proceptive behaviors are reduced by the dopamine agonist apomorphine, but not by amphetamine [4]. Paced mating behavior in ovariectomized hormone-primed rats is affected by amphetamine treatment [5]. Therefore, stimulant administration influences female sexual behavior. It remains to be tested whether differences in responsiveness of mesolimbic areas to cocaine and amphetamine are associated with changes in sexual receptivity. There is a wealth of data indicating that sex steroids, which vary throughout the estrous cycle of the female and can modulate proceptive and consummatory sexual behaviors, also modulate the behavioral and neurochemical responses to cocaine and amphetamine [6,7]. We used functional magnetic resonance imaging in

awake female rats [8] to test whether the neural response to cocaine varies the expression of lordosis. Lordotic behavior is characterized by a downward arching of the back and lifting of the tail in response to male rat mounts [9]. It was hypothesized that non-lordotic and lordotic females would show differential sensitivity to the stimulant actions of cocaine on the BOLD signal[8].

Methods

Sixteen Long Evans female rats (225-300 g) purchased from Charles River Laboratories (Wilmington, MA) were housed in pairs in a temperature and humidity controlled room and maintained on a 12 hour light-dark cycle (lights off at 19:00 hours). Water and Purina rat chow were provided ad libitum. All procedures adhere to office of laboratory animal welfare and Association for Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee.

For imaging studies, cocaine HCl (Sigma-Aldrich, St. Louis, MO) was injected intracerebroventricularly at a 20 μ g concentration dissolved in 10 μ L artificial cerebrospinal fluid (containing in mM: 145 NaCl, 2.7 KCl, 10 MgCl₂, 1.2 CaCl₂, 20 Na₂HPO₄•7H₂O, pH 7.4)[10]. Rats were randomly screened on mornings from 08:00 – 12:00 hrs for lordosis behavior [9] and were assigned to non-lordotic (n = 8) and lordotic groups (n = 8). During testing, two fingers were gently glided along the lower dorsal surface and rump of females. A reflexive downward arching of the back and lifting of the tail was observed in females that were lordotic. All imaging experiments were done in awake, unanesthetized rats [10]. Functional MRI experiments were carried out using a multi-concentric dual-coil small animal restrainer (Insight NeuroImaging Systems, Worcester, MA). Rats were acclimated to the restrainer and the imaging protocol a week before imaging [11]. Magnetic resonance imaging was conducted in a Bruker Biospec 4.7-T/40-cm horizontal magnet (Oxford Instrument, Oxford, U.K.). A high-resolution anatomical scan was collected for 6 minutes using a fast spin echo pulse sequence (field of view = 30 mm, 12 slices, 1.2 mm slice thickness, 256² data matrix for an in-plane resolution of 117 μ m²). Functional imaging was performed with a T₂-weighted multi-slice fast spin echo pulse sequence (effective echo time = 53.3 msec, repetition time = 1430 msec, 64² data matrix, echo train length = 16, echo spacing = 7 msec). A single acquisition of all 12 slices took 6 sec. A total of 100 brain images were collected for 10 minutes. Cocaine was administered following the initial 50 repetitions (5 minutes), which corresponded to the pre-cocaine baseline acquisition. Random-effects analyses using a fully segmented, 3-dimensional rat MRI atlas was used for statistical comparisons between treatment groups. Once each animal was fully registered and segmented in the atlas, the statistical responses for each was averaged on a voxel-by-voxel bases comparing control (32 scan repetitions) to experimental (32 scan repetitions) time periods. For each voxel (4,800 in number) in the brain a significance test and false discovery test were applied (independent of other voxels) to deem them as activated. Once these voxels for each animal were identified they were assigned to different regions of interest based on atlas information. The mean volume of activation (number of voxels occupying a given region) between treatment groups was statistically analyzed using a Mann-Whitney U-test (significance at p < 0.05).

Results

The number of voxels of BOLD activation (red) and deactivation (blue) in the mesolimbic and nigrostriatal areas for lordotic and non- lordotic females exposed to cocaine can be viewed as 3-dimensional models (Figure 1A). The maps are a composite of six subjects each. There is greater number of activated voxels in the non-receptive females in response to cocaine than in receptive rats. Conversely, the opposite was observed for the number of

deactivated (blue) voxels, particularly in the area of the ventral tegmental area. Receptive rats showed a greater deactivated volume than non-receptive rats (results of the statistical analysis are summarized in Figure legend 1).

