Title
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Journal
Molecular Psychiatry, 26(6)

ISSN
1359-4184

Authors
Petersen, Nicole
Rapkin, Andrea J
Okita, Kyoji
et al.

Publication Date
2021-06-01

DOI
10.1038/s41380-020-01000-1

Peer reviewed

HHS Public Access

Author manuscript

Mol Psychiatry. Author manuscript; available in PMC 2022 June 14.

Published in final edited form as:

Mol Psychiatry. 2021 June ; 26(6): 2038–2047. doi:10.1038/s41380-020-01000-1.

Striatal dopamine D_2-type receptor availability and peripheral 17β-estradiol

Nicole Petersen[1,2,3], **Andrea J. Rapkin**[4], **Kyoji Okita**[5,6], **Kaitlin R. Kinney**[1,2,3], **Tomi Mizuno**[1,2], **Mark A. Mandelkern**[3,7], **Edythe D. London**[1,2,3,8]

[1]Jane and Terry Semel Institute for Neuroscience and Human Behavior at UCLA, Los Angeles, CA 90095, USA

[2]Department of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA 90095, USA

[3]Veterans Administration of Greater Los Angeles Health System, Los Angeles, CA 90073, USA

[4]Department of Obstetrics and Gynecology, David Geffen School of Medicine, UCLA, Los Angeles, CA 90095, USA

[5]Department of Clinical Neuroimaging, Integrative Brain Imaging Center, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo 187-8551, Japan

[6]Department of Drug Dependence, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo 187-8551, Japan

[7]Department of Physics, University of California at Irvine, Irvine, CA 92697, USA

[8]Department of Molecular and Medical Pharmacology, UCLA, Los Angeles, CA 90095, USA

Abstract

Research using rodent models has established a relationship between the steroid hormone estrogen and dopamine function, by revealing changes throughout the estrous cycle and by directly manipulating neuroendocrine signaling through ovariectomy and administration of estrogen. However, a direct link between estrogen levels and dopamine signaling had not been established in humans. The goal of this study, therefore, was to assess the relationship between circulating 17β-estradiol and dopamine signaling in the human brain by testing for a relationship between two proxies for these variables: peripheral 17β-estradiol and striatal dopamine D_2-type receptor availability, measured with [^{18}F]fallypride and positron emission tomography (PET). Sixteen (23–45 years of age) women were tested on 2 days of the menstrual cycle estimated prospectively to occur during (a) the early follicular phase, when estrogen levels are near their nadir, and (b) the periovulatory phase, when estrogen levels peak. PET scans with [^{18}F]fallypride were performed on these 2 days, and serum 17β-estradiol was measured using radioimmunoassay. Dopamine D_2-type receptor availability did not differ significantly in the whole striatum or the caudate, putamen, or accumbens subregions during the high-estrogen vs. the low-estrogen phases of the menstrual cycle. We conclude that circulating estrogen levels do not affect dopamine D_2-type

✉Nicole Petersen, npetersen@ucla.edu, Edythe D. London, elondon@mednet.ucla.edu.

Conflict of interest The authors declare that they have no conflict of interest.

receptor availability in the human striatum although other indices of dopaminergic function may be affected.

Introduction

Sex differences have been observed in the etiology, epidemiology, and efficacy of treatment of neuropsychiatric conditions related to dopaminergic function. For instance, Parkinson's disease (PD), a disorder associated with loss of dopaminergic neurons, affects approximately twice as many men as women and presents with a somewhat different symptom profile in men vs. women [1–3]. Research into sex differences in addictive disorders has indicated that men are more likely than women to report using illicit drugs, tobacco products, and alcohol over the course of their lifespans [4], whereas women report some substance-specific challenges, including greater difficulty maintaining long-term abstinence from cigarettes [5], more psychosocial problems as a consequence of alcohol use disorder [6], and worse quality of life while dependent on opioids [7].

Sex differences in substance use disorders and their features are frequently attributed to the influence of ovarian hormones on responses to drugs of abuse. A 2012 review states, "There is substantial evidence that sex differences in the response to stimulants are due in large part to the fluctuations in estrogen (E2) and progesterone (P) that occur over the female reproductive cycle" [8]). Another review article from 2014 specifically links estrogen and dopamine (DA) function, arguing, "Sex differences in physiological and psychological responses to drugs of abuse are well documented and it is well established that estrogen effects on DA systems are largely responsible for these sex differences" [9].

These conclusions are well-justified by preclinical literature, which has consistently linked estrogen to dopaminergic neurochemical and behavioral markers [10–23], especially D_2-type (i.e., $D_2 + D_3$) receptors [19, 24–28]. Specifically, administration of estrogen reduced the density of striatal D_2-type receptors in ovariectomized female rats as measured by [^{3}H]methylspiperone binding [24], but increased striatal D_2-type receptor density in intact male rats [19]. Similarly, in cynomolgus monkeys, striatal D_2-type receptor availability (determined as binding potential referred to non-displaceable binding [BP_{ND}]), for [^{18}F] fluoroclebopride, was 11.7% higher in the luteal phase of the menstrual cycle compared to the early follicular phase, although estrogen levels were not assayed [29].

