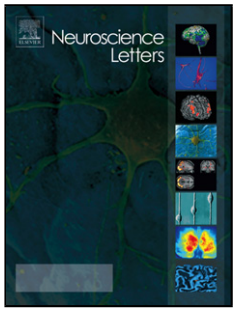

Title: 17β-estradiol locally increases phasic dopamine release in the dorsal striatum

Authors: Waqqas M. Shams, Marie-Pierre Cossette, Peter Shizgal, Wayne G. Brake

PII: S0304-3940(17)30948-5
DOI: https://doi.org/10.1016/j.neulet.2017.11.039
Reference: NSL 33247

To appear in: *Neuroscience Letters*

Received date: 27-7-2017
Revised date: 25-10-2017
Accepted date: 20-11-2017

Please cite this article as: Waqqas M.Shams, Marie-Pierre Cossette, Peter Shizgal, Wayne G.Brake, 17β-estradiol locally increases phasic dopamine release in the dorsal striatum, Neuroscience Letters https://doi.org/10.1016/j.neulet.2017.11.039

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RUNNING TITLE: Estradiol increases phasic dopamine release.

AUTHORS:
Waqqas M. Shams
Email: waqqas.shams@gmail.com

Marie-Pierre Cossette
Email: mpy_cossette@hotmail.com

Peter Shizgal
Email: peter.shizgal@concordia.ca

Wayne G. Brake (Corresponding Author)
Email: wayne.brake@concordia.ca

CORRESPONDENCE:
Department of Psychology, Centre for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H4B 1R6

ABBREVIATIONS[1]

FUNDING AND DISCLOSURE
This work was supported by discovery grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada [WGB: RGPIN-2016-06653; PS, RGPIN-308-11]. The authors declare no conflict of interest.

[1] E2 = 17β-estradiol; DA = dopamine; DS = dorsal striatum; OVX = ovariectomized; AMPH = amphetamine; SN = substantia nigra; FSCV = fast-scan cyclic voltammetry; CYC = cyclodextrin; AP = anteroposterior; ML = medial-lateral; DV = dorsoventral; ER = estrogen receptor; GABA = γ-aminobutyric acid; D2R = dopamine D2 receptor

Highlights

- 17β-estradiol has been shown to increase dopamine release in the dorsal striatum.
- It is not known if 17β-estradiol affects tonic and/or phasic dopamine release.
- We show that 17β-estradiol rapidly increases phasic striatal dopamine release.

ABSTRACT

Studies using *in vivo* microdialysis have shown that 17β-estradiol (E2) increases dopamine (DA) transmission in the dorsal striatum. Both systemic administration of E2 and local infusion into the dorsal striatum rapidly enhance amphetamine-induced DA release. However, it is not known to what degree these effects reflect tonic and/or phasic DA release. It was hypothesized that E2 acts directly within the DS to rapidly increase phasic DA transmission. In urethane-anaesthetized (1.5mL/kg) female rats, we used fast-scan cyclic voltammetry to study the effects of E2 on phasic, electrically-evoked release of DA in the dorsal striatum. Rats were ovariectomized and implanted with a silastic tube containing 5% E2 in cholesterol, previously shown to mimic low physiological serum concentrations of ~ 20-25 pg/ml. DA release was evoked every 1 min by delivering biphasic electrical stimulation in the substantia nigra. Local infusions of E2 (244.8 pg/µl) into the dorsal striatum increased the amplitude of the electrically evoked DA transients. Behaviorally significant stimuli and events trigger phasic release of DA. The present findings predict that E2 would boost such signaling in behaving subjects.

KEYWORDS

Estrogens, fast-scan cyclic voltammetry

INTRODUCTION

Striatal dopamine (DA) transmission is important for many functions including cognition, motor control, movement, learning, motivation, and reward [1]. Such functions are also influenced by 17β-estradiol (E2). It is well established that estrogens modulate DA signaling in the dorsal striatum (DS) [2-8]. E2 is the most highly concentrated circulating estrogen during reproductive years in females [9], and replacement of this hormone in ovariectomized (OVX) rats rapidly (within 30 min) restores and enhances amphetamine (AMPH)-induced striatal DA release [3-5,8,10]. Initially, such a rapid increase in striatal DA was shown in *in-situ* slice preparations or with systemic injection of E2 [2-8]. Recently, we have shown via *in-vivo* microdialysis that local infusions of E2 into the DS also result in a greater AMPH-induced release of DA in the dorsal striatum [11].

