Published in final edited form as:
Horm Behav. 2007 August ; 52(2): 146–155.

Evidence for Involvement of ERβ and RGS9-2 in 17-β Estradiol Enhancement of Amphetamine-Induced Place Preference Behavior

Jill L Silverman and **James I Koenig**
Maryland Psychiatric Research Center, Program in Neuroscience, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD 21228

Abstract

Estrogen enhances dopamine-mediated behaviors, which make women and female rats more sensitive to the effects of the psychostimulant drugs, cocaine and amphetamine. How cocaine and amphetamine elicit more robust behavioral responses in females remains unclear, but studies have shown that the Regulator of G-protein Signaling 9-2 (RGS9-2) protein is an important modulator of the behavioral responses to these drugs. Previously, we reported that 17-beta estradiol reduced RGS9-2 mRNA expression in the shell of the nucleus accumbens, but not the core. The present studies were designed to further evaluate the involvement of RGS9-2 in estradiol-enhancement of amphetamine-induced place preference behavior and to examine which estrogen receptor subtype mediates the effect of estradiol. Female Sprague-Dawley rats were ovariectomized and treated for fourteen days with an inert vehicle or 17-beta estradiol (by Silastic implant or injection [80μg/kg]). 17-beta-Estradiol-treated female rats had enhanced amphetamine-induced conditioned place preference behavior compared to vehicle-treated, ovariectomized female rats. In situ hybridization histochemistry and Western blotting identified an inverse relationship between RGS9-2 protein expression in the nucleus accumbens shell and the hormonal enhancement of amphetamine-induced place preference behavior. A similar relationship was not found between place preference behavior and RGS9-2 expression in the accumbens core. Moreover, treatment of ovariectomized female rats with the selective estrogen receptor-beta agonist, diarylpropionitrile (1 mg/kg), for two weeks also facilitated amphetamine-induced place preference behavior and selectively reduced nucleus accumbens shell RGS9-2 protein expression. These data provide insight into a potential mechanism by which estrogen and/or sex modulate mesoaccumbal dopamine receptor signaling and possibly, addictive behaviors.

Keywords

Estrogen; receptor; nucleus accumbens; psychostimulant; RGS9; place preference

Correspondence to: Jill L Silverman, Maryland Psychiatric Research Center, University of Maryland School of Medicine, PO Box 21247, Baltimore, MD 21228, Email: jsharifi@mprc.umaryland.edu, Phone: 410-402-6091, Fax: 410-402-6066.

Introduction

According to the National Institute of Drug Abuse, over nine million women have used illegal drugs in the past year. Previously, there was a large difference between the number of men and women in drug abuse treatment programs but this gap has been narrowing at an alarming rate (http://www.nida.nih.gov/WomenDrugs/Women-DrugAbuse.html). Substance abuse among females has grown rapidly because once females experience illicit drugs, they progress to the abuse state faster than males. During periods of elevated estrogen secretion, psychostimulant drugs generate enhanced euphoria and craving in women (White et al., 2002). Similarly, these drugs have profoundly increased effects in female rodents (for review Becker et al., 2001; Lynch et al., 2002; Carroll et al., 2004). Specifically, female rats display augmented locomotor sensitization to amphetamine (AMPH) (Robinson, 1984) and cocaine (van Haaren and Meyer, 1991) compared to males. 17β-estradiol (E2) appears to have mediated the more powerful effects of these drugs (Becker, 1999). Additionally, E2-treated ovariectomized (OVX) female rats became proficient at cocaine self-administration more rapidly than OVX female and male rats (Lynch and Carroll, 1999; Hu et al., 2004). Conditioned place preference (CPP) studies also suggested that female rats developed associations between contextual stimuli and the rewarding effects of cocaine at lower doses and with shorter latencies than male rats (Russo et al., 2003a). Female gonadal hormones appear to be responsible for this enhanced preference (Russo et al., 2003b; Russo et al., 2003a).

Given the hormonal influence on dependence patterns, an investigation of E2 and progesterone (P) effects on the neural substrates of reward behaviors, such as the mesolimbic dopamine (DA) system, is critical. Early anatomical experiments identified E2-concentrating cells in the limbic system (Pfaff and Keiner, 1973). Presynaptically, E2 potentiated psychostimulant-induced DA release in the nigrostriatal system (Becker et al., 1984) and enhanced DA turnover in the striatum and nucleus accumbens (NAc) (Di Paolo et al., 1985; Thompson and Moss, 1994). While DA release appears to be facilitated by E2, there is controversy about the downstream actions of E2 on DA receptor expression, re-uptake, and post-synaptic signaling suggesting that the nature of the E2 effect on DA-mediated behavior is unresolved. On the molecular level, E2 effects on gene transcription can be mediated by either estrogen receptor (ER)α or ERβ. These members of the steroid hormone receptor superfamily have distinctive neuroanatomical distribution patterns that probably underlie the differential behavioral effects of E2 (Ogawa et al., 1998; Ogawa et al., 1999; Lund et al., 2005; Walf and Frye, 2005). The localization of ERβ mRNA and immunoreactivity (Shughrue et al., 1997; Shughrue and Merchenthaler, 2001) in the ventral striatum and the ventral tegmental area (VTA) (Creutz and Kritzer, 2004) supports the hypothesis of ERβ-mediated augmentation of DA function and subsequent facilitation of enhanced reward behaviors in female rats.

Anatomically, VTA projection neurons terminate in the NAc. The NAc shell has been linked with emotional and motivational functions (Sellings and Clarke, 2003) while the NAc core appears to be involved in movement-related behaviors (Maldonado-Irizarry and Kelley, 1995; Sellings and Clarke, 2003). Interestingly, rats self-administered AMPH if it was injected into the medial shell but not core (Ikemoto et al., 2005). The NAc shell also is involved in naturally rewarding situations, such as pup retrieval in rats and mice, and pair bond formation in voles. These conclusions were based on the selective induction of the immediate early gene, FOS, in the NAc shell during these behaviors (Li and Fleming, 2003; Aragona et al., 2006). The VTA neurons projecting to the NAc elicit effects by releasing DA onto either D1 or D2 receptors, which are highly expressed in the NAc. D1-like receptors (D1, D5) are G-protein coupled receptors (GPCR) that stimulate adenylate cyclase and subsequently, the cAMP response element binding protein (CREB) (Nestler and Carlezon, 2006). On the other hand, D2-like receptors (D2, D3, and D4) are coupled to inhibitory G-proteins, Gαi/Gαo, that inactivate adenylate cyclase and decrease CREB (Kebabian and Calne, 1979). D2-like

receptors are important mediators of processes associated with psychostimulant abuse (Wise et al., 1990; Britton et al., 1991; Self et al., 1996).

One of the specific intracellular regulators of DA D2 receptor activation is a protein known as Regulator of G-protein Signaling9-2 (RGS9-2) (Rahman et al., 2003; Kovoor et al., 2005). It has been shown that RGS proteins modulate the function of GPCRs by acting as GTPase-activating proteins (GAPs) (Dohlman and Thorner, 1997; Berman and Gilman, 1998). GAPs increase the rate of GTP hydrolysis and accelerate the rate of the Gα inactivation. Consequently, RGS proteins actively control the duration of receptor-mediated intracellular signaling by dictating the rate of the GTP-GDP exchange reaction. RGS9-2 is selectively expressed in the NAc and dorsal striatum and co-localizes with the D2 receptor (Gold et al., 1997; Rahman et al., 1999). Acute and chronic psychostimulant administration modifies the expression of RGS9-2 mRNA in the male rat striatum (Burchett et al., 1998). Unfortunately, however, very little work has focused on this potentially important intracellular signaling protein in female rats. Previous work from our laboratory illustrated E2 selectively reduced RGS9-2 mRNA expression in the NAc shell. Interestingly, AMPH failed to alter RGS9-2 mRNA expression in contrast to studies in male rats (Sharifi et al., 2004). The present studies were performed to test the hypothesis that E2-potentiation of AMPH-induced place preference behavior in female rats is mediated by ERβ. Additionally, it is hypothesized that E2-facilitation of psychostimulant behaviors is associated with alterations in RGS9-2 levels. Thus, we examined whether potentiation of CPP was accompanied by a correlative reduction of RGS9-2 protein in the NAc.