Human imaging of healthy control research participants had provided some evidence of sex differences in striatal dopaminergic neurochemical markers. With [^{11}C]raclopride as the radioligand, PET showed higher striatal D_2-type receptor affinity (determined via the equilibrium dissociation constant, K_d) in men than in women [30], greater amphetamine-induced DA release, indicated by reduction in BP_{ND}, in men than in women [31], and no sex difference in baseline BP_{ND} [31]. A trend toward greater DA release in seven men as compared with six women was also obtained with [^{18}F]fallypride ($p < 0.1$) [32], and the finding was replicated in a group of young (mean age = 22.4 years) but not older (mean age = 41.36 years) adults [33]. In addition, smoking a cigarette [34] or drinking ethanol [35] induced greater ventral striatal DA release, measured as reduction in [^{11}C]raclopride BP_{ND}, in men than in women. Thus, it appears that ventral striatal DA release in response to drug

challenges is greater in men than in women, although the effect of sex may be tempered by age.

Sex differences, especially related to cigarette smoking, extend to other brain regions. The sex difference in smoking-induced striatal DA release may be mediated by greater midbrain D_2-type autoreceptor density in women than in men who smoke [36]. This difference does not appear in individuals who do not smoke, and may lead to a downregulation of striatal DA D2-like receptors due to smoking in men but women. Notably, men but not women who smoke have lower striatal D2-type receptor availability [37, 38]. In addition, women who smoke exhibit less amphetamine-induced DA release, measured with [^{11}C] FLB457, in the prefrontal cortex compared to their counterparts who do not smoke [39], whereas men do not show this smoking-related difference.

Although sex differences in DA neurochemistry have been repeatedly observed and generally attributed to ovarian hormones, studies directly evaluating effects of estrogen on dopaminergic neurochemical markers have produced equivocal results. A cross-sectional study using [^{11}C]FLB457 reported no relationship between ovarian hormones and DA release or DA D2-type receptor availability in women who did or did not smoke cigarettes [39]. Wong et al. [40] found a trend toward a higher rate constant of receptor binding (k3) of (3-N-[^{11}C]-methyl)spiperone in high-estrogen phases of the menstrual cycle in six women during six different menstrual cycle phases, while Nordström et al. [41] found no effect of menstrual phase on the ratio of putamen:cerebellar D_2-type receptor binding potential using [^{11}C]raclopride. Another study using [^{11}C] raclopride [31] found lower putamen (but not caudate or ventral striatal) D_2-type receptor availability in women during the luteal phase compared to the early follicular phase, but no significant correlation between estradiol and D_2-type receptor binding potential or DA release. At least part of the discrepancy in the findings may reflect the known contribution of variations in endogenous DA to the binding of [^{11}C]raclopride and other radiolabeled benzamide DA receptor antagonists [42].

It is difficult to draw an unambiguous conclusion regarding the relationship between 17β-estradiol and striatal D_2-type BP_{ND} from these existing studies. Notably, none were designed specifically to interrogate this relationship, and all but one involved between-subjects comparisons. Inter-individual variation in ovarian hormone levels is considerable [43], pointing to a need for a within-subjects design to address this question. Using best practices for menstrual cycle phase determination in human biobehavioral research [44], we therefore tested the hypothesis that higher circulating 17β-estradiol (E2) levels during periovulation would be associated with lower striatal D_2-type BP_{ND} measured with [^{18}F]fallypride PET than in the early follicular phase. Sixteen women underwent PET scanning during two menstrual cycle phases, once shortly after the onset of menses, when E2 levels are relatively low, and again when they were anticipated to be experiencing their periovulatory E2 peak. Striatal DA D_2-type receptor BP_{ND} was compared between these two menstrual phases.

Methods

All study procedures were approved by the Institutional Review Boards at both the University of California, Los Angeles, and the Greater Los Angeles Department of Veterans

Affairs. The experiment had a repeated-measures, quasi-experimental design. Sample size was determined a priori on the basis of effect size estimates from prior literature [31]. Eighteen women participated in two [^{18}F]fallypride PET scans, one when they were anticipated to be in the early follicular phase of the menstrual cycle (days 0–5), and again when they were projected to be in the periovulatory estrogen peak based on mid-cycle timing and urinary luteinizing hormone (LH) assessments. LH testing to predict ovulation has been established as a reliable measurement [45], and is both convenient enough to perform at home without sacrificing accuracy [46].

Participants and recruitment

Women between the ages of 18 and 44 were recruited from the Los Angeles community via Internet advertisements on Craigslist. After an initial phone screening interview to determine eligibility, all participants provided written, informed consent. They were required to have regular menstrual cycles every 24–32 days, and not to be pregnant or breastfeeding. Participants were excluded if they: were unable to undergo MRI due to claustrophobia or metal implants; self-reported a lifetime history of neurological or endocrine disorders; self-reported any substance use disorders within the previous 2 years; used hormonal contraception within the previous 3 months; or experienced menstrual-related affective and physical symptoms that met criteria for Premenstrual Dysphoric Disorder according to the Daily Record of Severity of Problems (DRSP) [47]. Mental health histories were assessed using the Mini International Neuropsychiatric Interview [48], and participants were excluded for mental health conditions other than a past major depressive episode (>6 months before study entry). Basic demographic information is provided in Table 1.

