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Sex differences in the effects of estradiol in the nucleus accumbens and striatum on the response to cocaine: Neurochemistry and behavior

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ABSTRACT

Background: Females exhibit more rapid escalation of cocaine use and enhanced cocaine-taking behavior as compared to males. While ovarian hormones likely play a role in this increased vulnerability, research has yet to examine the role of estradiol in affecting the behavioral and neurological response to cocaine in a brain region- and sex-specific way.
Methods: First, we examined stereotypy and locomotor sensitization after repeated cocaine administration (10 mg/kg i.p.) in intact (SHAM) and castrated (CAST) males, and ovariectomized (OVX) females treated with 5 μg estradiol benzoate (EB) or vehicle (OIL). Next, we used in vivo microdialysis to examine the effects of acute EB treatment on cocaine-induced DA in the regions mediating the display of these behaviors (i.e., the dorsolateral striatum, DLS; and the nucleus accumbens, NAc; respectively).
Results: We find that EB enhances sensitization of cocaine-induced stereotypy in OVX females after 12 days of cocaine treatment, and after a 10-day withdrawal. Similarly, the OVX/EB females show enhanced locomotor sensitization compared to the other three groups on the same days. Using in vivo microdialysis to assess the neurochemical response, we find that EB rapidly enhances cocaine-induced DA in DLS dialysate of OVX females but not CAST males, and has no effect in NAc of either sex.
Conclusions: With these experiments, we show that there are sex differences in the effects of estradiol to preferentially enhance the response to cocaine in the DLS over the NAc in females, which may contribute to the preferential sensitization of stereotypy in females.

1. Introduction

A variety of factors contribute to an individual's risk for developing drug addiction or compulsive drug taking in animal models. High impulsivity or novelty-seeking predisposes an individual toward an addictive phenotype, whereas individuals demonstrating low impulsivity and novelty-seeking are at reduced risk for developing addiction (Belin et al., 2008; Cummings et al., 2011). There are also striking sex differences in drug taking: women escalate through the stages of addiction more rapidly (Kosten et al., 1993), have shorter periods of abstinence (Lynch et al., 2002), and are more responsive to cocaine cues as compared to men (Robbins et al., 1999). This sex difference is also widely noted in laboratory rodents, with females acquiring drug taking behavior more rapidly and demonstrating an enhanced motivation for cocaine compared to males (Hu et al., 2004; Jackson et al., 2006; Larson et al., 2005; Roberts et al., 1989; Zhao and Becker, 2010).

In both clinical and preclinical settings, gonadal hormones have been found to play a key role in this sexual dimorphism. Women report an increased "high" with smoked cocaine during the follicular phase of the menstrual cycle when estradiol is elevated (Sofuoglu et al., 1999), and female rats work harder to obtain an infusion of cocaine and acquire cocaine-taking more rapidly when estradiol is high (Becker and Hu, 2008; Hu et al., 2004; Roberts et al., 1989). Together, these data demonstrate the importance of estradiol in the development of drug dependence and compulsive drug taking in females.

The nucleus accumbens (NAc) and dorsolateral striatum (DLS) are thought to be crucial sites in the transition from drug taking to compulsive drug seeking, where the NAc is important for acquisition and escalation, and the DLS is necessary for compulsive or habitual drug taking (Belin-Rauscent et al., 2012; Willuhn

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et al., 2012). When drug intake transitions from casual use to compulsive drug abuse, DA transmission becomes enhanced in the DLS and reduced in the NAc (Willuhn et al., 2012). Importantly, ovarian hormones can modulate the neurochemical response to psychomotor stimulants by enhancing DA neurotransmission in reward-associated brain regions. Pretreatment of ovariectomized (OVX) female rats with estradiol 30 min prior to the psychostimulant amphetamine enhances DA in dialysate of DLS (Becker, 1990; Castner et al., 1993), and administration of cocaine to intact (i.e., cycling) females results in increased DA release and reduced DA clearance in dorsal striatum compared to OVX females (Walker et al., 2012). While it is clear from these data that ovarian hormones enhance psychostimulant-induced DA neurotransmission in DLS, it has yet to be determined whether it is estradiol alone that induces rapid changes in cocaine-induced DA in DLS, or how estradiol affects DA neurotransmission in the NAc—a phenomenon that we hypothesize is integral for expression of sex differences in the progression from drug use to abuse.

