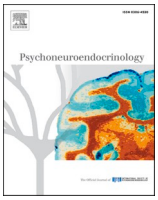
Psychoneuroendocrinology 156 (2023) 106320

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Effects of 24-hour oral estradiol-valerate administration on hormone levels in men and pre-menopausal women

Gabriele M. Rune [a], Gina Joue [b], Tobias Sommer [b,*]

[a] Institute of Cell Biology and Neurobiology, Charité Anatomy, Charitéplatz 1, 10117 Berlin, Germany
[b] Institute of Systems Neuroscience, University Medical Center Hamburg-Eppendorf, Martinistr. 52, D-20248 Hamburg, Germany

ARTICLE INFO

Keywords:
Estrogen
Luteinizing hormone
Follicle stimulating hormone
Testosterone
Insulin-like growth factor 1

ABSTRACT

In order to translate the findings from the vast animal literature on the effect of 17β-estradiol (E2) on brain and behavior to humans, a placebo-controlled pharmacological enhancement of E2 levels for at least 24 h is necessary. However, an exogenous increase in E2 for such a prolonged period might affect the endogenous secretion of other (neuroactive) hormones. Such effects would be of relevance for the interpretation of the effects of this pharmacological regimen on cognition and its neural correlates as well as be of basic scientific interest. We therefore administered a double dose of 12 mg of estradiol-valerate (E2V) to men and of 8 mg to naturally cycling women in their low-hormone phase, and assessed the concentration of two steroids critical to hormone regulation: follicle stimulating hormone (FSH) and luteinizing hormone (LH). We also assessed any changes in concentration of the neuroactive hormones progesterone (P4), testosterone (TST), dihydrotestosterone (DHT) and immune-like growth factor 1 (IGF-1). This regimen resulted in similar E2 levels in both sexes (saliva and serum). FSH and LH levels in both sexes were down-regulated to the same degree. P4 concentration decreased in both sexes only in serum but not saliva. TST and DHT levels dropped only in men whereas sex-hormone binding globulin was not affected. Finally, the concentration of IGF-1 decreased in both sexes. Based on previous studies on the effects of these neuroactive hormones, only the degree of downregulation of TST and DHT levels in men might have an impact on brain and behavior, which should be considered when interpreting the effects of the presented E2V regimes.

1. Introduction

A large body of evidence has been collected in animal experiments on the manifold effects of 17β-estradiol (E2) mainly in the hippocampus, but also in the prefrontal cortex and on the dopaminergic system, with observable changes in behavior (Luine, 2014; Taxier et al., 2020). E2 exerts rapid effects on neural circuits within hours via membrane-bound E2 receptors (ER) ER-α, ER-β, and G-protein coupled ERs. It also has slower 'classical', genomic effects that take place after about 24 h via cytosolic ER-α and ER–β (Graves et al., 2011).

Although E2 is often considered a female sex hormone, E2 and ERs are found in the brains of both sexes. However, the levels of E2 as well as ER expression differ in a region-specific manner between the sexes (Hojo and Kawato, 2018; Yagi and Galea, 2019). Similarly, the effects of the exogenous application of E2 on neural morphology and plasticity, as well as on behavior, show sex differences (Barker and Galea, 2009; Brandt and Rune, 2020; Frick et al., 2018). In the female hippocampus, for instance, E2 is synthesized *de novo* and acts in a paracrine manner on synaptogenesis and in facilitating long-term potentiation (LTP; Fester and Rune, 2015), an electrophysiological parameter of memory. E2 also facilitates LTP in the male hippocampus but via a different pathway than in females, which in turn affects different hippocampal mnestic functions compared to females (Frick et al., 2018). In the dopaminergic system, E2 enhances phasic dopamine release and regulates dopamine receptor expression, but differently in the sexes (Yoest et al., 2018).

Translational studies on the effects of E2 in humans require a placebo-controlled, double-blind, randomized pharmacological administration of E2. Eisenegger et al. described a transdermal application of E2 in men that raised E2 levels for several hours, long enough for rapid effects of E2 to take place (Eisenegger et al., 2013). The classic, genomic actions of E2, however, need E2 to be elevated for a longer time period. Accordingly, we administered E2-valerate (E2V) on two consecutive days to both healthy, naturally cycling women in their low-hormone phase and men in a series of experiments and observed effects on the

* Corresponding author.
E-mail address: tsommer@uke.de (T. Sommer).

https://doi.org/10.1016/j.psyneuen.2023.106320
Received 31 January 2023; Received in revised form 6 June 2023; Accepted 7 June 2023
Available online 9 June 2023

hippocampus, prefrontal cortex and the dopaminergic system that were very similar to what has been previously described in animals (e.g., Bayer et al., 2018; Joue et al., 2022).