Methods

Animals

Female adult Sprague-Dawley rats (175-200g) were purchased from Charles River Laboratories (Kingston, NY). The animals were housed in a temperature-controlled animal facility on a 12h-12h light-dark cycle (lights on from 0700 hr to 1900 hr). Animals had access to food and water *ad libitum*. All animals procedures were approved by University of Maryland School of Medicine Institutional Animal Care and Use Committee and adhered to the NIH Guide for the care and use of laboratory animals (National Institutes of Health Publications, n. 80-23, revised 1978).

Experimental Design

Preliminary testing—Two separate control experiments were performed to evaluate bias in the place preference apparatus. First, twelve adult female rats weighing between 225-250g were anesthetized with chloral hydrate (300 mg/kg; Sigma Aldrich, St Louis, MO) and the ovaries were removed using a ventral approach. On the morning of the seventh day after surgery, the rats were treated with a 0.9% solution of sodium chloride i.p. (1 ml/kg; saline, Fisher Scientific, Pittsburg, PA). Following the injection, the rats were placed on one side of the place preference apparatus (see details below) for thirty minutes. In the afternoon, after a second saline injection, the rats were placed in the opposite chamber of the place preference apparatus for thirty minutes. This procedure was repeated for two additional days. At approximately 1200 hours on the fourth day, rats were placed in the place preference box for twenty minutes and allowed access to both chambers. The session was videotaped and at a later time, the tapes were scored for time spent in both chambers of the apparatus by a trained observer.

In a second experiment, twelve ovary-intact, random-cycle female rats weighing between 225-250g received an injection of 0.9% saline (1 ml/kg, i.p.) at 1000 hrs and were placed in one side of the place preference apparatus for thirty minutes. In the afternoon of the same day, the rats were given a second injection of saline and placed in the other chamber of the place

preference apparatus for thirty minutes. As above, these female rats were treated with saline for two additional days followed by exposure to the place preference apparatus, and on the fourth day their place preference behavior was video recorded. At a later time, the videotapes were scored for time each rat in each of the chambers of the place preference apparatus by a trained observer.

Experiment 1: Effects of Hormonal Milieu on AMPH induced Place Preference in Female Rats: Fifteen adult female rats were surgically OVX under aseptic conditions using a ventral approach following an i.p. injection of chloral hydrate (300 mg/kg). While under anesthesia, ten of the OVX animals received a subcutaneous (s.c.) Silastic implant (5mm in length) filled with crystalline E2 (Steraloids, Inc., Newport, RI) (Bridges, 1984) in order to maintain proestrus levels of circulating E2 (OVX+E2; n=10). Five of the ten E2-replaced female rats were also given a daily s.c. injection of P (2.5 mg/kg; Steraloids, Inc., Newport, RI) dissolved in sesame oil (E2+P; n=5). P replacement at this dose has been routinely used to induce reproductive behaviors in rats (Hiermke et al., 1987; Russell et al., 2005). The remaining OVX female rats (n=5) received an empty s.c. implant and were designated as non-hormone receiving control rats. The OVX+E2 and the OVX females received a daily injection of sesame oil. Eleven days after OVX, the rats received AMPH and saline injections to CPP behaviors. Following three days of conditioning, all rats were tested for AMPH-induced place preference followed immediately by euthanasia to harvest the brains for analysis if RGS9-2 protein levels using Western blotting.

Experiment 2: Effects of Selective Agonists on Place Preference in Female Rats: Thirty-six female rats were anesthetized with chloral hydrate (300 mg/kg, ip) and their ovaries were removed using a ventral approach (OVX). Twenty-four hours after surgery, each rat received a s.c. injections of either E2 (80 μg/kg; Steraloids, Inc., Newport, RI; n=9), the ERα selective receptor agonist, propylpyrazoletriol (PPT; 1 mg/kg; Tocris Cookson, Ellisville, MO; n=9) or the ERβ selective agonist diarylpropionitrile (DPN 1mg/kg; Tocris Cookson, Ellisville, MO; n=9). These compounds were administered daily by a s.c. injection for fourteen days because of their limited availability. E2, DPN and PPT were dissolved in sesame oil. The remaining OVX female rats (n=9) were treated for fourteen days with the sesame oil vehicle. Eleven days after OVX, rats received AMPH and saline injections to condition place preference behaviors. Following three days of conditioning, all rats were tested for AMPH-induced place preference followed immediately by euthanasia to collect brain tissue.

Drug Administration

d-AMPH sulfate (Sigma Aldrich, St Louis, MO) was dissolved in sterile saline and injected i.p. at a dose of 1.0 mg/kg. Animals also received an i.p. injection of sterile saline (0.9%NaCl; Fisher Scientific, Pittsburg, PA).

Conditioned Place Preference

Place preference cages consisted of a rectangular plexiglass box containing two distinct chambers separated by a removable guillotine door. One conditioning chamber (28 cm × 28 cm × 12cm) was black in color and contained horizontal steel rod floor. The other conditioning chamber (28 cm × 28 cm × 12 cm) was black and white striped with a steel mesh floor. The conditioning procedure consisted of three phases and was conducted according to previously published protocols (Russo et al., 2003b). First, rats were pre-conditioned for a five minute period during which the animals had unlimited access to both chambers. The pre-conditioning period provides for acclimation to the CPP apparatus. Second, a three-day series of conditioning sessions were conducted in which rats were randomly assigned to separate conditioning groups of AMPH (1.0mg/kg) or saline. Experimental animals were injected with AMPH each day for three days in either a morning or afternoon session. They were then

immediately placed in one chamber of the CPP apparatus for 30 minutes. Saline was also administered each day and the rats were subsequently confined to the other chamber of the CPP apparatus. Conditioning sessions were conducted over three days and six sessions. Third, on the test day (>12-16 hours after the last training session), animals were allowed access to both chambers of the CPP apparatus for twenty minutes during which a video recording was collected for later analysis. A blind observer analyzed the tapes for the total time spent in each chamber. CPP scores were calculated by subtracting the time spent in the saline-paired chamber from the time spent in the AMPH-paired chamber (Russo et al., 2003a).

Western Blotting

Immediately after behavioral testing, the rats were taken to an adjoining room and sacrified by decapitation. The brains were removed from the skull and rapidly frozen on powdered dry ice. To isolate the NAc and dorsal striatum, the brains were cut into 1-mm coronal sections on a cryostat (Leica, Deerfield, Il) and the NAc shell and core were isolated by micropunches using a 12-gauge needle (Gold et al., 2003). A representative coronal rat brain section illustrating the location of the micropunches (1.45 mm anterior to bregma) is shown in Figure 1 (Swanson, 2004). In Experiment 1, a total of four bilateral micropunches were taken from the NAc shell and core from two adjacent coronal sections. In Experiment 2, each brain was bisected after removal from the rat's skull. One half of the brain was frozen for in situ hybridization histochemical analysis. The other half of the brain was cryosectioned into 1-mm coronal sections and two punches were obtained from adjacent sections for Western blotting of RGS9-2. The brain punches were homogenized by sonication in a buffer containing 50 mM Tris (pH 8.0), 150mM NaCl, 5mM EDTA, 1%SDS, 10μg/mL leupeptin and 10μg/mL pepstatin. The homogenates were briefly sonicated, and centrifuged at 15,000 rpm for 15 minutes. Protein concentration in the supernatant was measured using a Bio-Rad protein assay kit (Bio-Rad, Carson, CA). Brain tissue extracts were diluted in Laemmli sample buffer (Biorad, Carson, CA) to a concentration of 15 μg of protein per lane and resolved by electrophoresis on a 10% polyacrylamide gel (Biorad, Carson, CA). Proteins were electrotransferred from the gel onto PVDF membrane (Bio Rad, Carson, CA) in cold transfer buffer containing 25mM Tris, 192mM glycine. The PVDF membrane was blocked with 5% nonfat dry milk dissolved in 1X Tris buffered saline (TBS) and was then incubated with goat anti-rat RGS9 antiserum (Santa Cruz Biotechnology T-19; 1:1000, Santa Cruz, CA) diluted in TBS/0.1% Tween (TBS-T) with 1% milk and 1% bovine serum albumin (BSA) overnight. The next day the membrane was rinsed 3 times for 10 min with TBS-T in 1 % milk. The sample membrane was then incubated in rabbit anti-goat alkaline phosphatase-conjugated secondary antibody (Chemicon; 1:5000, Temecula,CA), diluted in TBS-T, for one hour and rinsed again in TBS-T. The blot was washed once with TBS and the protein detected using the Biorad Immuno-Star chemiluminescent kit (Biorad, Carson, CA). Integrated densitometeric measurements of the protein bands were made using a Sony CCD camera attached to a Power Macintosh computer running NIH Image 1.62 (Bethesda, MD). Following collection of the RGS9-2 images, blots were stripped and reprobed for beta-actin using a mouse monoclonal antiserum (Chemicon; 1:10000, Temecula, CA). The procedures described above were repeated. Relative RGS9-2 expression levels were normalized using beta-actin staining intensity to ensure equal loading of all lanes.