Release of DA varies along a continuum of timescales [12] ranging from sustained "tonic" to episodic "phasic" release [13]. Tonic DA release maintains concentrations of extracellular DA that depend, among other influences, on baseline firing. Phasic DA release, on the other hand, is driven by burst firing and occurs in response to behaviorally salient stimuli [13]. The temporal resolution of the *in-vivo* microdialysis method is low. Thus, to determine at what points along the tonic-phasic continuum E2 modulates dopaminergic neurotransmission in the DS, it is necessary to complement the existing microdialysis data with observations obtained by means of a method with higher temporal resolution.

Although the local effect of estrogens on phasic DA release in the DS has not been reported previously, the effects of estrogens on DA release in the nucleus accumbens [14] and the central nucleus of the amygdala [15] have been examined using *in vivo* voltammetry. Direct

infusion of E2 into the nucleus accumbens results in a rapid increase in phasic K^+-stimulated DA release [14], whereas subcutaneous estradiol benzoate (another estrogen) increases electrically-evoked phasic DA release in the central nucleus of the amygdala [15] in OVX rats. In the current study, the substantia nigra (SN) was electrically stimulated and striatal phasic DA release was measured in response to a local infusion of E2 or its vehicle (cyclodextrin) in the DS. Phasic DA release was measured using fast-scan cyclic voltammetry (FSCV) in anesthetized OVX female rats. It was hypothesized that E2 acts directly within the DS to rapidly increase phasic DA transmission.

METHODS

Animals

Female Long-Evans rats (Concordia University in-house breeding colony) weighing 220-260 g and 60-65 days old, were single housed at 21°C in polypropylene shoebox cages with beta-chip bedding. A 12:12 h reverse light/dark cycle was used with lights off at 9:00 AM. Rats had access to *ad libitum* Purina rat chow and water. All handling, testing and surgical procedures were performed during the dark phase of the diurnal cycle. All protocols were previously approved by Concordia University's animal research ethics committee and were in accordance with the guidelines of the Canadian Council on Animal Care.

Surgery

In preparation for the ovariectomy surgery, twelve rats were anesthetized using Isoflurane (4% for induction; 2% for maintenance: Inhalation Anesthetic, Richmond Hill, ON, Canada) and bilaterally ovariectomized through a dorsal incision (1 cm). Post-ovariectomy, all rats were implanted with a silastic tube (1 cm long: 1.96 mm OD, 1.47 mm ID) containing 5% E2 (Sigma

Chemical Co., St. Louis, MO, USA) in cholesterol (Sigma) in the nape of the neck. The silastic tube containing E2 was previously shown to mimic low physiological serum concentrations of ~ 20 – 25 pg/mL consistent with estrus [16]. The purpose of such a low chronic E2 administration was to keep the E2 receptors upregulated so as to maintain their sensitivity to later microinfusions of E2. These capsules have been shown to produce consistent E2 serum levels up to 4 weeks following implantation [16]. For post-surgical care, all rats were administered the anti-inflammatory drug, Anafen (0.1 mL/rat, SC; MERIAL Canada Inc., Morgan Baie d'Urfe, QC, Canada) and the antibiotic Penicillin G (0.2mL/rat, intramuscular; CDMV, St. Hyacinthe, QC, Canada); antibiotic ointment (By Pharmaceuticals Inc., Brampton, ON, Canada) was applied to the incision. Following surgery, all rats were allowed at least one week to recover in their home cages. The rats were then pseudorandomly assigned to one of the two groups, E2 (n = 6) or cyclodextrin (CYC; n = 6) alone and were tested between days 8 and 21 post ovariectomy.

FSCV recordings

Each rat was anesthetized using urethane (1.5 mL/kg, IP), given an injection of atropine sulphate (0.05 mg/kg SC; Sandoz Canada Inc., QC, Canada) to reduce bronchial secretions during surgery, and given an injection of saline (1.0mL SC; Baxter, Baxter Corporation Mississauga, ON) every 2 hours to prevent dehydration. Prior to placing the rat in the stereotaxic apparatus (David Kopf Instruments; Tujunga, CA, USA), tear gel (Alcon Canada Inc., Mississauga, ON, Canada) was applied to the surface of the eyes, to avoid dryness, and the topical anesthetic, xylocaine (AstraZeneca Canada Inc., Canada), was applied to the external auditory meatus of the rat to reduce discomfort from the ear bars. The two stimulation electrodes were stereotaxically implanted, secured with dental acrylic, and anchored with jeweler's screws.