Cocaine sensitization is the escalation in behavioral response exhibited by an animal after repeated exposures to the same dose of the drug (Robinson and Becker, 1986), and is thought to be indicative of cocaine-induced neural changes that are significant in the development of drug abuse, addiction, and craving (Robinson and Berridge, 1993). The behaviors that are expressed during psychostimulant-induced sensitization (e.g., rotational behavior, locomotor hyperactivity, and stereotyped behavior) tap into the sensitization of DA neurotransmission in different regions of the mesotelencephalic dopaminergic circuitry; while dopaminergic activity in NAc and DLS drive locomotor behavior (Delfs et al., 1990), it is DA action in the DLS that is primarily responsible for the expression of psychostimulant-induced stereotypy (Dickson et al., 1994; Perrot et al., 2009). The influence of ovarian hormones on various aspects of psychomotor-induced sensitization has been studied (Hu and Becker, 2003; Sell et al., 2000; Walker et al., 2012), but again the role of estradiol in modulating these DLS- and NAc-driven behaviors in the context of cocaine-sensitization had not been studied in a sex-specific way.

We hypothesize that a female's vulnerability for drug addiction is enhanced due in part to estradiol's selective action in the female DLS, which can rapidly enhance DA neurotransmission and facilitate the transition from recreational to compulsive drug use by altering behavioral outcomes. To this end, we separately examined the two facets of behavioral sensitization (i.e., locomotor and stereotyped behavior) that are modulated primarily by the NAc and DLS respectively, and found that estradiol's preferential enhancement of DLS DA is reflected in enhanced cocaine-induced stereotyped behavior as opposed to locomotor behavior in females. To determine if EB has selective, sex-specific effects in the DLS that may contribute to these changes in behavior, we used in vivo microdialysis in gonadectomized male and female rats to demonstrate that acute EB selectively affects the cocaine-induced increase in dialysate DA from the DLS, but not the NAc, in female but not male rats.

2. Methods

2.1. Animals

Male and female Sprague Dawley rats (Charles River Breeding Laboratory; Portage, MI) 50–55 days of age were maintained on a 14:10 L:D cycle (lights on at 8:00 AM), housed in same-sex pairs in standard laboratory cages, and allowed free access to water and phytoestrogen free rat chow (2014 Teklad Global, 14% protein rodent maintenance diet, Harlan rat chow; Harlan Teklad, Madison, WI). All procedures were carried out in accordance with the National Institutes of Health guidelines on laboratory animal use and care, using a protocol approved by the University Committee on Use and Care of Animals.

2.2. Experiment 1: cocaine sensitization

Fifty-three females were ovariectomized (OVX) and 57 males were either castrated (CAST) or received a sham (SHAM) operation under 2% isoflurane anesthesia. Briefly, OVX was performed via a single dorsal incision along the midline below the ribs, where the ovary from each side is externalized and then removed. For CAST, the testes are removed via a ventral approach in which the scrotal sac is opened and the testes are visualized and removed. Wounds are closed via 11 mm wound clips, and animals are given one week to recover. Additional details regarding surgeries are published in Hu and Becker (2003).

On test days, animals were placed in testing chambers (heavy circular plastic tubs (20 cm $W \times 42$ cm $L \times 21$ cm H) where all testing was videotaped. After a 30 min habituation they received either 5 μg estradiol benzoate ((EB), Sigma Aldrich, MO, in 0.1 ml peanut oil) or 0.1 ml peanut oil only (OIL), followed 30 min later by either cocaine (10 mg/kg, i.p., in 0.9% sterile saline) or saline vehicle. Testing concluded 1 h after cocaine/saline administration. Cocaine exposure and EB/OIL treatment continued 4 days a week for 3 consecutive weeks for a total of 12 days followed by a 10 day withdrawal from drug and hormone treatments. After withdrawal, animals were placed in the testing chambers for Challenge Day, when all animals (cocaine and saline) received OIL, and 30 min later, cocaine (10 mg/kg, i.p.).

