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Estradiol facilitation of cocaine-induced locomotor sensitization in female rats requires activation of mGluR5

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Abstract

In comparison to men, women exhibit enhanced responsiveness to the stimulating and addictive properties of cocaine. A growing body of evidence implicates the steroid hormone estradiol in mediating this sex difference, yet the mechanisms underlying estradiol enhancement of behavioral responses to cocaine in females are not known. Recently, we have found that estrogen receptor alpha (ER α) functionally couples with the metabotropic glutamate receptor 5 (mGluR5) to mediate the effects of estradiol on both cellular activation as well as dendritic spine plasticity in brain regions involved in cocaine-induced behavioral sensitization. Thus, we sought to determine whether mGluR5 activation is required for the facilitative effects of estradiol on locomotor responses to cocaine. To test this hypothesis, ovariectomized (OVX) female rats were tested for locomotor activity on the first and fifth days of daily systemic injections of cocaine. For the two days prior to each locomotor test, animals were injected with the mGluR5 antagonist MPEP (or vehicle) and estradiol (or oil). MPEP treatment blocked the facilitative effects of estradiol on cocaine-induced locomotor sensitization, without affecting acute responses to cocaine or the inhibitory actions of estradiol on weight gain. Considered together, these data indicate that mGluR5 activation is critical for the actions of estradiol on cocaine-induced behavioral sensitization.

Keywords

nucleus accumbens; estrogen; drug addiction; behavioral sensitization

Abuse of psychostimulants such as cocaine differs between men and women. Women begin using psychostimulants at an earlier age than men, escalate their use more quickly, and report greater subjective effects of these drugs [1]. Notably, responses to cocaine in women vary across the menstrual cycle with elevated responsiveness during the follicular phase [2],

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highlighting the contribution of estradiol to this differential response to cocaine. Indeed, studies in rodents have demonstrated that sex differences in the behavioral response to cocaine are eliminated following ovariectomy, and are restored following treatment with estradiol [3,4]. Despite such observations, little is known regarding the underlying neural mechanisms that mediate the facilitative effects of estradiol on cocaine-induced behaviors.

There is a growing body of evidence implicating metabotropic glutamate receptors (mGluRs) as an underlying mechanism for many of the neuroanatomical and behavioral effects of estradiol [5]. Recently, we found that estrogen receptor alpha (ER α) is functionally coupled to mGluR5 in striatal medium spiny neurons (MSNs) [6], an interaction that appears to be critical for plasticity within brain areas that regulate behavioral sensitization to cocaine. Similar to several recent studies examining the effects of cocaine [7,8], estradiol decreases dendritic spine density of MSNs in the core subdivision of the nucleus accumbens 24-48 hours after hormone administration [9]. Furthermore, pretreatment with the mGluR5 antagonist MPEP blocks this effect of estradiol [10]. Given that mGluR5 signaling has previously been implicated in the locomotor responses to cocaine [11,12], we hypothesized that in females ER α /mGluR5 signaling is required for estradiol facilitation of cocaine-induced locomotor sensitization.

Ovariectomized Sprague-Dawley rats were purchased from Harlan Laboratories (Madison, WI, USA) at 175-199 g, and pair housed upon arrival in polycarbonate cages with wire mesh tops. Females were maintained on a 12:12 hr light:dark cycle (lights on at 6 am), with all behavior testing occurring between the hours of 9 am and 2 pm. Food and water were available *ad libitum*. Animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (8th Ed.) and approved by the University of Minnesota Institutional Animal Care and Use Committee.