However, an increase in E2 levels after exogenous application of the hormone, in particular over 24 h, might affect the endogenous secretion of other (neuroactive) hormones. These side effects might, in turn, confound the results of such a pharmacological regime and potential effects on cognition and its neural correlates cannot be unambiguously attributed to changes in E2 levels. Moreover, it is also of basic science interest to describe the effects of a 24-hour exogenous E2 increase on the hormonal system.

So far, the effects of long-term E2 administration on the levels of relevant hormones have only been studied in the context of hormone therapy in postmenopausal and transwomen. In postmenopausal women such a treatment results in a decrease of progesterone (P4), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, and accompanied by an increase in sex hormone-binding globulin (SHGB) (Larsson-Cohn et al., 1978; Ligniers et al., 1986). In transwomen, long-term E2 treatment similarly affects these hormones and results moreover in a decrease in testosterone (TST) levels (Mueller et al., 2006; Wierckx et al., 2014). Additionally, it has been shown that E2 treatment in men can lead to a decrease in LH and FSH levels only after several hours (Finkelstein et al., 1991). However, in this study, no other hormone levels were assessed.

The goal of the current study was, therefore, to comprehensively describe the effects of exogenously increasing E2 levels over 24 h on the hypothalamo-pituitary-gonadal (HPG) axis, i.e., on FSH and LH, as well as on P4, TST, and dihydrotestosterone (DHT) levels. We expected changes in FSH and LH levels to affect the levels of neuroactive hormones P4, TST and DHT, which have been shown to influence cognition and its neural correlates (Barros et al., 2015; Heany et al., 2016; Tobiansky et al., 2018). Therefore, knowing the potential effects of the E2V treatment on their levels is critical.

We additionally assessed insulin-like growth factor 1 (IGF-1) and human growth hormone (hGH). Both hormones are of interest because it has been suggested that chronic oral treatment with E2 reduces IGF-1 levels by impairing hGH actions in the liver (Leung et al., 2004). IGF-1 interacts with E2 and mediates some of its effects on brain and behavior (Nelson et al., 2014). Moreover, IGF-1 alone, independent of E2, also modulates synaptic plasticity, neurotransmission, and cognition in animals (Fernandez and Torres-Alemán, 2012; Nyberg and Hallberg, 2013).

We assessed E2, P4, and TST levels in both serum and saliva because serum levels reflect total hormone levels, including the fraction bound to transporter proteins such as SHGB, while saliva levels reflect only the bioavailable fraction (Bellem et al., 2011). Accordingly, we also assessed serum SHBG levels. Moreover, SHBG levels have been found to decrease after chronic E2 treatment and would therefore serve as a point of comparison of how the current pharmacological regimen compares to these chronic E2 treatment studies.

From studies on chronic E2 treatments in postmenopausal and transwomen, we expected decreases in FSH, LH, P4, TST, DHT, and IGF-1 levels as well as increases in SHBG and hGH in response to 24 h of exogenously increased E2. As no study has directly compared the effects of E2 treatment in women and men, it could not be hypothesized whether the expected decrease would be of the same magnitude in both sexes. One goal of the current study was to determine whether E2 treatment affects the HPG axis similarly between men and women. As ER distribution and E2 signaling vary between the sexes, we reasoned that inducing similar E2 levels in both sexes was necessary to be able to attribute any ensuing observed divergences in E2′s effects to sex differences. We conducted a pilot study in men to determine the E2V dosage needed to increase their E2 levels to target levels in young women (see also Bayer et al., 2018).