Study design (see Fig. 1 for graphical overview)

Eligible participants were asked to monitor the onset of their menstrual cycle and menstrual-related symptoms as described on the DRSP using a custom-built website each day until two complete menstrual cycles had taken place. During this cycle-monitoring phase, participants were also asked to use LH urine tests (Clearblue®, Geneva, Switzerland) at home to identify when their periovulatory estrogen surge was likely to take place.

If LH tests were positive more than three cycle days apart (e.g., positive on cycle day 10 during the first monitored cycle, and positive on cycle day 14 during the second monitored cycle), participants were asked to complete one or more additional months of cycle monitoring until a consistent pattern emerged; participants with consistently too much (>3 days) inter-cycle variability were excluded.

Once two positive LH tests within three cycle days of one another were observed, participants were scheduled for MRI and PET scanning. Participants were scheduled for one session that was anticipated to take place on or one day after a positive LH test and one session that was anticipated to take place during the early follicular phase (within the first 8 days of the cycle, erring on the earlier side for participants with shorter cycles). This scheduling procedure was designed to maximize the low- vs. high-estrogen difference (see Table 2 for details on cycle position).

High-resolution structural MRI scans were obtained for co-registration with PET images for anatomical sampling; these scans were performed on a separate day from PET scans to reduce the burden on participants.

Test day procedures

On the days when participants were scheduled for PET scans, they arrived in the laboratory for breath and urine testing to verify absence of pregnancy and abstinence from alcohol, cigarettes, and drugs of abuse. Subsequently, ~4 mL of blood was drawn by venipuncture for hormone analysis.

MRI acquisition

High-resolution structural MRI images were acquired using a 3-Tesla Siemens Prisma scanner equipped with a 32-channel head coil. A magnetized-prepared gradient echo sequence was used, with slice thickness = 0.8 mm, TR = 2400 msec, TE = 2.24 msec, flip angle = 8°, acquisition time = 395 s, and FOV = 240 × 256 mm^2.

Positron emission tomography (PET) acquisition

PET and CT images were acquired with a Philips Gemini Tru Flight PET/CT (Philips Medical Systems, Eindhoven, Netherlands). CT transmission scan images were acquired immediately before PET images for use in attenuation correction. A bolus injection of [^{18}F]fallypride (5 mCi ± 10%), specific activity ⩾ 1 Ci $\mu$$mol^{-1}$ at time of injection was administered to participants coincident with the onset of PET emission scan acquisition. PET images were acquired in three-dimensional mode, then reconstructed using the 3D row action maximum likelihood algorithm (RAMLA) [49] to obtain images with 2 × 2 × 2 mm^3 voxel resolution in 160 1 min frames. The data were acquired in two 80 min scanning blocks, with a 20 min break when participants were removed from the tomograph and voided to reduce radiation exposure to the bladder. The total scan session had a duration of 180 mins.

PET analysis

Reconstructed, attenuation-corrected PET images in 1 min frames were merged into sixteen 10 min frames, then processed using in-house pipelines applying FSL tools. Images were motion-corrected using MCFLIRT [50] and then co-registered to each participant's T1-weighted structural MRI using FSL's nonlinear registration tool. Volumes of interest were anatomically defined with FIRST [51], and a manually-drawn cerebellum VOI created in MNI-152 space was transformed to native space for each participant for use as a reference region [52]. Time-activity data were extracted for each VOI and fit using the simplified reference tissue model [53] in PMOD (PMOD 3.1, Zurich, Switzerland) to calculate BP_{ND} with time-activity curves from VOIs as follows: $C_T(t) = R1C_R(t) + (k2 - R1k2/(1 + BP_{ND}))C_R(t) * \exp(-k2t/(1 + BP_{ND}))$ where $C_T(t)$ is the total radioactivity concentration in the striatum VOI measured by PET, R1 is the ratio of K1 to K1′ (K_1, influx rate constant for the striatum; K1′, influx rate constant for the cerebellum), $C_R(t)$ is the radioactivity concentration in the reference region (cerebellum), and * denotes the convolution integral.

The parameters R1, k2, and BP_{ND} in this model were estimated by a nonlinear curve-fitting procedure.

Statistical analysis

The effect of peripheral 17β-estradiol levels on striatal BP_{ND} was measured using a linear mixed model considering BP_{ND} in the whole striatum as well as three major subdivisions: the nucleus accumbens, caudate nucleus, and putamen.

Covariates

Effects of age, years of education, and body mass index (BMI) on BP_{ND} were tested by a linear mixed model to determine empirically if these variables carried influence on striatal BP_{ND} and therefore needed to be included in the planned statistical hypothesis tests. Age was significantly related to BP_{ND} in the whole striatum ($p = 0.0026$), caudate nucleus ($p = 0.0038$) and putamen ($p = 0.0009$), but not the nucleus accumbens ($p = 0.29$). Years of education and BMI did not have significant contributions, all ps > 0.2. In the interest of thoroughness, we tested for an age-by-E2 interaction on BP_{ND} in these VOIs and the effect was not significant in any of them (ps > 0.1). Progesterone was not independently related to any BP_{ND} measurements, nor did covarying for it change the outcome of any analyses; therefore, it was not included in the results reported above.