The stimulating electrode, consisting of a 0.25 mm stainless steel insect pin was insulated with Formvar enamel to within 0.5 mm of the tip. It was aimed at the following coordinates in the right SN (referenced to bregma); anteroposterior (AP): -4.8 mm; medial-lateral (ML): 2.4 mm; dorsoventral (DV): -7.9 mm [17]. A second electrode was made in the same manner as the first, but 3 mm of insulation was stripped at the tip. This electrode was aimed at the lateral orbital cortex (AP: 4.3 mm; ML: 2.4 mm; DV: -5.5 mm) to serve as the anode of the stimulation circuit. The coordinates for the anode were chosen in a similar manner to previously reported methods [18]. Briefly, the placement of the anode was chosen to place the recording site mid-way between the cathode (stimulating electrode) and the anode of the stimulation circuit. The reference electrode was then placed on or near the zero potential surface of the electrical field around the stimulation current to reduce electrical artifacts in the voltammetric recordings.

Hormone administration

During the experiment, all rats received a local (1.0 µl) intracerebral microinfusion, at a rate of 0.2 µl/min, of either the E2-CYC complex (244.8 pg/µl; Sigma), or the vehicle, CYC (5.196 ng/µl; Sigma), mixed in sterile saline (Hospira, IL, USA). E2 was encapsulated in CYC to make it water-soluble. We have shown this dose of E2 to be effective when infused into the DS to increase AMPH-induced striatal DA release [11] and to alter memory system bias when infused into the medial prefrontal cortex [19].

Voltammetry

The carbon fiber (Thorne, Amoco Corporation, Greenville, SC, USA) was glass-encased, and a seal was produced by heating the glass capillary with a pipette puller (PUL-1, WPI, Sarasota, FL, USA). A wire covered with silver paint (GC Electronics, Rockford, IL, USA) was inserted in the capillary to make contact with the carbon fiber and secured with shrink tubing

coated with epoxy (150-200 µm exposed tip length, 7 µm diameter). A 21 Ga stainless steel tube (30 mm in length) was secured with shrink tubing to the carbon fiber electrode and spaced 1 mm laterally and 1 mm above the exposed carbon fiber. This tube served as an injector needle holder. The carbon fiber electrode (working electrode) was aimed at the DS (AP: -0.3 mm, ML: 2.4 mm, DV: -4.0 mm – -5.6 mm). FSCV was computer-controlled as described previously [20]. Briefly, background-subtracted cyclic voltammograms were generated at 10 Hz by applying an 8.5 ms triangular waveform that ramped from -4.0 V to +1.3 V and back to -0.4 V at a scan rate of 400 V/s. The potential was held at -0.4 V between scans to promote cation absorption at the surface of the FSCV electrode. All potentials were measured with respect to the Ag/AgCl reference electrode. The waveform was generated using LabVIEW (National Instruments, Austin, TX) and a multifunction data acquisition board (PCI-6052E, National Instruments, Austin, TX).

A PCI-6711E board (National Instruments, Austin, TX) was used to perform waveform acquisition, and data collection. A synchronization signal from the PCI-6711E board was sent to the external input of a multi-channel pulse generator (Master-8, A.M.P.I. Jerusalem) and used to trigger the electrical stimulation 5 s after the start of each recording. The stimulation was patterned to prevent overlap with the voltammetric scans. This was accomplished by confining pulse generation to the 91.5 ms intervals separating the triangle waves. Voltages generated by the Master-8 pulse generator were converted to constant currents via a stimulus isolation unit (AM-2200, AM-Systems, Carlsborg, WA).

Once the carbon fiber electrode was in place, six baseline voltammograms were collected. DA release was evoked every 1 min by delivering electrical stimulation in the SN (500 ms trains duration, 2 ms pulse duration, 200 – 400 μA, 60Hz). Following collection of the baseline voltammograms, an intracerebral microinfusion of either E2 or CYC was administered

at a rate of 0.2 µl/min to yield a total volume of 1.0 µl. The injector remained in place for the duration of the collection period to prevent any additional disruption close to the recording site. Immediately at the end of the infusion, four blocks of six voltammograms were collected, with a 5 min inter-block interval. During the inter-block intervals, the carbon fiber was cycled at 60 Hz to maintain sensitivity to changes in DA concentration.

Statistical Analyses

The electrically evoked DA transients obtained during baseline testing were compared to electrically evoked DA transients recorded following vehicle or E2 infusion. To render these responses comparable, the peak of each DA transient was normalized to its respective averaged baseline. This was achieved by dividing each peak by the average of the six baseline peaks. Matlab (Natick, MA, USA) was used to perform resampling with replacement (bootstrapping with 1000 iterations) so as to assess the statistical reliability of the effects [21]. The criterion for a statistically reliable effect in the resampling analysis was an absence of overlap between the 95% confidence intervals around the results from the E2 and vehicle conditions.