2. Methods

2.1. Participants

Data from 20 male and 20 naturally cycling female participants in their low-hormone menstrual /early follicular phase (when E2 and P4 levels are comparable to those in men), were included in this study. Male and female participants did not differ in age ($t(33.8) = 1.29$, $p = .21$), but men weighed more ($t(30.67) = 4.87$, $p < .0001$) and had higher BMI ($t(30.9) = 2.63$, $p = .01$) (demographics in Table 1). They were taken from a larger project where 137 participants were randomly assigned double-blind, within each sex to receive E2V or placebo (PBO) capsules (Joue et al., 2022; Nouri et al., 2022). The 40 participants here were randomly selected from the E2V groups of this larger sample. The sample size was based on the power needed to detect an effect of E2V on TST levels, as determined in the pilot study in men (see below). Menstrual cycle timing was determined by self-reports, then verified by hormone concentrations. Three women had relatively high P4 levels (serum level > 3 ng/ml, median = 8.82; saliva > 100 pg/ml, median = 158.80) usually observed in the luteal rather than follicular phase. However, as their E2 levels were low (median levels of serum 54.8 pg/ml and saliva 4.51 pg/ml) and they reported to be in their menstrual phase, they were only excluded from the analyses of P4 levels. In verification of this decision, the pattern of results for all analyses reported here did not change when these participants were also excluded. Only naturally cycling women who had not taken any oral contraceptives or were not pregnant in the 6 months prior to the study were included. Participants reported to have not been diagnosed with a mental disorder and to not be using illegal drugs in a general questionnaire. None of the participants had contraindications for taking E2 (e.g., obesity or at risk for cardiovascular problems). Participants were asked to sleep properly and not to drink alcohol the night after the first E2V dose. The study was approved by the Ethics Committee of the Hamburg Medical Association (PV4738). All volunteers gave written, informed consent and received monetary reimbursement. One female participant was excluded because her E2 levels suggested that she was not in the early follicular phase.

2.2. Procedure

Estradiol-valerate (E2V; from Progynova 21 UTA, Schering, Germany) was administered orally over two consecutive days. E2V is the synthetic ester of natural E2, with an average t_{max} of approximately 3–6 h and a half-life of 14 h (Kuhl, 2005). On Day 1 of the experiment, the first dose of E2V was administered by the experimenter. Participants took the second dose on their own the next day, approximately 7.5 h before blood was drawn. Blood and saliva were not collected when E2 levels were expected to peak because the participants were in the fMRI scanner performing experiments during that time window. The male E2V group received two 6-mg capsules of E2V on each of the two days (total 12 mg per day), and the female E2V group two 4-mg capsules on each of the two days (total 8 mg per day). These dosages were chosen to bring the E2 levels of men and women to within the same range, based on our previous study (Bayer et al., 2018) and a pilot experiment reported below.

On each testing day, three saliva samples were collected (over about an hour on both Day 1 and Day 2) and pooled for analysis (~3 ml in total) in order to achieve stable hormone level measurements. Blood was drawn (~1 ml) on days 1 and 2 and analyzed in the 20 participants of the E2 groups. Blood was collected in Sarstedt Monovette tubes, gently mixed with the silicate clotting activator in the tubes, and allowed to clot for at least 30 min at room temperature before being centrifuged at room temperature at 3000 g for 15 min. The supernatant serum was pipetted into a separate vial. Saliva and serum samples were stored at − 18 °C until analysis by the company IBL (Hamburg, Germany) using luminescence (saliva E2, P4, and TST) and ELISAs (serum of all hormones). The sensitivities of the luminescence immunoassays were 0.3 pg/ml for

Table 1
Hormone levels, mood and side effects, and demographics.