E2 as a categorical variable

Because two 17β-estradiol measurements were obtained for each participant, PET imaging sessions were categorized post hoc as low- or high E2 for each individual participant relative to the other reading (e.g., an estradiol level of 100 pg/mL would be classified as “low estrogen” for a participant whose other session was 200 pg/mL, but would be classified as “high estrogen” for a participant whose other session was 40 pg/mL). Effects of 17β-estradiol on striatal BP_{ND} were tested using a linear mixed model, with E2 treated as a categorical variable (low/high) and entered as a fixed effect, and participant as a random effect. Age was entered as a covariate.

E2 as a continuous variable

A second analysis was performed using the same linear mixed model, but treating E2 as a continuous variable instead, with raw values of E2 entered as fixed effects.

We also performed analyses to test whether the amount of change in E2 and was related to the amount of change in BP_{ND} in the striatal VOIs. Change scores were calculated as follows:

$$\textit{E2 change score} = [\text{E2 concentration during high E2 session}] - [\text{E2 concentration during low E2 session}]$$

$$BP_{ND}\ \textit{change score}(\text{calculated separately for each VOI}) = [\text{BPND during high E2 testing session}] - [BP_{ND}\ \text{during low E2 testing session}.$$

The two change scores were entered into a linear regression model to test their association with to one another in each VOI. Although our hypotheses centered on potential effects of E2, serum progesterone concentrations were determined. To rule out whether changes in progesterone affected the E2 effect on striatal BP_{ND}, we also tested for association of the ratio of progesterone to E2 concentration with striatal BP_{ND}.

Bayes factors

Because the sample size was small, confidence in null hypothesis statistical testing was limited. Therefore, we decided post hoc to include Bayes factor estimation to evaluate the effect of E2 on BP_{ND} in the three main VOIs of interest (accumbens, caudate, putamen). Bayesian repeated measures ANOVAs were calculated using JASP [54] (v. 0.14, Amsterdam, Netherlands). Estrogen level (low vs. high) was entered as a repeated measures factor with age as a covariate.

Exploratory analysis (extrastriatal VOIs)

Because [^{18}F]fallypride PET allows determination of extrastriatal binding to DA D2-type receptors, we performed an exploratory evaluation testing the effect of low vs. high E2 on BP_{ND} in eight extrastriatal VOIs. The VOIs were defined and BP_{ND} calculated using the same procedure we used to calculate striatal VOI BP_{ND} (see above). The main effect of low vs. high E2 was evaluated using an identical linear mixed model approach to that described above for striatal regions.

Hormone analysis

Serum 17β-estradiol and progesterone levels were measured by electrochemiluminescence (Roche Elecsys Immunoassay System, F. Hoffman-La Roche, Basel, Switzerland) at the UCLA Clinical Laboratory and Pathology Services.

Results

Hormone levels and session timing

The change in serum concentration of 17β-estradiol varied for each participant, with a minimum percent increase from the lower to higher reading of 35.6%, and a maximum increase of 744.7% (Fig. 2). Three participants had serum progesterone concentrations above 2 ng/mL, indicating that ovulation had likely occurred (Table 3). Including or excluding these participants did not alter any results subsequently reported. The difference between the low-estrogen and high-estrogen sessions was highly statistically significant, $F_{(1,17)} = 36.65$, $p = 0.00001$.

Efforts were made to schedule one session during the early follicular phase and one session during the periovulatory estrogen surge for each participant, but the scan session used for the low- vs. high-estrogen analysis was assigned post hoc on the basis of serum estrogen levels. Thus, scan scheduling was tailored to each individual participant's cycle. The cycle days on which the low- and high-estrogen testing sessions occurred are shown in Table 2.

Striatal dopamine D2-type receptor BPnd when peripheral estrogen was relatively low vs. relatively high

There were no differences between the sessions in the mean injected dose [low-estrogen scan = 5.10 ± 0.30 (mean ± SD in mCi); high-estrogen scan = 5.10 ± 0.33 (mean ± SD in mCi); $p = 0.62$], specific activity at time of injection [low estrogen scan = 17.5 ± 12.2 (mean ± SD in Ci/μmol); high-estrogen scan = 16.8 ± 11.0 (mean ± SD in Ci/μmol); $p = 0.88$), or injected dose [low-estrogen scan = 5.08 ± 0.31 (mean ± SD in mCi); high-estrogen scan = 5.05 ± 0.33 (mean ± SD in mCi); $p = 0.62$].

A linear mixed model treating E2 as a categorical variable revealed no significant effect of E2 level (low vs. high) on striatal BP_{ND}. This was true for the whole striatum, $F_{(1,15)} = 0.048$, $p = 0.829$, Cohen's $f = 0.0171$, and for all subdivisions tested: whole accumbens, $F_{(1,15)} = 0.013$, $p = 0.910$, Cohen's $f = 0.117$; whole caudate, $F_{(1,15)} = 0.320$, $p = 0.580$, Cohen's $f = 0.235$; and whole putamen, $F_{(1,15)} = 0.108$, $p = 0.747$, Cohen's $f = 0.198$ (Fig. 3). Effect sizes were small in whole striatum and nucleus accumbens, approaching medium in the caudate nucleus and putamen. A post hoc power analysis indicated that with the observed effect size in the whole striatum, 117 women would need to be tested to achieve $\beta = 0.8$.