Histology

After the completion of the experiment, a lethal injection of sodium pentobarbital (120mg/rat, IP) was administered. The location of the stimulating and recording electrodes was marked by passing 1 mA of anodal current for 20 s. The animals were then perfused intracardially with 0.9% sodium chloride, followed by a formalin-Prussian Blue solution (10% formalin, 3% potassium ferricyanide, 3% potassium ferrocyanide, and 0.5% trichloroacetic acid) that forms a blue reaction product from the iron deposited at the tip of the stimulating electrode. Next, the brains were removed and fixed with 10% formalin solution. 40 µm thick, coronal sections were cut with a cryostat (Thermo Electron Corporation) and examined to confirm

placements of the FSCV electrode and injector in the DS and the stimulating electrode in the SN (*see* Figure 1).

RESULTS

DA transients were time-locked to the electrical stimulation, and all voltammograms had a signature characteristic of DA, with an oxidation peak at roughly 0.65 V and a reduction peak at roughly -0.2 V (*see* Figure 2 for a representation of DA transients prior to and following E2 or CYC administration).

The peaks of the DA responses following E2 infusion were systematically higher than the peaks in the corresponding baseline voltammograms (Figure 3A) and were maximal by the end of the 5-min infusion. There is no overlap between the 95% confidence intervals surrounding the DA responses observed during the baseline, thus meeting our criterion for a statistically reliable effect. In contrast, no differences were found between the peaks of the DA responses following CYC infusion and the corresponding baseline voltammograms (Figure 3B).

DISCUSSION

Infusions of E2 increased the amplitude of electrically-evoked DA transients in the DS, as measured by FSCV, whereas the CYC vehicle was without effect. These findings support the hypothesis that E2 acts directly within the DS to rapidly increase phasic DA transmission. In previous studies employing microdialysis [2,4,5,7,8,11], increases in the extracellular concentration of DA in the DS were observed, but due to the low temporal resolution of the measurement method, it was not possible to determine the degree to which changes in tonic or phasic release were responsible. The present results suggest that potentiation of phasic DA release by E2 could have contributed to the changes in DA concentration observed previously in

microdialysis samples following administration of E2 to the DS. To our knowledge, this study is the first to show that direct infusion of E2 into the DS increases phasic DA release *in vivo*.

The effect of E2 on phasic DA release was maximal by the end of the 5-min infusion. This short latency suggests that E2 must have acted non-genomically in the DS. Electron microscopy has shown that estrogen receptor (ER)α, ERβ, and G-protein coupled estrogen receptor-1 are localized at the membrane of neurons within the DS [22]. These receptors are rarely found in the nuclei of neurons within the DS. In fact, striatal ERs are most frequently observed in the axons and terminals of γ-aminobutyric acid (GABA)ergic [23] and cholinergic neurons, as well as in glia [22]. Interestingly, ERs are not seen on DA terminals in the DS. However, $GABA_B$ receptors are located on presynaptic DA terminals, and play a critical role in the inhibition of striatal DA release (*for review, see* [24]). Given that systemic injections of E2 rapidly reduce striatal GABA concentrations [25], the presence of E2 in the DS could indirectly increase striatal DA release through attenuation of GABA release. Such effects could be mediated by membrane-bound ERα on medium-spiny GABA neurons [24]. Supporting evidence is provided by recent findings by our laboratory showing that ERs are present on the membrane of GABAergic neurons [22,23]. Thus, the rapid effects of E2 on phasic DA release in the DS reported here could be due to E2 action at membrane-associated ERs on the terminals of GABA neurons. Membrane-associated ERs are also found on cholinergic neurons in the DS [22] which could also have contributed.

The findings of the current study show that in addition to any effects of E2 on tonic DA signaling in the DS, E2 boosts phasic DA signaling. These results have implications for the influence of estrogens on processes dependent on phasic DA signaling, such as learning, movement, and motivation.

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FIGURE LEGENDS

Figure 1. Schematic representation of (A) carbon fiber and injector placements in the dorsal striatum and (B) electrode placements in the substantia nigra. Black lines represent all E2 group carbon fiber placements, while grey lines represent all CYC group carbon fiber placements. Black circles represent all E2 group injector or electrode placements, while grey circles represent all CYC group injector or electrode placements. E2 = 17β-estradiol; CYC = cyclodextrin.

Figure 2. Dorsal striatal dopamine transients prior to and following drug administration. A) Dopamine traces prior to and following E2 administration. B) Dopamine traces prior to and following administration of the vehicle, CYC. E2 = 17β-estradiol; CYC = cyclodextrin.

Figure 3. Dorsal striatal dopamine concentrations in response to E2 or the vehicle, CYC, administration. A) Response to E2, and B) CYC administration in comparison to baseline, normalized using all baseline measures. It should be noted that error bars in panel B are smaller than the symbols. Error bars represent 95% confidence intervals around the mean. E2 = 17β-estradiol; CYC = cyclodextrin.

Dorsal Striatum

Substantia Nigra