Behavior was scored from the videotapes that were analyzed for individual stereotyped movements and horizontal locomotion (the cage was divided into quadrants on the video screen; when the animal's hindquarters crossed the line a quadrant crossing was scored). Tapes were scored for 60 min on Day 1, Day 12, and Challenge Day. Locomotor behavior (i.e., number of quadrants crossings) and stereotypy (i.e., total number of stereotyped headbobs and forelimb movements/h) were analyzed by observers blind to animal treatments. All testing was conducted between 12:00 and 3:00 PM (during the light phase).

Previous experiments have not found an effect of acute EB on locomotor behavior and stereotypy in castrated males (Becker et al., 2001; Castner et al., 1993), and no difference between CAST and intact males on cocaine-induced rotational behavior (Hu and Becker, 2003) so we did not include a CAST group treated with acute EB. Instead, we chose to include intact males that received a sham operation in addition to the CAST group. All males received OIL. The final group assignments and numbers were: OVX/EB coc ($n = 18$), OVX/OIL coc ($n = 15$), CAST/OIL coc ($n = 17$), SHAM/OIL coc ($n = 19$), OVX/EB sal ($n = 10$), OVX/OIL sal ($n = 10$), CAST/OIL sal ($n = 11$), SHAM/OIL sal ($n = 10$).

2.3. Experiment 2: in vivo microdialysis

Twenty females were OVX and 20 males were CAST as described above. Two weeks later animals were anesthetized using ketamine (40 mg/kg i.p.; Henry Schein Animal Health, Dublin, OH) and dexmedetomidine (0.3 mg/kg i.p.; Henry Schein Animal Health, Dublin, OH) and guide cannulae (CMA/Microdialysis AB, Chelmsford, MA) were implanted bilaterally: one was aimed at NAc (8 mm length; coordinates from bregma: 1.8 AP ±1.4 ML, from bottom of skull: −1.0 DV), and one was aimed at DLS (5 mm length; 0.2 AP,±3.0 ML, −1.2 DV) on the contralateral side. The cannulae were fixed in place using skull screws and dental cement, and a stylet was inserted to maintain patency. Dialysis probes (CMA/11, 2 mm and 4 mm; CMA/Microdialysis AB, Chelmsford, MA) were tested for in vitro recovery <1 week prior to the experiment, as described previously (Becker and Rudick, 1999). Only probes with greater than 10% (for 2 mm probes) and 20% (for 4 mm probes) recovery were used.

Eighteen hour prior to testing, probes were inserted into the guide cannulae as described previously (Jenkins and Becker, 2003). Samples were collected every 10 min. After three baseline samples, animals received an s.c. injection of either 5 μg EB or OIL. Collection continued for 30 min, after which animals received an i.p. injection of 10 mg/kg cocaine in 0.9% saline. Sample collection continued for an additional 90 min. DA in dialysate was determined by HPLC-EC (Becker and Rudick, 1999).

2.4. Histology

After microdialysis, animals received an overdose of FatalPlus (Vortech Pharmaceuticals, Dearborn, MI) and were decapitated. Brains were fixed in 4% paraformaldehyde, sectioned at 60 μm, and stained with cresyl violet to determine probe placement. Data from animals with probe placements outside the NAc or DLS were excluded. Probes were considered in the DLS if they were >2.5 mm lateral from midline. Data from the striatum of one animal was excluded for having a probe too medial. Due to the size and placement of the NAc probes, measurements from NAc included readings from the core and shell simultaneously. Data were excluded for four animals because of a leak in the probe or trouble with the equipment on the day of testing. The final numbers in each group were as follows, for DLS: CAST/EB, $n = 8$; CAST/OIL, $n = 8$; OVX/EB, $n = 7$; OVX/OIL, $n = 7$; and for NAc: CAST/EB, $n = 9$; CAST/OIL, $n = 7$; OVX/EB, $n = 7$; OVX/OIL, $n = 7$.

2.5. Statistics

Statistical analyses were performed using IBM SPSS Statistics 19. Data were analyzed using repeated measures ANOVA. *Post hoc* tests with Bonferroni correction were performed when significant main effects were present.