The correlation between the BP_{ND} values at low and high E2 BPs were as follows: whole striatum, $r = 0.627$; nucleus accumbens, $r = 0.540$; caudate nucleus, $r = 0.445$; and putamen, $r = 0.599$.

The results were the same when entering E2 as a continuous variable instead. No significant relationships between E2 and whole striatum, accumbens, putamen, or caudate BP_{ND} were observed, all ps > 0.2.

Bayes factors

A Bayesian repeated measures ANOVA was used to evaluate whether the data better fit the null hypothesis (no effect of E2 on BP_{ND}) or the alternative hypothesis (effect of E2 on BP_{ND}). The Bayes factor for the alternative over the null (BF_{10}) was 0.387 for caudate BP_{ND}, 0.347 for accumbens BP_{ND}, and 0.348 for putamen BP_{ND}. The Bayes factor for the null over the alternative was the inverse for each, or (BF_{01}) 2.693 for caudate BP_{ND}, 4.146 FOR accumbens BP_{ND}, and 2.792 for putamen BP_{ND}.

Exploratory analyses of extrastriatal VOIs

Linear mixed models indicated that BP_{ND} did not differ between testing sessions in any exploratory VOI (Table 4).

Discussion

This study tested the hypothesis that striatal DA D_2-type receptor availability decreases in response to rising 17β-estradiol levels as the menstrual cycle proceeds from the early follicular to the periovulatory phase. Earlier studies used the menstrual cycle as a proxy for hormone levels, compared 17β-estradiol between subjects as a secondary component of an existing sex differences analysis, or evaluated women at random intervals throughout the

menstrual cycle. This is the largest study to measured 17β-estradiol directly in the same women twice, coinciding with measurements of D_2-type receptor availability.

There were no significant changes in D_2-type receptor availability in the whole striatum or the subregions tested (caudate, putamen, accumbens) comparing early follicular and periovulatory phases of the cycle. Thus, in spite of ample evidence from preclinical literature using rodent models, and limited evidence in six humans [40] and seven nonhuman primates [29], varying estradiol levels in women may not be an evidence-based explanation for observed sex differences in dopaminergic function that are linked to neuropsychiatric disorders. This is consistent with one previous study in which five women were evaluated during two time points in the menstrual cycle, with cycle phase varying between participants (e.g., one was scanned during the follicular phase and again during the follicular phase; another was scanned once during the follicular phase and again during the luteal phase; [41]). Studies evaluating the relationship between estradiol and other measures of DA function, such as DA release and DA transporter availability, are still needed, although existing studies also do not support such a relationship [33, 39, 55].

There are several reasons why these PET imaging findings in human research participants may diverge from those reported in rodents. One possibility is that the time-course of E2 effects on D_2-type receptors was not captured; estrogen-induced changes in receptor density may occur either so rapidly (hours, as observed in [24]) or so slowly (4–8 days, observed in [19]) that single measurements on 2 days of the cycle are not informative, and serial measurements over 4–8 day period following the periovulatory estrogen peak may be needed. Another possibility is that the supraphysiological doses of estrogens (e.g., estradiol benzoate), administered to rodents, exceeded comparable concentrations produced by menstrual-related fluctuations, although at least one study observed differences in rodent striatal D_2-type receptor binding sites over the estrous cycle [25]. A third possibility is that the experimental paradigm engaged by the majority of animal studies, in which animals are ovariectomized and given add-back hormones, does not capture the neuroendocrine dynamics that take place in a naturalistic setting for reasons that are currently unknown. A fourth possibility is that serum 17β-estradiol concentrations do not reflect striatal 17β-estradiol levels, which are currently not measurable in vivo in humans, although at least one review describes a tight temporal relationship between peak striatal 17β-estradiol and peak peripheral 17β-estradiol in rodents [56]. Finally, the discrepancy may simply reflect a species difference in the association of circulating estradiol with dopaminergic function.

Some strengths and limitations influence the interpretation of this study. Strengths include the within-subject design, use of LH tests to estimate day of ovulation, and measurement of 17β-estradiol and progesterone in serum. In addition, test-retest variation in BP_{ND} measured with [^{18}F] fallypride is similar to that of other D_2 radioligands, and is generally small in receptor-rich regions [57], making it unlikely that such variation would obscure changes due to estrogen. Specifically, data from 16 healthy subjects (mostly men) collected at intervals of 3–9 week demonstrated an intraclass correlation coefficient (ICC) of 0.85 for whole striatum, with BP_{ND} changes of 2.74 ± 3.27% (mean ± SEM), with similar effects in striatal subdivisions (data not shown).