Throughout the experiment, females were injected s.c. with 2 μ g of estradiol (17 β -estradiol; Sigma, St. Louis, MO, USA) in 0.1 ml cottonseed oil (or cottonseed oil alone), on a two days on, two days off schedule (Figure 1). Females were also injected i.p. with 1 mg/kg/ml MPEP (2-methyl-6-(phenylethynyl)pyridine hydrochloride; Abcam, Cambridge, MA, USA) in saline (or saline vehicle alone) 30 min prior to each hormone injection. We found previously that this dose of MPEP blocks estradiol-induced changes in dendritic spines within the nucleus accumbens core when measured 24 hrs later, but has no effect on spine density in the absence of estradiol treatment [10]. Beginning on the third day of the experiment (the day immediately following the second day of hormone injections), females were injected i.p. for five consecutive days with 15 mg/kg/ml of cocaine (cocaine hydrochloride; Sigma) in saline. This pattern of cocaine injections was chosen because it induces modest (if any) sensitization in untreated OVX female rats [1,4], thereby allowing us to more clearly identify the facilitative effect of estradiol on sensitization as well as any potential blockade of that effect following MPEP treatment.

Locomotor activity was assessed in clear, polycarbonate open field chambers (47.5 $\times$ 25.5 $\times$ 20.5 cm) containing corncob bedding. Females were initially placed into a chamber for 30 min (Habituation session), injected with cocaine, and then immediately returned to that chamber for 60 min (Test session). Each chamber was situated within a sensing frame

(Kinder Scientific, Poway, CA, USA) that generated an X-Y grid of photobeams within the chamber. Data from the sensing frames were transmitted to a computer running Motor Monitor software (Kinder Scientific). This software further discriminated beam breaks into ambulations and fine movements. Ambulations were defined as a change of the animal's entire body position on the X-Y grid. Fine movements were defined as all beam breaks that did not meet the criterion for an ambulation. Fine movements therefore comprised a range of behaviors, including grooming and sniffing. The number of rears (elevation of the animal onto its hind paws with fore paws placed upon the wall of the chamber) was also quantified, by a researcher blind to the experimental condition of the animal. At the conclusion of the Test session, females were returned to their home cages.

All data were analyzed using SPSS for Macintosh, version 20.0 (IBM Corp, Armonk, NY, USA). Data were first examined to determine if the assumptions of parametric statistical tests were met. For all statistical tests, results were considered to be significant if $p < .05$. Body weight was subjected to a mixed-design factorial ANOVA, with time (initial weight, weight at first cocaine test, and weight at fifth cocaine test) as a repeated factor, and drug (MPEP or vehicle) and hormone (estradiol or oil) as independent factors. Significant time x hormone interactions were further examined for the effect of time within each hormone treatment group using paired-samples t -tests (error term derived from interaction analysis), with Bonferroni correction for multiple comparisons. The number of ambulations, fine movements, and rears during the first and fifth Habituation and Test sessions were also examined via factorial ANOVAs, and significant time x drug x hormone interactions were further examined for the effect of time within each treatment group using paired-samples t -tests (error term derived from interaction analysis).

MPEP injections did not significantly affect locomotor responses during Habituation sessions. Females performed fewer ambulations ($F(1,45) = 90.61, p = .000$), fine movements ($F(1,46) = 60.35, p = .000$), and rears ($F(1,43) = 45.63, p = .000$) in the fifth vs. the first session (data not shown). However, there were no significant effects of drug, hormone, or drug x hormone interactions.

MPEP treatment blocked the estradiol facilitation of sensitized ambulatory activity following repeated cocaine injections. The effects of estradiol treatment and test session on ambulatory activity differed across drug treatment groups, as evidenced by a significant time x drug x hormone treatment interaction ($F(1,43) = 4.49, p = .040$) (Figure 2). Specifically, females treated with estradiol + vehicle had significantly higher ambulations in the fifth vs. first Test sessions ($t(11) = -2.61, p = .024$), an effect that was not observed in any other treatment group. There were no significant effects of drug, hormone, or significant drug x hormone interaction on the initial ambulatory responses to cocaine.

Treatment with MPEP did not significantly affect measures of non-ambulatory locomotor activity. During the Test sessions, females exhibited more fine movements during the fifth vs. the first session ($F(1,44) = 12.08, p = .001$) and more fine movements in response to estradiol compared to oil treatment ($F(1,44) = 5.03, p = .030$) (Figure 3A). These effects did not significantly differ as a function of MPEP treatment. There were also no significant

effects of time, drug, or hormone treatment on rearing during the Test sessions, or significant interaction of these factors (Figure 3B).