In Situ Hybridization

In experiment 2, following bisection of the brains and freezing on powdered dry ice, the brains were stored at -70°C. Subsequently, each hemisected brain was cut into twelve-micron coronal sections using a cryostat and mounted on SupraFrost Plus slides (Fisher Scientific, Pittsburg, PA). Tissue fixation and hybridization were conducted according to the previously published protocol (Sharifi et al., 2004). Briefly, tissue sections were fixed with 4% paraformaldehyde, acetylated with acetic anhydride (0.25%) in triethanolamine (0.1M, pH 8), and dehydrated with

chloroform and a series of ethanol rinses. Hybridization buffer containing 1.0×10^6 cpm of ^{35}S-labeled cRNA probe (synthesized by the riboprobe method) was applied. Antisense cRNA probes were generated using DNA templates for rat RGS9-2 that spans nucleotides 437-639 (GenBank Accession U32433). Processed, mounted sections received 25 microliters per section of hybridization buffer (1.0×10^6 cpm). Labeled sense strand probes served as control for background hybridization. Following hybridization (18h at 55°C), slides were rinsed in 4× SSC, incubated with RNase A (20 mg/ml), rinsed under high stringency conditions (0.1× SSC at 68°C), and dehydrated with an ethanol series. The slides were exposed to Kodak BioMax MR film (Eastman Kodak Co., Rochester, NY) for autoradiographic image analysis. Films were developed using GBX developing chemicals (Eastman Kodak Co., Rochester, NY). Autoradiographic images were captured using a Sony CCD video camera interfacing with a Power Macintosh computer via a Quick Time digitizer and Frame Grabber (Data Translation, Marlboro, MA). The data collected from the films were processed using NIH Image 1.62 (Bethesda, MD) to yield relative hybridization values for the regions of interest, expressed as a percentage of control hybridization and statistical analysis performed.

Statistical Analysis

For each experiment, CPP scores obtained for each separate hormone treatment were analyzed using a one-way analysis of variance (ANOVA) with a Tukey's post-hoc analysis. These statistical tests were also employed for RGS9-2 protein measurements, following a beta-actin correction, in which data is presented as the mean value ± standard error of the mean (S.E.M). A p-value less than 0.05 represented a significant difference between groups in all analysis. All analyses were conducted using Graphpad Prism (Graphpad Software Inc., San Diego, CA).

Results

Preliminary Findings

OVX female rats treated with saline spent 413.3 ± 51.74 seconds (mean ± SEM) in the black chamber and 420.5 ± 52.2 seconds in the black and white striped chamber of the place preference apparatus (Student's $t = 0.0975$, $n = 12$, $p > 0.05$). Saline-treated, ovary-intact, random-cycle female rats spent 476.1 ± 122.9 seconds in the black chamber and 418.33 ± 118.0 seconds in the black and white chamber of the place preference apparatus (Student's $t = 0.3394$, $n = 12$, $p > 0.05$). Because no side bias was revealed in these preliminary experiments, AMPH and saline were randomly paired with the black or black and white striped side of the chamber in Experiments 1 and 2.

Experiment 1: Effects of Hormonal Milieu on AMPH induced Place Preference in Female Rats

In the following experiments, the saline-paired and AMPH-paired sides were assigned in a randomized, counter-balanced manner. During the three days of conditioning in the CPP apparatus, one training session consisted of the rats being placed in one chamber of the apparatus following injection of a low dose (1.0 mg/kg) of AMPH. During the second daily training session, the rats were placed in the other chamber following injection of the saline vehicle. These placements were randomized with regard to time of day. Figure 2A shows that OVX female rats spent approximately equal amounts of time in the AMPH-paired chamber and the saline-paired chamber with the resultant CPP score approximating zero. However, OVX rats that received either E2 or a combination of E2+P replacement therapy were able to discriminate between the effects of a low dose of AMPH (1.0 mg/kg) and the saline vehicle. The ability to distinguish AMPH from saline resulted in the rats in these two treatment groups spending significantly more time in the AMPH-paired chamber than the saline-paired chamber and a positive CPP score (one-way ANOVA, $F_{(2,12)} = 6.115$, $p<0.05$). Tukey's post-hoc multiple comparison test revealed a significant difference between OVX and OVX+E2 treatments ($q = 4.025$, $p<0.05$) as well as OVX and OVX+E2+P treatments ($q = 4.363$, $p<0.05$).

Using Western blotting techniques to quantify RGS9-2 protein expression in the NAc shell and core, we subsequently tested the hypothesis that a significant AMPH-induced place preference would be associated with decreased RGS9-2 protein levels in the NAc shell but not core. Figure 1 shows a diagrammatic representation of the NAc regions sampled for shell and core tissue. A representative Western blot of RGS9-2 protein expression in the NAc shell is shown in the inset of Figure 2B. RGS9-2 is a 78 kilodalton (kDa) protein and Western blotting reveals a single band of immunoreactive material at this molecular weight in the NAc shell protein extracts. Lanes 1 and 2 show representative RGS9-2 expression in extracts of the NAc shell of OVX female rats, lanes 3 and 4 show expression in OVX+E2 replaced rats and lanes 5 and 6 show RGS9-2 expression in OVX rats following E2+P replacement. Beneath the RGS9-2 bands are the corresponding beta-actin expression profiles, which were used as loading controls. Figure 2B shows the quantitative comparison of RGS9-2 protein expression across all groups. OVX+E2 and OVX+E2+P-treated rats had significantly lower RGS9-2 expression in the Nac shell (ANOVA, $F_{(2,12)} = 13.99$, $p<0.05$). Tukey's post-hoc comparison revealed that OVX rats had significantly higher levels of RGS9-2 in the NAc shell than the OVX+E2 ($q = 4.747$, $p<0.05$) or OVX+E2+P-treated rats ($q = 6.443$, $p<0.01$). In contrast to the NAc shell, the level of RGS9-2 protein in the NAc core was unaffected by E2 or E2+P administration (Figure 2C). In the inset of Figure 2C, lane 1 represents RGS9-2 immunoreactive material in the core of an OVX female rat, lane 2 shows RGS9-2 expression in the NAc core of a representative OVX+E2-treated rat and lane 3 depicts RGS9-2 expression in a representative OVX+E2+P treated rat. No significant differences were observed in RGS9-2 protein levels in the NAc core (ANOVA, $F_{(2,12)} = 0.2940$, $p>0.05$, Figure 2C).

Experiment 2: Effects of Selective Agonists on Place Preference in Female Rats

Since E2 potentiated AMPH-induced place preference in OVX female rats, examination of the contribution of the ER subtypes in E2-enhanced CPP became of great interest. In these studies the ERα and ERβ agonists, PPT and DPN, were employed. A previous study revealed the ability of the ERα agonist, PPT, to reduce post-OVX weight gain in rat uterus as well as overall body weight (Le Saux and Di Paolo, 2005; Roesch, 2006). In our studies, OVX female rats treated for fourteen days with the sesame oil vehicle had a body weight of 285 ± 12.0 g. Treatment with E2 for fourteen days reduced the body weight of OVX rats to 249 ± 7.0 g. OVX female rats that received PPT for fourteen days had a body weight of 256 ± 6.7 g while OVX rats treated with DPN had a weights of 283 ± 10 g (one-way ANOVA, $F_{(3,32)} = 42.6$, $p<0.001$). Tukey's post-hoc statistical analysis of these data revealed a highly significant difference between the body weights of the OVX rats treated with sesame oil compared to the rats treated with OVX+E2 ($q = 15.27$, $p<0.001$) or OVX+PPT ($q = 12.83$, $p<0.001$) but no difference between OVX and OVX+DPN ($q = .7080$, $p>0.05$) or OVX+E2 and OVX+PPT ($q = 2.374$, $p>0.05$) suggesting that the doses of PPT and DPN employed were effective.