Limitations include the relatively small sample size (although more than twofold larger than previous investigations), measurements at only two time points, and relatively small changes in 17β-estradiol in some participants, and the resolution of PET, which precluded separation of the shell and core regions of the nucleus accumbens, which are functionally different. In light of these constraints and those outlined above, conclusions regarding the relationship between 17β-estradiol and D_2-type receptors must be cautious, and these findings remain in need of replication. If there is an effect of peripheral estrogen on striatal DA BP_{ND}, the effect appears to be small at best (Cohen's d [effect of estrogen on whole striatum] = 0.08), unlike preclinical studies, which find significant effects with Ns of 5–15 animals [24, 58, 59]. Moreover, Bayes factors suggest that the data are better explained by the null hypothesis (no effect of estrogen on D_2-type receptor binding potential) than by the alternative hypothesis (that estrogen is related to D_2-type receptor binding potential) [60].

The fact remains, however, that disorders linked to dopaminergic dysfunction manifest differently in men and women. Taken in the larger context of existing literature, these findings suggest that it may be important to attend to factors other than estrogen and striatal neurochemical markers to address these sex differences. Those factors may include (1) other relevant neuroendocrine variables, namely progesterone and its neuroactive metabolite allopregnanolone, (2) other brain regions, and (3) non-biological explanatory variables, such as psychosocial factors and the experience-induced neuroplastic changes they produce. Future directions for exploring the neurobiological factors that underlie sex differences in behavior and psychiatric diseases may benefit from a greater focus on these variables.

Acknowledgements

This paper was made possible by the dedicated contributions of support staff including Maritza Johnson and Will Chu, and undergraduate student researchers including Brendan Bych, Sabina Fridman, and Jacqueline Rohde.

Funding

This work was supported by F32DA039715 (NP), the Marjorie Greene Family Trust, and the Thomas P and Katherine K Pike Chair in Addiction Studies (EDL).

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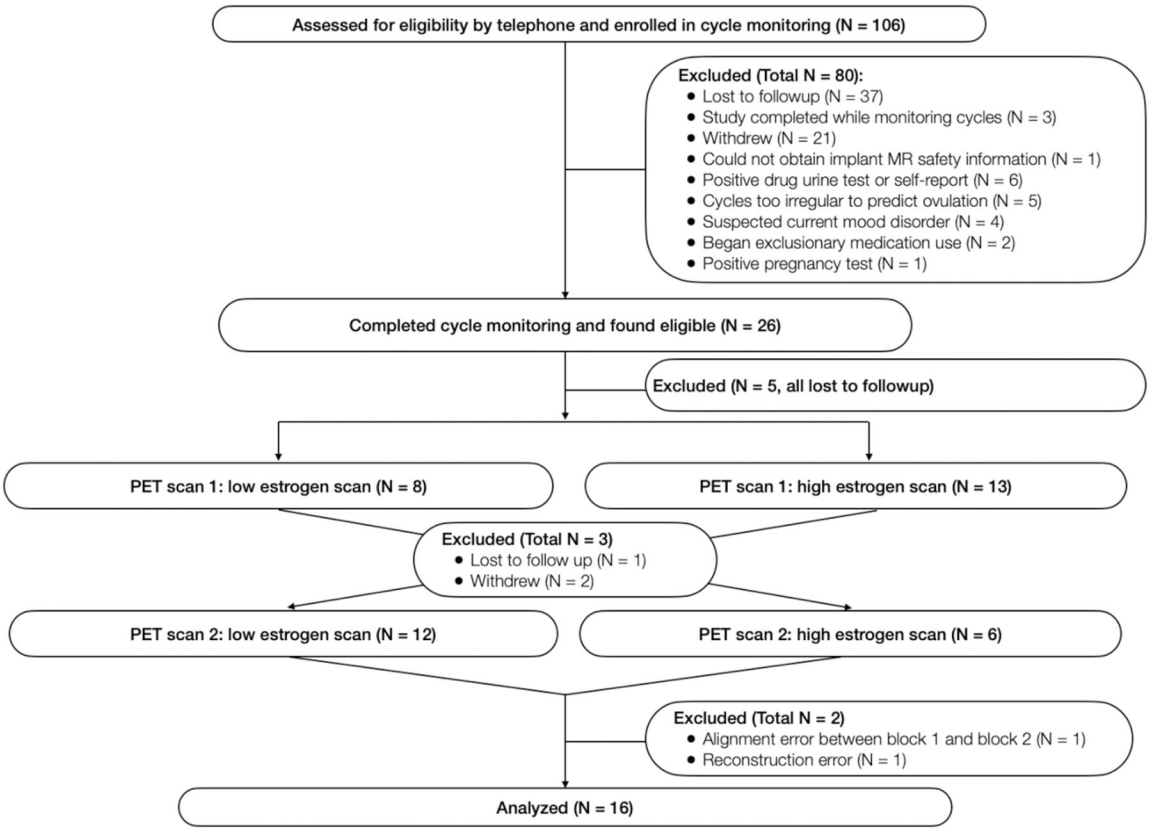

Fig. 1. CONSORT diagram graphically depicting the overall flow of the study.