Treatment with estradiol significantly attenuated weight increases in OVX females. The effect of time on weight gain differed depending on hormone treatment group ($F(2,90) = 107.03, p = .000$), but did not significantly differ across drug treatment groups or across both drug and hormone treatments. Weight increased significantly from the start of the experiment to the first session ($t(24) = -6.15, p = .000$), and from the first to the fifth session ($t(24) = -12.65, p = .000$), in oil-treated females irrespective of drug condition (Bonferroni correction for multiple comparisons). This pattern of weight increase with time was not observed in estradiol-treated females.

Our findings demonstrate that estradiol facilitation of cocaine-induced locomotor sensitization depends critically on activation of the metabotropic glutamate receptor subtype mGluR5. This effect was specific to ambulatory responses, since injections of the mGluR5 antagonist MPEP did not block the effects of estradiol treatment on other motor responses or on weight gain. In light of previous work implicating mGluR5 in mediating estradiol-dependent neural plasticity within the nucleus accumbens core (NAcC), our data identify a novel mechanism whereby estradiol can potentiate cocaine-induced locomotor sensitization in females, as well as a potential therapeutic target for treating psychostimulant addiction in women.

The effects of MPEP on estradiol facilitation of locomotor responses were specific to sensitized, rather than acute, responses to cocaine. Furthermore, MPEP treatment alone did not significantly alter ambulatory responses. This is likely due to the timing and dose of our drug injections. We chose to administer 1 mg/kg MPEP only on the two days before each locomotor test, given previous studies demonstrating that higher doses inhibit the acute cocaine-induced locomotor responses when administered just prior to testing [11,12]. This highlights one of the advantages of the present work. Using a lower dose and shifting the timing of injections allowed the effects of MPEP to be more directly attributed to blocking the downstream effects of estradiol, a critical point given the focus of the present work on elucidating the mechanisms underlying estradiol effects on locomotor sensitization. But consequently, the role of mGluR5 signaling independent of estradiol remains to be more clearly established within our model (e.g., the role of mGluR5 agonism on structural plasticity and locomotor sensitization in females).

In addition to the locomotor-facilitating effects of estradiol, we also observed an inhibition of weight gain in estradiol-treated females that is consistent with previous work [13]. Therefore, one could posit that the observed effects of MPEP represent indirect actions on estradiol-dependent physiological responses (e.g., food intake and/or metabolism). This seems unlikely for two reasons. First, weight gain was inhibited in estradiol-treated females that received either MPEP or vehicle. Second, the degree of locomotor sensitization did not correlate with changes in weight, regardless of hormone or drug treatment condition (data not shown). These results suggest that MPEP acted to specifically inhibit the locomotor-enhancing effects of estradiol, without broadly disrupting physiological processes related to locomotion.

Our findings implicate mGluR5 activation in the signaling pathway whereby estradiol ultimately facilitates cocaine-induced locomotion. This may occur via an estrogen receptor alpha (ER α)-dependent mechanism. In OVX female mice, activation of ER α through systemic administration of PPT increases ambulations and stereotypy scores compared to untreated females, an effect that is not mimicked by administration of DPN, an estrogen receptor beta agonist [14]. Furthermore, treatment with PPT, but not DPN, in OVX females increased responding on the inactive lever (an indication of general locomotor activation) during reinstatement of cocaine self administration [15].