As in Experiment 1, OVX female rats that received sesame oil injections for fourteen days when exposed to three-days of place conditioning did not develop an AMPH place preference (Figure 3A). However, the OVX rats treated with E2 or the ERβ selective agonist, DPN, for fourteen days were able to discriminate the AMPH-paired context from the saline-paired context and developed a significant preference for the AMPH-paired environment (one-way ANOVA with Tukey's post-hoc analysis ($F_{(3,32)} = 4.305$, $p<0.05$; $q_{OVX+E2} = 3.853$, $p<0.05$; $q_{OVX+DPN} = 4.907$, $p<0.05$). However, OVX rats treated with the ERα selective agonist, PPT, did not develop a preference for the AMPH-paired context (Figure 3A).

Following behavioral testing, animals were sacrificed for determination of the effect of PPT and DPN on RGS9-2 protein levels in the NAc shell and core. One-way ANOVA followed by Tukey's post-hoc comparison revealed a statistically significant decrease ($F_{(3,12)} = 5.761$, $p<0.05$) in RGS9-2 protein expression in the NAc shell of OVX+E2-treated rats ($q_{OVX+E2} =$

4.816, $p<0.05$) and the OVX+DPN-treated rats ($q_{OVX+DPN} = 5.218$, $p<0.05$) compared to the OVX-sesame oil vehicle rats (Figure 3B). These findings were replicated in a second set of NAc shell samples also with 4-5 animals per experimental group. Figure 3C shows representative examples of the expression of RGS9-2 in the NAc shell of an OVX-sesame oil control rat (Lane 1), an OVX+E2-treated rat (Lane 2), an OVX+PPT-treated rat in Lane 3 and in Lane 4 the expression of RGS9-2 in the NAc shell of an OVX+DPN-treated rat. The beta-actin loading controls are shown in the lower portion of the figure. There was no significant effect of these hormonal treatments on RGS9-2 expression in the NAc core (data not shown). The brains from five randomly selected rats from each group were used for RGS9-2 in situ hybridization histochemistry. Table 1 depicts the values of RGS9-2 mRNA expression in the NAc shell were significantly reduced by both E2 and DPN replacement therapies ($F_{shell\ (3,16)}=7.763$, $p<0.05$; $q_{OVX+E2} = 6.194$, $p<0.01$; $q_{OVX+DPN} = 4.122$, $p<0.05$) as compared to control OVX female rats. The data obtained by in situ hybridization histochemistry correlated with the effects of E2 and DPN on the NAc RGS9-2 protein expression. In addition, no changes in RGS9-2 mRNA expression were found in the NAc core after any hormone treatment regimen (data not shown).

Discussion

Clinical and preclinical evidence provide a strong basis for the sexually differentiated pattern in behavioral responses generated by psychostimulants (Quinones-Jenab, 2006). Recent studies have documented an earlier initiation of cocaine use among young females compared to males as well as a higher frequency of cocaine use (Chen and Kandel, 2002). Human female functional imaging studies revealed positive correlations between E2 level and brain activity in the reward circuit (Dreher et al., 2007). In rodent models, females exhibited enhanced locomotor responses to cocaine (van Haaren and Meyer, 1991), accelerated acquisition of cocaine self-administration behavior (Lynch et al., 2002; Hu et al., 2004) and greater cocaine-induced place preference (Russo et al., 2003a) which was accompanied by enhanced NAc DA release (Carr and White, 1983; Pettit and Justice, 1989; Peris et al., 1991). The cyclical pattern of estrogen secretion in females appeared to be responsible for the exaggerated behavioral responses to psychostimulants because surgical removal of the ovaries yields behavioral responses in self-administration tasks that are similar to male rats (Hu et al., 2004; Lynch and Taylor, 2005).

Evidence indicated an E2 induced increase in tyrosine hydroxylase activity in the VTA (Kritzer, 2003) and in the amount of DA released from striatal and accumbens nerve terminals following psychostimulant administration (Thompson and Moss, 1994; Xiao and Becker, 1998). However, with enhanced DA release, changes in DA receptor densities in the terminal regions that compensate for the augmented release of DA would be expected. However, inconsistent E2-induced changes in D2 receptor density have been found with both increased and decreased receptor density being reported (Di Paolo et al., 1982; Hruska, 1986; Lammers et al., 1999). In humans, no D2 receptor changes have been found across the menstrual cycle according to a recent positron emission tomography study (Nordstrom et al., 1998). Other studies suggested that alterations in D2 receptor coupled G-protein function may be altered by E2 administration and could account for E2-enhancement of psychostimulant-induced behaviors (Thompson et al., 2001; Febo et al., 2003). Earlier findings from our laboratory also suggested that an alteration in the recycling of G-proteins by a reduction of RGS9-2 mRNA expression may be a novel site of E2 action (Sharifi et al., 2004). The work presented in this paper extended our previous finding in several ways.

First, OVX female rats failed to develop a place preference for AMPH using a three-day, six session training paradigm while OVX rats receiving E2 replacement from either an implanted silastic capsule containing crystalline E2, E2 implant in combination with P injection or daily

E2 injections successfully learn to discriminate AMPH from saline. In each case of learning the association of AMPH and a specific context, the rats expressed less RGS9-2 in the NAc shell. Consistent with our findings are data from male mice with a null mutation of the RGS9-2 gene, which display heightened locomotor responses to AMPH (Rahman et al., 2003), the D2 agonist quinpirole (Kovoor et al., 2005) and enhanced AMPH-induced place preference (Rahman et al., 2003). E2-induced enhancement of cocaine-induced locomotor activity (Hu and Becker, 2003) has also been reported. Thus, there is a strong possibility that the accompanying reduction of RGS9-2 in the NAc shell underlies E2-enhancement of AMPH-induced behavior. Russo and colleagues (2003b) reported E2 treatment of OVX female rats failed to enhance cocaine-induced CPP following a four-day training period and a high dose of cocaine as compared to OVX females not treated with E2. Because we employed a threshold dose of AMPH (1.0mg/kg) and an abbreviated training schedule, we were able to differentiate the facilitating effects of E2. If a higher dose of AMPH and a longer training paradigm had been used we might easily have generated results similar to Russo et al., (2003b). Previously, P and its metabolites were found to enhance place preference for locations associated with mating (Gonzalez-Flores et al., 2004). Frequently, however, P antagonizes E2 effects. For example, co-administration of P with E2 prevents an enhancement of the acquisition of cocaine self-administration in females normally associated with E2 treatment (Jackson et al., 2006) and P reduces locomotor counts following cocaine (Quinones-Jenab et al., 2000). While P may impede some effects of E2, several studies support the notion that the E2 interaction with DA mechanisms in brain are not disrupted by P (Becker and Rudick, 1999). Currently, the results of our experiments are consistent with this notion. Yet, it is important to note that in our experiments, P treatment was given s.c. for fourteen days. We recognize that this regimen does not mimic physiological P secretion across the rat estrus cycle. However, other investigators have implanted silastic capsules releasing P for seven to twenty-one days (Sell et al., 2000; Russo et al., 2003b) to study putative proestrus P modulation of E2 effects. We did not find that P altered E2-amplified AMPH-induced place preference, which was rather surprising considering the P enhancement of mating (Gonzalez-Flores et al., 2004) and cocaine-induced place preference (Russo et al., 2003b). It is possible that P had no effect in our experiments because the E2 influence on place preference behavior could have been maximally stimulated precluding further enhancement. This seems reasonable in light of the findings of Russo et al (2003b). It could also be that with our repeated daily P treatment masked a possible enhancement of preference behavior by the combination of E2 and P.