Figure 1B shows a significant higher number of activated voxels in non-lordotic females in the nucleus accumbens, ventral pallidum, substantia nigra, and dorsal striatum. In contrast, these same areas plus the ventral tegmental area showed a significant higher number of deactivated voxels in sexually receptive females (Mann-Whitney U-Test, $p < 0.05$). Time course data showing the composite percent change in BOLD signal intensity for the mesolimbic dopaminergic system is shown in Figure 2. A repeated measures 2-way ANOVA comparing positive BOLD signal changes from the time of cocaine administration showed a significant main effect for condition ($F_{(1,79)} = 10.52$; $p < 0.01$) and time ($F_{(49,3920)} = 2.21$; $p < 0.001$). Non-lordotic females showed a greater change in BOLD signal intensity over time as compared to sexually receptive females. In contrast, receptive females showed a greater reduction in BOLD signal intensity following cocaine administration as compared to non-lordotic females. There was a significant main effect for negative BOLD for condition ($F_{(1,79)} = 10.37$; $p < 0.01$) and time ($F_{(49,3920)} = 3.66$ ($p < 0.001$)).

Discussion

Centrally administered cocaine resulted in increased BOLD activity in dopamine-enriched regions of the mesolimbic, mesocortical and nigrostriatal regions in all females. However, the BOLD activation varied with the expression of lordosis. Females that displayed lordotic responses also showed a lower response to cocaine than females that did not express this behavior. It is interesting to note that while non-lordotic females showed a significantly greater positive BOLD activity (signal increase) in response to cocaine, lordotic females showed more signal decreases or negative signal changes. This might reflect underlying differences in the neurochemical actions of cocaine; however, this remains to be further tested.

Similar to variations in sexual motivation, the locomotor stimulant actions of cocaine vary throughout the estrous cycle [12]. Females in the proestrous stage show greater locomotor responses to cocaine than those in diestrous II [12]. Intravenous self-administration of cocaine also varies throughout the estrous cycle and according to pregnancy status [13]. Ovariectomized rats treated with estrogen show a higher number of infusions of cocaine per hour than males and ovariectomized rats without estrogen treatment [14]. Increases in estrogen and progesterone levels in the bloodstream 24-48 hrs prior to late proestrous, morning of estrous, initiates a cascade of molecular events that adjusts the responsiveness of local neural networks to the transitory goal of sexual reproduction. It is perhaps the changes in these gonadal steroids that mediate the behavioral changes leading to differential responsiveness to cocaine observed in the present study. There is an impressive body of literature on the biological actions of estrogen, progesterone and androgenic steroids within the mesolimbic and associated reward and motor circuitry. In the specific case of estrogen, it has been shown to enhance amphetamine stimulated dopamine release in the dorsal striatum [7]. Estrogen has also been demonstrated to alter striatal dopamine transport [15] and alter gene expression for dopamine and serotonin receptor subtypes across several limbic brain regions in the rat [16]. Previous work by Febo et al (2003) indicates that cocaine induced changes in dopamine D₂ associated G-protein activation was dependent on estrogen treatment [6]. Estrogen receptors have been identified in major sources of dopamine within the central nervous system of the rat, the ventral tegmental area and substantia nigra [17] while additional direct effects of estrogen have been observed in the nucleus accumbens [18] and medial prefrontal cortex [19]. It is important to note a few important pitfalls of the study. First, steroid hormones were not assayed here but were assumed to vary according

previous literature. Second, the extent of lordosis was not monitored or used as a grouping factor. Only two extremes were used, non-lordotic and lordosis. There should also be caution taken in interpreting changes in BOLD using fMRI methods as purely neuronal in origin since the signal itself relies on cerebrovascular changes partly related to localized changes in blood flow rates. There is evidence of direct effects of estrogen treatment on cerebral blood flow that should be taken into consideration when interpreting MRI results that implicate hormonal changes[20]. Recent work in male rats and findings in clinical research support an interaction between sexual motivation and the effects of stimulant drugs. Recent work [21] shows that prefrontal cell firing in male rats is directly associated with approaching a sexually receptive female. Cocaine appears to increase activity of neurons of the prefrontal cortex during approach responses. A recent clinical study noted that a single intravenous dose of the stimulant drug methylphenidate increased ratings of sexual desire in control and cocaine-abusing subjects, strongly suggesting an association between sexual desire and stimulant abuse [22].

Conclusion

A novel finding of the present experiment is that females that are receptive and show greater levels lordosis, also show reduced positive BOLD signal response to cocaine. On the other hand, females that were negative for lordosis showed a greater BOLD activation in response to the stimulant drug.

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Figure 1.

A) Three-dimensional composite brain volumes showing areas of increase (red) and decrease (blue) BOLD activity in response to cocaine. Shown are the mesocorticolimbic and nigrostriatal dopaminergic systems of non-receptive and sexually receptive females. B) Number of voxels showing increased (red) or decreased (blue) BOLD activity in response to cocaine. Data are presented as median with ranges in parenthesis. * $p < 0.05$, ** $p < 0.01$.

Figure 2. Changes in BOLD activity over time in response to cocaine. Data are presented as percent change from baseline (mean $\pm$ SEM).