Participants were initially assessed by eligibility by telephone, then asked to complete at least 2 months of menstrual cycle monitoring. Those who completed this and were still eligible afterward were scheduled for their first of two of [^{18}F]fallypride PET scans during a specific menstrual cycle window (either a low-estrogen scan during the early follicular phase, or a high-estrogen scan during the periovulatory window). After completing the first PET scan, participants crossed over to the opposite arm of the study to complete their second and final scan.

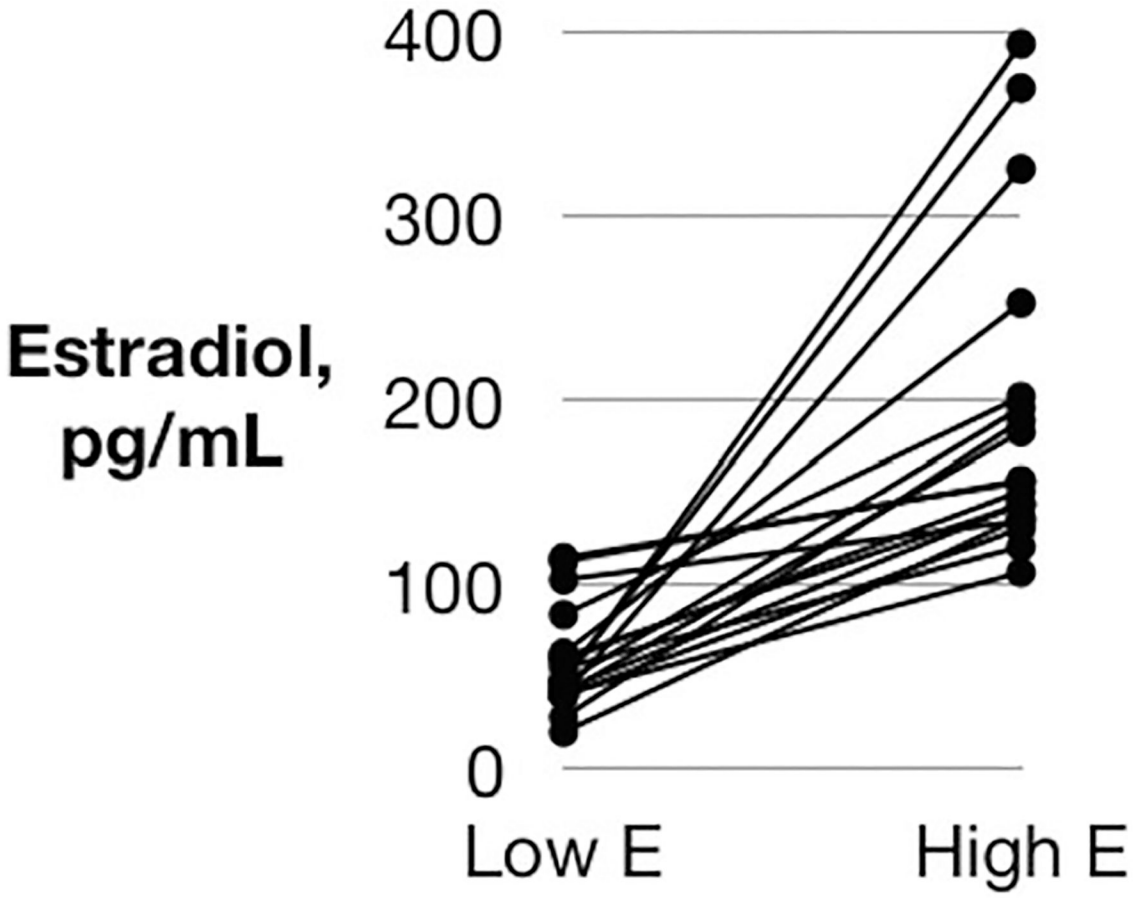

Fig. 2. Serum 17β-estradiol levels for each participant, each session, are depicted here.
The mean estrogen levels for the low-estrogen and high-estrogen sessions differed significantly, $p < 0.0001$.