A second finding of this work is the demonstration that E2 augmentation of AMPH-induced place preference appeared to be due to activation of ERβ. Our studies utilized a fourteen-day E2 treatment regimen following OVX, which decreases the likelihood that the action of E2 involved a membrane-bound receptor.. However, because we employed pharmacological agents which are highly selective for ERα and ERβ but not absolutely specific, the possibility remains that non-genomic effects of E2 could be involved (Thompson and Moss, 1994). Coincident with DPN-induction of AMPH place conditioning was a reduction of RGS9-2 expression in the NAc shell, similar to OVX+E2-treated female rats. PPT, which activates ERα selectively, did not have a significant effect on either RGS9-2 levels in the NAc or on AMPH-induced CPP. It is likely that both E2 and DPN exert their effects within the NAc since peripheral administration of E2 increases concentrations of E2 within the NAc (Frye and Rhodes, 2006). Additionally, an infusion of ER antisense oligonucleotides into the NAc prevented E2-induced place preference (Walf and Frye, 2005). Because of the presence of ERβ in brain regions known to be involved in rewarding behaviors, such as the NAc and the VTA (Shughrue and Merchenthaler, 2001; Creutz and Kritzer, 2002), it is likely that the effects of DPN and E2 involved activation of NAc ERβ. However, other studies demonstrated that ERβ receptor stimulation with DPN selectively affect D2 receptor binding in the NAc core and not the shell (Le Saux et al., 2006). Unfortunately, there is no obvious explanation for these somewhat dichotomous findings. Micropunches were used to separate two immediately

adjacent structures in the present study and it is possible that our finding of DPN-induced changes in the NAc shell could be attributable to contamination with NAc core tissue but based on the accuracy of the micropunching exemplified in Figure 1, this explanation would not appear to be relevant. It is also possible that the repeated administration of AMPH coupled with the associative learning our animals received during CPP training may have changed the dynamic interaction of DA, D2 receptors and ERβ within the NAc subterritories, subsequently yielding these somewhat discordant findings. Furthermore, it should be noted that mice with a null mutation of RGS9-2 have been shown to display enhanced psychostimulant induced place preference compared to wild-type mice, and these animals have no significant differences in D2 receptor binding (Rahman et al., 2003). These data provide a basis for investigating the role of ERβ in other aspects of reward related behaviors.

Previously, we reported that E2 decreased RGS9-2 mRNA expression in the NAc shell (Sharifi et al., 2004). In the present studies, we demonstrated that E2 also decreases RGS9-2 protein levels in the NAc shell, but not core, and that rats showing psychostimulant-induced place preference have significant reductions in the NAc shell RGS9-2 protein levels compared with animals not exhibiting place preference behavior. The NAc shell is the site most commonly associated with the rewarding effects of drugs of abuse. The NAc shell has been demonstrated to be the neural substrate for AMPH self-administration (Ikemoto et al., 2005) and it plays a major role in AMPH place preference (Carr and White, 1983). Considering the abundant research that has implicated DA signaling in reward related behaviors (Koob, 1996; Nestler, 2004) and the critical role of the D2 receptor in these behaviors, a likely target of investigation would be RGS9-2, because of its strikingly specific expression to the dorsal and ventral striatal areas and its exclusive co-localization with the D2 receptor (Rahman et al., 2003; Kovoor et al., 2005). Interestingly, RGS9-2 mRNA is not expressed in VTA neurons (Gold et al., 2003) suggesting that the actions of E2 on RGS9-2 expression are targeted to postsynaptic D2 receptor expressing cells in the NAc. RGS proteins regulate GTP-GDP recycling and are known to have a GTPase activity (Dohlman and Thorner, 1997; Berman and Gilman, 1998). Estrogens could have additional effects on the levels of G-proteins directly in the NAc and previous studies have shown that E2 treatment decreased Gαi protein levels in the striatum (Thompson and Certain, 2005). Decreasing Gαi could also augment CPP behavior if similar effects of E2 were present in the NAc. Alternatively, Ikeda et al., (2000) reported that a steroid binding protein with an RGS domain interacted with the estrogen response element. Further investigation will be needed to determine whether the observed decreases of RGS9-2 in E2-treated OVX females are the result of a direct interaction between RGS9-2 and ERβ.

The CPP paradigm is based on the concepts of classical conditioning and associative learning to generate a linkage between the rewarding effects of a drug and the contexual stimuli of the environment. A variety of studies have demonstrated that E2 augments learning and memory (Woolley et al., 1997; Daniel and Dohanich, 2001). E2 enhanced working memory (Daniel et al., 1997) and exhibited robust effects on behavioral and neurochemical measures of striatal function (Becker, 1999), which are critical for procedural memory. Recently, E2 has been shown to increase the sensitivity of D2 antagonist effects in learning tasks (Daniel, 2006; Daniel et al., 2006a; Daniel et al., 2006b). The present findings in combination with the work reported by Russo et al. (2003b) and Walf and Frye (2006; 2007) would support the thesis that E2 facilitates other types of non-hippocampal learning, i.e. associative learning. In the current study, OVX rats treated with E2, E2+P or the ERβ agonist DPN learned to discriminate the AMPH-paired context from the saline-paired context and developed a significant preference for the AMPH-paired environment. Each of these groups significantly differed from the OVX controls and had attenuated RGS9-2 expression in the NAc shell. The correlative nature of these data provides evidence for the possibility that as animals improve performance on the CPP task, RGS9-2 protein levels decrease in the NAc shell, but not the NAc core. Because E2 facilitates AMPH-induced CPP behavior, we can conclude that E2 enhances the heightened

state of association between environmental context and drug reward and that this effect may involve NAc RGS9-2. In fact, increased DA in the NAc shell, but not core, is associated with development of addictive processes, while the core is selectively involved in rekindling drug taking behavior during episodes of relapse (Pontieri et al., 1995; McFarland and Kalivas, 2001; Kalivas and McFarland, 2003; Sellings and Clarke, 2003; Di Chiara et al., 2004).

In summary, studies have demonstrated AMPH-induced place conditioning in male rats and mice but we may be among the first to show that E2 augments the development of AMPH place preferences in female rats. In addition, evidence is presented for ERβ mediation of E2's effects on reward enhancement. Finally, these studies point to the possible involvement of RGS9-2 in E2-enhanced behaviors and provide evidence for its role in augmented behavioral responses that might be account for the more rapid development of addictive behavior in women.

Acknowledgements

This work was supported by NIH grant # F31 DA017027-01.

References

Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang Z. Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci 2006;9:133–139. [PubMed: 16327783]

Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 1999;64:803–812. [PubMed: 10593204]

Becker JB, Rudick CN. Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming: a microdialysis study. Pharmacol Biochem Behav 1999;64:53–57. [PubMed: 10494997]

Becker JB, Beer ME, Robinson TE. Striatal dopamine release stimulated by amphetamine or potassium: influence of ovarian hormones and the light-dark cycle. Brain Res 1984;311:157–160. [PubMed: 6488037]

Becker JB, Molenda H, Hummer DL. Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. Ann N Y Acad Sci 2001;937:172–187. [PubMed: 11458536]

Berman DM, Gilman AG. Mammalian RGS proteins: barbarians at the gate. J Biol Chem 1998;273:1269–1272. [PubMed: 9430654]

Bridges RS. A quantitative analysis of the roles of dosage, sequence, and duration of estradiol and progesterone exposure in the regulation of maternal behavior in the rat. Endocrinology 1984;114:930–940. [PubMed: 6697968]

Britton DR, Curzon P, Mackenzie RG, Kebabian JW, Williams JE, Kerkman D. Evidence for involvement of both D1 and D2 receptors in maintaining cocaine self-administration. Pharmacol Biochem Behav 1991;39:911–915. [PubMed: 1684870]

Burchett SA, Volk ML, Bannon MJ, Granneman JG. Regulators of G protein signaling: rapid changes in mRNA abundance in response to amphetamine. J Neurochem 1998;70:2216–2219. [PubMed: 9572311]

Carr GD, White NM. Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sci 1983;33:2551–2557. [PubMed: 6645814]

Carroll ME, Lynch WJ, Roth ME, Morgan AD, Cosgrove KP. Sex and estrogen influence drug abuse. Trends Pharmacol Sci 2004;25:273–279. [PubMed: 15120494]

Chen K, Kandel D. Relationship between extent of cocaine use and dependence among adolescents and adults in the United States. Drug Alcohol Depend 2002;68:65–85. [PubMed: 12167553]

Creutz LM, Kritzer MF. Estrogen receptor-beta immunoreactivity in the midbrain of adult rats: regional, subregional, and cellular localization in the A10, A9, and A8 dopamine cell groups. J Comp Neurol 2002;446:288–300. [PubMed: 11932944]

Creutz LM, Kritzer MF. Mesostriatal and mesolimbic projections of midbrain neurons immunoreactive for estrogen receptor beta or androgen receptors in rats. J Comp Neurol 2004;476:348–362. [PubMed: 15282710]

Daniel JM. Effects of oestrogen on cognition: what have we learned from basic research? J Neuroendocrinol 2006;18:787–795. [PubMed: 16965297]

Daniel JM, Dohanich GP. Acetylcholine mediates the estrogen-induced increase in NMDA receptor binding in CA1 of the hippocampus and the associated improvement in working memory. J Neurosci 2001;21:6949–6956. [PubMed: 11517282]