Our group has previously shown that in the female striatum, ER α can directly transactivate mGluR5 [6]. The present data are consistent with this model. Interestingly, activation of group I mGluR signaling by estradiol has both immediate and long-term consequences within the nervous system. Insight can be drawn from studies examining the neural regulation of female sexual receptivity. Within this model, stimulation of membrane estrogen receptors in the hypothalamus is required to elicit lordosis behavior days later [16]. Initial actions of ER α /mGluR1a signaling result in the rapid (within 30 min) activity-induced internalization of μ -opioid receptors in the medial preoptic nucleus [17], followed by a slower (within 4 hrs) but lasting increase in dendritic spine density in the arcuate nucleus [18]. This parallels what is seen within the striatum. Specifically, stimulation of estrogen receptors [19] or group 1 mGluRs [20] rapidly induce striatal dopamine release, via disinhibition of local dopaminergic terminals [21]. These pathways appear integrated, as estradiol facilitation of dopamine release via inhibition of GABAergic signaling is dependent on mGluR5 [22]. Hours later, this same signaling pathway results in the reduction in dendritic spine density within the NAcC [9,10]. Given that estradiol can potentiate cocaine-induced locomotor sensitization when administered repeatedly beginning 30 min [3] or several days prior to the first cocaine injection [4,23,24], stimulation of the ER α /mGluR5 signaling pathway likely results in an coordinated sequence of acute and longer-lasting effects within the NAcC, which may ultimately lead to the behavioral outcomes observed here.

A substantial body of evidence supports a role for the NAcC in the behavioral and neuroanatomical responses to cocaine. Lesions of the NAcC increase novelty- and cocaine-induced locomotor responses in male rats [25], whereas lesions of the nucleus accumbens shell have no effect. Structural plasticity within the NAcC as well as changes in excitability [26] may be critical for mediating the sensitizing effects of cocaine on locomotor activity. Initial studies suggested an increase in dendritic spine density within the NAcC following chronic cocaine [e.g., 27,28]. However, more recent studies using advanced staining techniques and 3-D imaging, thought to more accurately capture neuroanatomical structure [29], have observed a decrease in spine density within this brain region following cocaine administration [7,8], similar to the acute effects of estradiol. The cooperative properties of estradiol and cocaine regulating neuroplasticity within the NAcC may therefore provide the basis for females exhibiting heightened responsiveness to psychostimulants.

Considered in light of previous research, our data suggest that estradiol may act via an mGluR5-dependent mechanism to potentiate the neuroanatomical and locomotor-stimulating effects of cocaine. Despite strong commonalities between the development of behavioral

sensitization and addiction susceptibility [30], the behavioral sensitization model cannot capture the full extent of psychostimulant use/abuse. Further research is required to determine whether the effects of estradiol on psychostimulant self-administration in females are also dependent on interactions between ER α and mGluR5.

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Highlights

- mGluR5 blockade disrupted estradiol-enhancement of locomotor sensitization
- There was no effect on non-ambulatory locomotor responses (e.g., rearing)
- Physiological responses to estradiol were also not disrupted by mGluR5 blockade

Figure 1.

Timeline of experimental manipulations. OVX female rats were injected with MPEP or vehicle (Veh), followed by estradiol (E) or oil, on days 1, 2, 5, and 6 ($n = 12-13$ per group). Cocaine (Coc) was injected on days 3-7. Locomotor activity was assessed on the first and fifth Coc injection days.

Figure 2.

Estradiol enhancement of cocaine-induced ambulations is dependent on mGluR5. In estradiol (E) treated females, ambulations (mean $\pm$ SEM) were higher in response to the fifth cocaine (Coc) vs. first Coc injection (Test 2 vs. Test 1, $*p < .05$ (paired-samples *t*-test)). This hormone effect was not observed when MPEP was administered 30 minutes prior to estradiol. In addition, MPEP alone did not affect Coc-induced ambulations.

Figure 3.

Increase in fine motor movements following estradiol treatment and across test day. (A) Females exhibited more fine movements following the fifth cocaine (Coc) vs. the first Coc injection (Test 2 vs. Test 1), and more fine movements when injected with estradiol (E) vs. oil. (B) There were no differences within/across groups on rearing. $*p < .05$ (ANOVA main effect).

Figure 4.

Estradiol attenuation of weight gain is unaffected by mGluR5 antagonism. In oil-treated OVX females, weight increased across the experimental time course, an effect that was not observed following estradiol (E) treatment. The inhibitory effects of E were not altered by MPEP treatment. $*p < .05$ (paired-samples *t*-tests with Bonferroni correction for multiple comparisons).