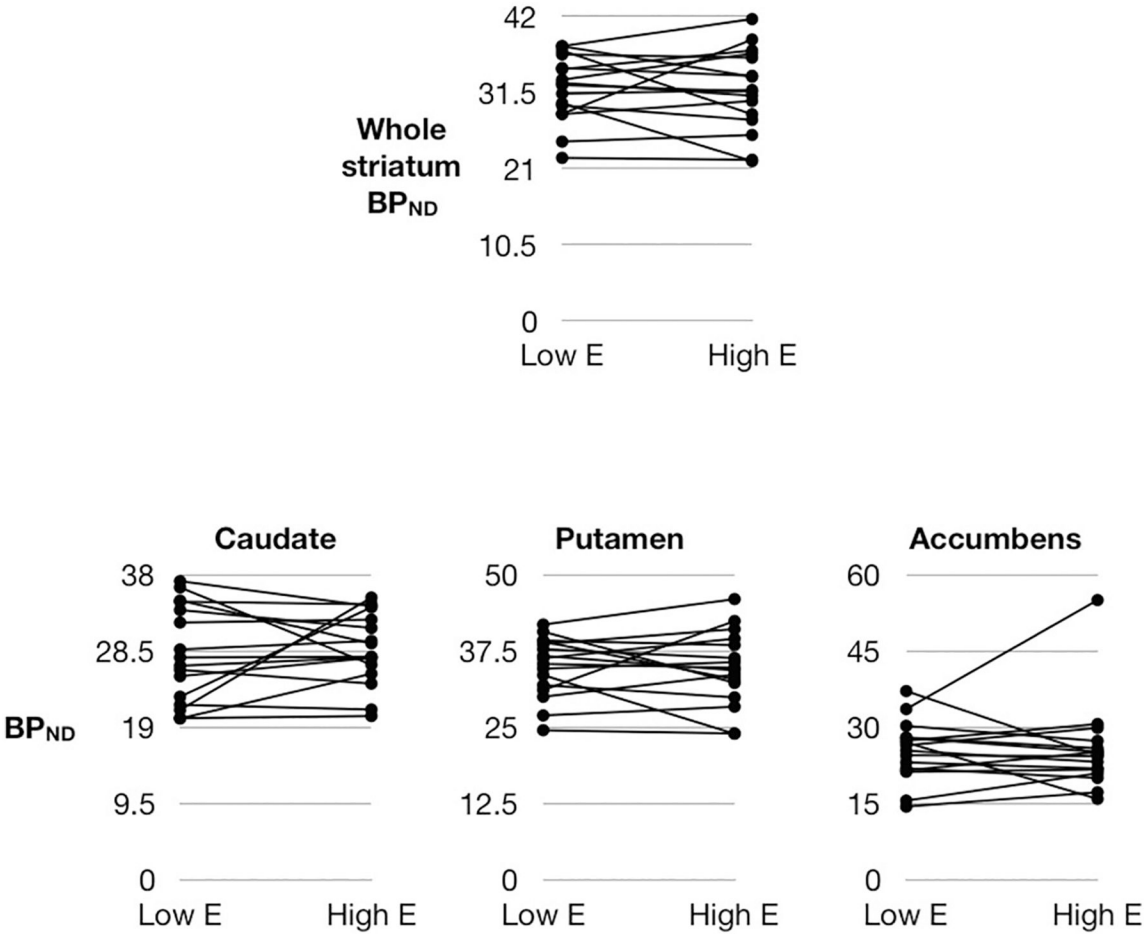

Fig. 3. BP_{ND} values for each subject, each session, are shown here.

The top panel shows BP_{ND} values for the whole striatum, and below it, values for the caudate (left), putamen (center), and accumbens (right) are given. Despite highly significant differences in estradiol levels, no statistically significant differences in [^{18}F]fallypride PET BP_{ND} were found in the whole striatum or the three subregions tested.

Table 1

Participant demographics.

Age (mean ± SD)	33.3 ± 6.45 years
Ethnic background (number of participants in each group)	
Black	4
Hispanic	8
White	3
More than one	1
BMI (mean ± SD)	26.4 ± 7.34 kg/m^2
Years of education (mean ± SD)	15.8 ± 1.59

Table 2

Timing of scans relative to menstrual cycle position.

	Low estrogen session	High estrogen session
Average cycle day (mean ± SD)	9.7 ± 7.65	12.6 ± 2.60
Range of cycle days	2–30	5–16

Scan timing differed considerably between participants due to idiosyncracies in menstrual cycles.

Table 3

Estradiol and progesterone concentrations.

	Low 17β-estradiol session	High 17β-estradiol session	Low 17β-estradiol vs. high 17β-estradiol session comparison (*p* value)
Serum 17β-estradiol			
Mean ± SD	60.0 ± 27.1 pg/mL	191.6 ± 84.7 pg/mL	<0.0001
Range	20–115 pg/mL	107 to 397 pg/mL	
Serum Progesterone			
Mean ± SD	0.85 ng/mL ± 1.3	1.06 ng/mL ± 2.2	0.72
Range	0[a]-5 ng/mL	0.1–9.8 ng/mL	

Estradiol differed significantly between the two sessions while progesterone did not.

[a]Denotes a concentration that was below the minimum detectable limit of the radioimmunoassay equipment.

Table 4

D2-type BP_{ND} in extrastriatal VOIs.

VOI	BP at low E2 scan (mean ± SD)	BP at high E2 scan (mean ± SD)	*p* value: low vs. high E2 comparison
Amygdala	2.86 ± 0.50	2.86 ± 0.44	0.65
Hippocampus	1.16 ± 0.28	1.15 ± 0.28	0.47
Pallidum	13.1 ± 2.10	13.0 ± 2.33	0.63
Thalamus	2.75 ± 0.46	2.74 ± 0.34	0.40
Lateral orbitofrontal cortex	0.63 ± 0.24	0.67 ± 0.29	0.62
Medial orbitofrontal cortex	0.70 ± 0.24	0.74 ± 0.21	0.27
Anterior cingulate cortex	0.68 ± 0.20	0.69 ± 0.20	0.88
Insula	1.46 ± 0.41	1.55 ± 0.42	0.16

Dopamine receptor availability was measured in 8 extrastriatal VOIs when E2 was relatively low and also when E2 was relatively high. BP_{ND} was not significantly different at these two time points in any of the extrastriatal VOIs tested.