Daniel JM, Hulst JL, Berbling JL. Estradiol replacement enhances working memory in middle-aged rats when initiated immediately after ovariectomy but not after a long-term period of ovarian hormone deprivation. Endocrinology 2006a;147:607–614. [PubMed: 16239296]

Daniel JM, Sulzer JK, Hulst JL. Estrogen increases the sensitivity of ovariectomized rats to the disruptive effects produced by antagonism of D2 but not D1 dopamine receptors during performance of a response learning task. Horm Behav 2006b;49:38–44. [PubMed: 15936760]

Daniel JM, Fader AJ, Spencer AL, Dohanich GP. Estrogen enhances performance of female rats during acquisition of a radial arm maze. Horm Behav 1997;32:217–225. [PubMed: 9454673]

Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, Acquas E, Carboni E, Valentini V, Lecca D. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 2004;47(Suppl 1):227–241. [PubMed: 15464140]

Di Paolo T, Rouillard C, Bedard P. 17 beta-Estradiol at a physiological dose acutely increases dopamine turnover in rat brain. Eur J Pharmacol 1985;117:197–203. [PubMed: 4076343]

Di Paolo T, Bedard PJ, Dupont A, Poyet P, Labrie F. Effects of estradiol on intact and denervated striatal dopamine receptors and on dopamine levels: a biochemical and behavioral study. Can J Physiol Pharmacol 1982;60:350–357. [PubMed: 7200388]

Dohlman HG, Thorner J. RGS proteins and signaling by heterotrimeric G proteins. J Biol Chem 1997;272:3871–3874. [PubMed: 9064301]

Dreher JC, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. Proc Natl Acad Sci U S A 2007;104:2465–2470. [PubMed: 17267613]

Febo M, Gonzalez-Rodriguez LA, Capo-Ramos DE, Gonzalez-Segarra NY, Segarra AC. Estrogen-dependent alterations in D2/D3-induced G protein activation in cocaine-sensitized female rats. J Neurochem 2003;86:405–412. [PubMed: 12871581]

Frye CA, Rhodes ME. Administration of estrogen to ovariectomized rats promotes conditioned place preference and produces moderate levels of estrogen in the nucleus accumbens. Brain Res 2006;1067:209–215. [PubMed: 16388786]

Gold SJ, Ni YG, Dohlman HG, Nestler EJ. Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. J Neurosci 1997;17:8024–8037. [PubMed: 9315921]

Gold SJ, Han MH, Herman AE, Ni YG, Pudiak CM, Aghajanian GK, Liu RJ, Potts BW, Mumby SM, Nestler EJ. Regulation of RGS proteins by chronic morphine in rat locus coeruleus. Eur J Neurosci 2003;17:971–980. [PubMed: 12653973]

Gonzalez-Flores O, Camacho FJ, Dominguez-Salazar E, Ramirez-Orduna JM, Beyer C, Paredes RG. Progestins and place preference conditioning after paced mating. Horm Behav 2004;46:151–157. [PubMed: 15256304]

Hruska RE. Elevation of striatal dopamine receptors by estrogen: dose and time studies. J Neurochem 1986;47:1908–1915. [PubMed: 3772381]

Hu M, Becker JB. Effects of sex and estrogen on behavioral sensitization to cocaine in rats. J Neurosci 2003;23:693–699. [PubMed: 12533629]

Hu M, Crombag HS, Robinson TE, Becker JB. Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology 2004;29:81–85. [PubMed: 12955098]

Ikeda M, Hirokawa M, Satani N, Kinoshita T, Watanabe Y, Inoue H, Tone S, Ishikawa T, Minatogawa Y. Molecular cloning and characterization of a steroid receptor-binding regulator of G-protein signaling protein cDNA. Gene 2001;273:207–214. [PubMed: 11595167]

Ikemoto S, Qin M, Liu ZH. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the accumbens core, shell, and olfactory tubercle valid? J Neurosci 2005;25:5061–5065. [PubMed: 15901788]

Jackson LR, Robinson TE, Becker JB. Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. Neuropsychopharmacology 2006;31:129–138. [PubMed: 15920500]

Kalivas PW, McFarland K. Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology (Berl) 2003;168:44–56. [PubMed: 12652346]

Kebabian JW, Calne DB. Multiple receptors for dopamine. Nature 1979;277:93–96. [PubMed: 215920]

Koob GF. Hedonic valence, dopamine and motivation. Mol Psychiatry 1996;1:186–189. [PubMed: 9118342]

Kovoor A, Seyffarth P, Ebert J, Barghshoon S, Chen CK, Schwarz S, Axelrod JD, Cheyette BN, Simon MI, Lester HA, Schwarz J. D2 dopamine receptors colocalize regulator of G-protein signaling 9-2 (RGS9-2) via the RGS9 DEP domain, and RGS9 knock-out mice develop dyskinesias associated with dopamine pathways. J Neurosci 2005;25:2157–2165. [PubMed: 15728856]

Kritzer MF. Long-term gonadectomy affects the density of tyrosine hydroxylase- but not dopamine-beta-hydroxylase-, choline acetyltransferase- or serotonin-immunoreactive axons in the medial prefrontal cortices of adult male rats. Cereb Cortex 2003;13:282–296. [PubMed: 12571118]

Lammers CH, D'Souza U, Qin ZH, Lee SH, Yajima S, Mouradian MM. Regulation of striatal dopamine receptors by estrogen. Synapse 1999;34:222–227. [PubMed: 10523759]

Le Saux M, Di Paolo T. Chronic estrogenic drug treatment increases preproenkephalin mRNA levels in the rat striatum and nucleus accumbens. Psychoneuroendocrinology 2005;30:251–260. [PubMed: 15511599]

Le Saux M, Morissette M, Di Paolo T. ERbeta mediates the estradiol increase of D2 receptors in rat striatum and nucleus accumbens. Neuropharmacology 2006;50:451–457. [PubMed: 16309717]

Li M, Fleming AS. The nucleus accumbens shell is critical for normal expression of pup-retrieval in postpartum female rats. Behav Brain Res 2003;145:99–111. [PubMed: 14529809]

Lund TD, Rovis T, Chung WC, Handa RJ. Novel actions of estrogen receptor-beta on anxiety-related behaviors. Endocrinology 2005;146:797–807. [PubMed: 15514081]

Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl) 1999;144:77–82. [PubMed: 10379627]

Lynch WJ, Taylor JR. Decreased motivation following cocaine self-administration under extended access conditions: effects of sex and ovarian hormones. Neuropsychopharmacology 2005;30:927–935. [PubMed: 15647749]

Lynch WJ, Roth ME, Carroll ME. Biological basis of sex differences in drug abuse: preclinical and clinical studies. Psychopharmacology (Berl) 2002;164:121–137. [PubMed: 12404074]

Maldonado-Irizarry CS, Kelley AE. Excitotoxic lesions of the core and shell subregions of the nucleus accumbens differentially disrupt body weight regulation and motor activity in rat. Brain Res Bull 1995;38:551–559. [PubMed: 8590077]

McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 2001;21:8655–8663. [PubMed: 11606653]

Nestler EJ. Molecular mechanisms of drug addiction. Neuropharmacology 2004;47(Suppl 1):24–32. [PubMed: 15464123]

Nestler EJ, Carlezon WA Jr. The Mesolimbic Dopamine Reward Circuit in Depression. Biol Psychiatry. 2006

Nordstrom AL, Olsson H, Halldin C. A PET study of D2 dopamine receptor density at different phases of the menstrual cycle. Psychiatry Res 1998;83:1–6. [PubMed: 9754700]

Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. Endocrinology 1998;139:5070–5081. [PubMed: 9832446]

Ogawa S, Chan J, Chester AE, Gustafsson JA, Korach KS, Pfaff DW. Survival of reproductive behaviors in estrogen receptor beta gene-deficient (betaERKO) male and female mice. Proc Natl Acad Sci U S A 1999;96:12887–12892. [PubMed: 10536018]

Peris J, Decambre N, Coleman-Hardee ML, Simpkins JW. Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [3H]dopamine release. Brain Res 1991;566:255–264. [PubMed: 1814541]

Pettit HO, Justice JB Jr. Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. Pharmacol Biochem Behav 1989;34:899–904. [PubMed: 2623043]

Pfaff D, Keiner M. Atlas of estradiol-concentrating cells in the central nervous system of the female rat. J Comp Neurol 1973;151:121–158. [PubMed: 4744471]

Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the “shell” as compared with the “core” of the rat nucleus accumbens. Proc Natl Acad Sci U S A 1995;92:12304–12308. [PubMed: 8618890]

Quinones-Jenab V. Why are women from Venus and men from Mars when they abuse cocaine? Brain Res. 2006

Quinones-Jenab V, Krey LC, Schlussman SD, Ho A, Kreek MJ. Chronic ‘binge’ pattern cocaine alters the neuroendocrine profile of pregnant rats. Neurosci Lett 2000;282:120–122. [PubMed: 10713411]

Rahman Z, Gold SJ, Potenza MN, Cowan CW, Ni YG, He W, Wensel TG, Nestler EJ. Cloning and characterization of RGS9-2: a striatal-enriched alternatively spliced product of the RGS9 gene. J Neurosci 1999;19:2016–2026. [PubMed: 10066255]

Rahman Z, Schwarz J, Gold SJ, Zachariou V, Wein MN, Choi KH, Kovoor A, Chen CK, DiLeone RJ, Schwarz SC, Selley DE, Sim-Selley LJ, Barrot M, Luedtke RR, Self D, Neve RL, Lester HA, Simon MI, Nestler EJ. RGS9 modulates dopamine signaling in the basal ganglia. Neuron 2003;38:941–952. [PubMed: 12818179]

Robinson TE. Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. Psychopharmacology (Berl) 1984;84:466–475. [PubMed: 6441946]

Roesch DM. Effects of selective estrogen receptor agonists on food intake and body weight gain in rats. Physiol Behav 2006;87:39–44. [PubMed: 16181647]

Russo SJ, Jenab S, Fabian SJ, Festa ED, Kemen LM, Quinones-Jenab V. Sex differences in the conditioned rewarding effects of cocaine. Brain Res 2003a;970:214–220. [PubMed: 12706263]

Russo SJ, Festa ED, Fabian SJ, Gazi FM, Kraish M, Jenab S, Quinones-Jenab V. Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience 2003b;120:523–533. [PubMed: 12890521]

Self DW, Barnhart WJ, Lehman DA, Nestler EJ. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists. Science 1996;271:1586–1589. [PubMed: 8599115]

Sell SL, Scalzitti JM, Thomas ML, Cunningham KA. Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. J Pharmacol Exp Ther 2000;293:879–886. [PubMed: 10869388]

Sellings LH, Clarke PB. Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neurosci 2003;23:6295–6303. [PubMed: 12867514]

Sharifi JL, Brady DL, Koenig JI. Estrogen modulates RGS9 expression in the nucleus accumbens. Neuroreport 2004;15:2433–2436. [PubMed: 15640770]

Shughrue P, Scrimo P, Lane M, Askew R, Merchenthaler I. The distribution of estrogen receptor-beta mRNA in forebrain regions of the estrogen receptor-alpha knockout mouse. Endocrinology 1997;138:5649–5652. [PubMed: 9389555]

Shughrue PJ, Merchenthaler I. Distribution of estrogen receptor beta immunoreactivity in the rat central nervous system. J Comp Neurol 2001;436:64–81. [PubMed: 11413547]

Swanson, LW. Brain Maps: Structure of the Rat Brain. 3. London: Academic Press; 2004.

Thompson TL, Moss RL. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. J Neurochem 1994;62:1750–1756. [PubMed: 8158125]

Thompson TL, Certain ME. Estrogen mediated inhibition of dopamine transport in the striatum: regulation by G alpha i/o. Eur J Pharmacol 2005;511:121–126. [PubMed: 15792779]

Thompson TL, Bridges SR, Weirs WJ. Alteration of dopamine transport in the striatum and nucleus accumbens of ovariectomized and estrogen-primed rats following N-(p-isothiocyanatophenethyl) spiperone (NIPS) treatment. Brain Res Bull 2001;54:631–638. [PubMed: 11403989]

van Haaren F, Meyer ME. Sex differences in locomotor activity after acute and chronic cocaine administration. Pharmacol Biochem Behav 1991;39:923–927. [PubMed: 1763112]

Walf AA, Frye CA. ERbeta-selective estrogen receptor modulators produce antianxiety behavior when administered systemically to ovariectomized rats. Neuropsychopharmacology 2005;30:1598–1609. [PubMed: 15798780]

White TL, Justice AJ, de Wit H. Differential subjective effects of D-amphetamine by gender, hormone levels and menstrual cycle phase. Pharmacol Biochem Behav 2002;73:729–741. [PubMed: 12213517]

Wise RA, Murray A, Bozarth MA. Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. Psychopharmacology (Berl) 1990;100:355–360. [PubMed: 2315433]

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J Neurosci 1997;17(5):1848–59. [PubMed: 9030643]

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA. Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J Neurosci 1997;17:1848–1859. [PubMed: 9030643]

Xiao L, Becker JB. Effects of estrogen agonists on amphetamine-stimulated striatal dopamine release. Synapse 1998;29:379–391. [PubMed: 9661256]

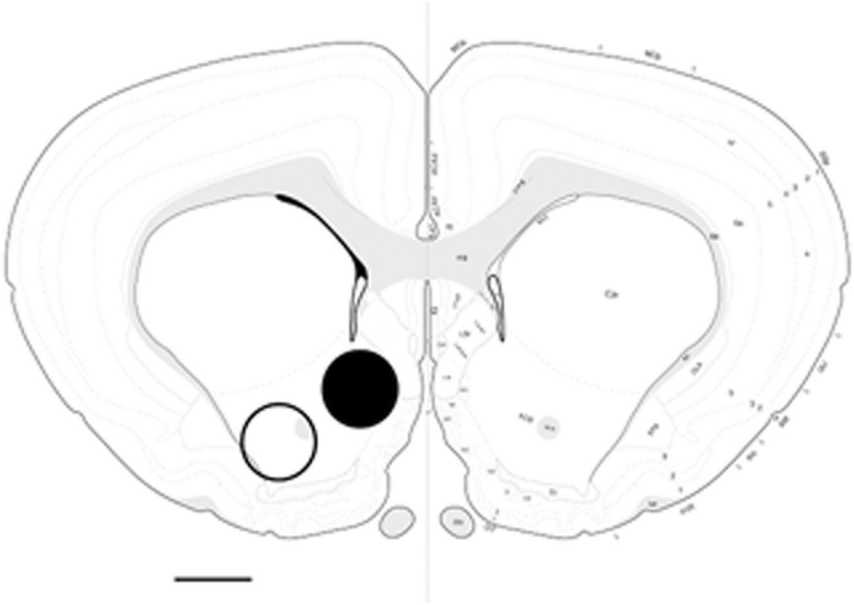

Figure 1. Histological Example of Nucleus Accumbens

A representative coronal section depicting the NAc shell and core. The scale bar is 1-mm. The 1-mm micropunches extracted from the slice are represented by a filled circle (shell) and an open circle (core).

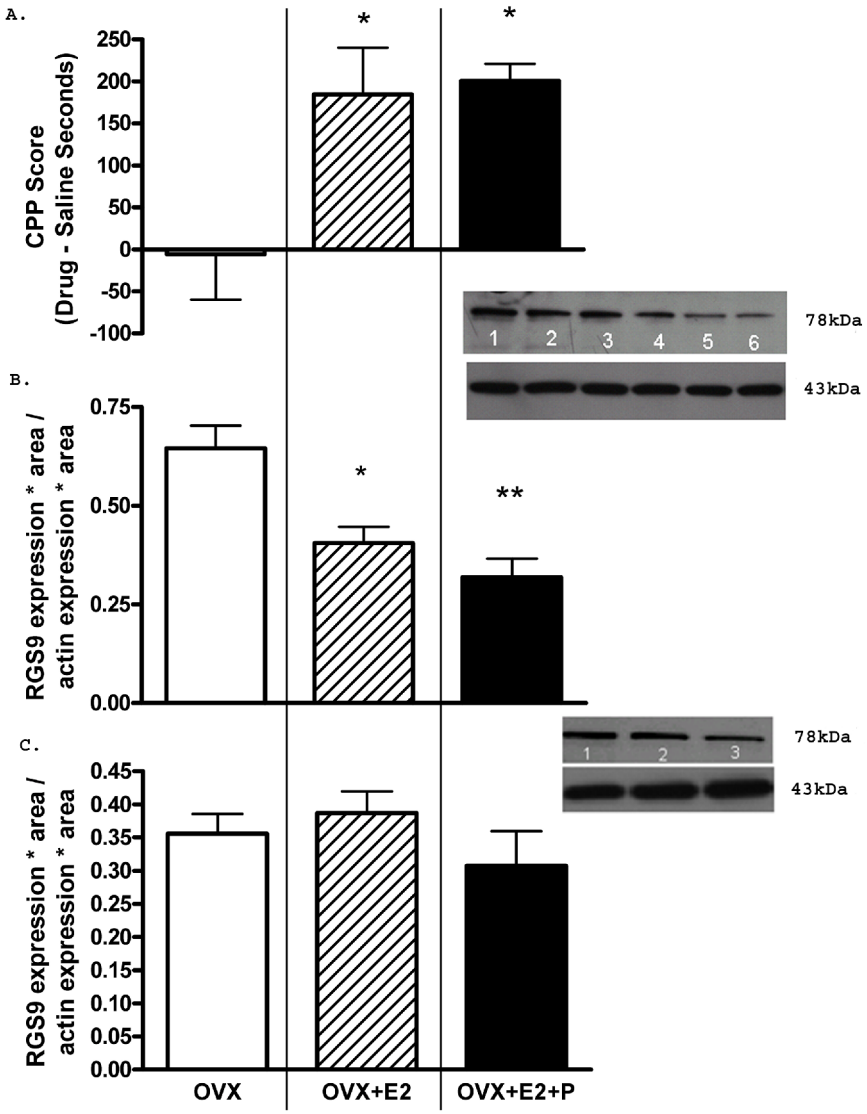

Figure 2.

Figure 2A. Place Preference to AMPH in OVX rats with and without Gonadal Hormone Replacement

The effects of OVX, OVX+E2 and OVX+E2+P replacement on AMPH-induced CPP in female rats are shown. Data are represented as the mean (±S.E.M.) time spent in the drug paired chamber minus the time spent in the saline paired chamber (CPP scores). * indicates a statistically significant difference from OVX controls ($p<0.05$ by one-way ANOVA and Tukey's post-hoc comparison). There were 5 female rats in the OVX, OVX+E2 and OVX+E2 +P groups.

Figure 2B. Effects of Gonadal Hormones on RGS9-2 Following AMPH-induced CPP in the NAc Shell

Data are presented as the mean (±S.E.M.) integrated density of RGS9-2 protein expression in the NAc shell of OVX, OVX+E2 and OVX+E2+P treated females sacrificed after CPP testing. There were 5 rats in each treatment group. Significant reductions in RGS9 expression in the NAc shell are indicated by * ($p<0.05$) or **($p<0.01$). Inset of figure shows a representative

Western blot of RGS9-2 expression in the NAc Shell using 15 micrograms of tissue extracted utilizing micropunches. RGS9-2 is a 78 kDa protein shown in the upper image. The lower image shows beta-actin expression (43 kDa) expression.

Figure 2C. Effects of Gonadal Hormones on RGS9-2 Following AMPH-induced CPP in the NAc Core

Quantification of the RGS9-2 expression in of the NAc core tissue. Data are presented as the mean (±S.E.M.) of integrated density. The inset of the figure displays a representative immunoblot for Experiment 1 on NAc core samples. There were 5 rats in each treatment group.

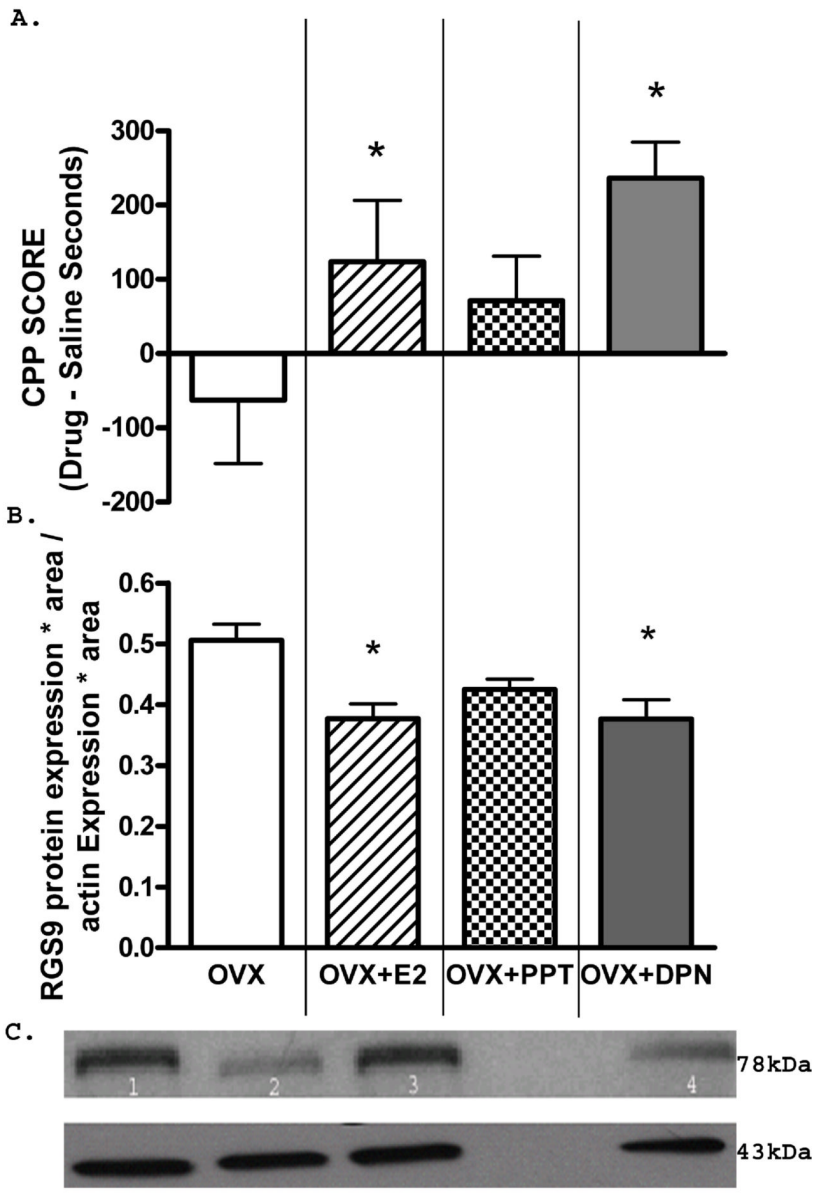

Figure 3.

Figure 3A. The Effects of OVX and Selective Agonists on AMPH-induced CPP in Female Rats

Place preference is expressed by the mean (±S.E.M.) time spent in the drug paired chamber minus the time spent in the saline paired chamber (CPP scores). * indicates a statistically significant difference compared to OVX control rats ($p<0.05$) by one-way ANOVA and Tukey's post-hoc comparison. There were 9 animals in the OVX, OVX+E2, OVX+PPT and OVX+DPN groups.

Figure 3B. Effects of Selective Agonists on RGS9-2 expression in the NAc Shell

Data are presented as the mean (±S.E.M.) integrated density of RGS9-2 protein expression in the NAc shell of the four groups OVX, OVX+E2, OVX+PPT and OVX+DPN (n=4/group). Significantly reductions in RGS9-2 expression in the NAc shell are indicated by * ($p<0.05$).

Figure 3C. Representative Immunoblots from Experiment 2 in the NAc Shell

Expression of RGS9-2 in the NAc shell using 15 micrograms of tissue extracted utilizing micropunches. The upper image shows expression of the 78 kDa protein, RGS9-2. The lower image shows beta-actin expression (43 kDa) expression. Lane 1 depicts RGS9-2 protein expression in the NAc shell of OVX female rat. Lanes 2 shows expression in an OVX+E2 female rat. Lane 3 depicts expression in an OVX+PPT treated female rat and lane 4 depicts expression in a DPN-treated OVX female rat.

Table 1

Effects of SERMS on RGS9-2 mRNA Expression in the NAc Shell and Core

Data shown are mean RGS9-2 mRNA relative optical density measures (±S.E.M.) in the NAc shell and core of OVX, OVX+E2, OVX+PPT and OVX+DPN treated rats.

	OVX	OVX+E2	OVX+PPT	OVX+DPN
SHELL	51.7±0.8	34.4±3.0**	46.5±4.0	39.3±0.6*
CORE	46.6±3.9	45.2±4.8	42.8±5.0	39.8±4.2

* indicates statistical significance $p<0.05$ or ** $p<0.01$ versus OVX control female rats. There were 9 animals treated with hormone regimen and 5 animals of each group were randomly selected for in situ hybridization histochemical analysis.