		women		men	
		Day1	Day2	Day1	Day2
E2 saliva^{D}		3.62 (2.75) [19]	25.4 (12.5) [19]	2.27 (1.94) [19]	28.5 (12.4) [17]
serum^{D}		58.8 (24.8) [17]	321 (110.0) [18]	52.6 (21.2) [20]	267.0 (120.0) [20]
P4 saliva^{D}		50.1 (40.1) [16]	41.0 (32.0) [16]	42.7 (19.3) [19]	40.7 (30.5) [19]
serum^{D}		0.55 (0.59) [15]	0.45 (0.16) [15]	0.58 (0.33) [20]	0.39 (0.13) [20]
TST saliva$^{D,S,\ D*S}$		15.4 (19.3) [19]	12.2 (12.3) [19]	83.0 (36.4) [19]	25.4 (39.8) [19]
serum$^{D,\ S,\ D*S}$		0.52 (0.24) [18]	0.48 (0.15) [18]	3.90 (1.8) [20]	1.64 (0.94) [20]
DHT$^{D,\ S,D*S}$		239.0 (98.3) [18]	225.0 (68.8) [18]	519.0 (295.0) [20]	351.0 (155.0) [20]
FSH^{D}		8.57 (4.32) [18]	3.69 (1.78) [18]	5.40 (4.77) [20]	2.76 (2.76) [20]
LH^{D}		6.01 (3.08) [18]	1.81 (2.22) [18]	6.97 (3.48) [20]	2.51 (1.72) [20]
SHBG^{S}		74.6 (26.5) [18]	75.0 (37.1) [19]	37.5 (26.3) [20]	35.9 (34.4) [20]
IGF-1^{D}		279.0 (106.0) [18]	277.0 (57.3) [18]	291 (67.2) [20]	283.0(82.7) [20]
hGH		1.88 (2.15) [9]	3.15 (3.69) [12]	1.60 (0.87) [3]	1.81(1.30) [7]
MDMQ	calm/nervous^{S}	18 (4.50)	17 (3.50)	18 (4.0)	18 (3.0)
	good/bad mood^{D*S}	13 (4.00)	12 (2.00)	15 (5.25)	13 (2.25)
	awake/tired	15 (6.00)	18 (5.50)	18 (3.25)	17 (3.00)
side effects	headache	0 (1)	0 (1)	0 (1)	0 (1)
	abdominal pain^{S}	0 (3)	0 (1)	0 (1)	0 (0)
	breast soreness^{S}	0 (1)	0 (2)	0 (0)	0 (0)
	nausea^{S}	0 (0.5)	0 (1)	0 (0)	0 (0)
	dizziness^{S}	0 (1)	0 (1.5)	0 (0.25)	0 (0)
	back pain	0 (2)	0 (1)	0 (1)	0 (0)
age (y)		27.2 ± 4.30		25.6 ± 3.30	
weight (kg)^{S}		62.6 ± 7.57		79.2 ± 13.1	
BMI (kg/m^2)^{S}		22.0 ± 2.18		24.4 ± 3.37	
menses to Day 2 (d)		1.53 ± 2.22		NA	
age at menarche (y)		13.3 ± 1.24		NA	
time E2 doses		17:10 ± 1:75	10:30 ± 1:12	17:30 ± 1:54	10:30 ± 1:09
time diff betw doses (h)		17.20 ± 1.45		17.00 ± 1.44	
saliva sample		16:55 ± 1:42	19:08 ± 1:25	16:58 ± 1:25	18:41 ± 1:10
saliva Day2 - dose 2 (h)		8.38 ± 0.42		8.25 ± 1.10	
serum sample		16:52 ± 2:01	17:70 ± 1:97	17:13 ± 1:35	18:0 ± 1:60
serum Day 2 - dose 2		7.43 ± 1.68		7.74 ± 1.39	

The units of hormone levels in saliva: E2, P4 and TST [pg/ml]. The units of hormone levels in serum: E2 and DHT [pg/ml], P4, TST, IGF-1 and hGH [ng/ml]; LH and FSH [mIU/ml]; SHBG [nmol/l]. Hormone levels, MDMQ and side-effect ratings were not normally distributed, so median and interquartile range (in round brackets) are reported. Hormone assays failed sometimes for various reasons and sample size for each hormone level is shown in square brackets. Age, weight, BMI, days to menses, age of menarche and times when saliva and serum samples were collected are reported as mean ± standard deviation, Time of day in HH:MM. Significant effects of the mixed effect models with the factors Sex and Day (Day, Sex, and interaction of both factors) are indicated as superscripts, i.e., D, S, D*S.

E2, 2.6 pg/ml for P4, and 1.6 pg/ml for TST. The sensitivities of the ELISAs were 10.6 pg/ml for E2, 0.045 ng/ml for P4, 0.18 ng/ml for TST, 17 pg/ml for DHT, 0.856 mIU/ml for FSH, 1.27 mIU/ml for LH, 0.09 ng/ml for IGF-1, 0.23 nmol/l for SHGB, and 0.04 ng/ml hGH.

On both testing days, participants filled out standardized questionnaires about their mood (Steyer et al., 1997) and potential physical side effects (headaches, abdominal pain, breast soreness, nausea, dizziness, and back pain). On Day 2, participants completed questionnaires after the fMRI tasks. At the very end, they were asked to guess whether they received PBO or E2V.

Before the main study, we conducted a pilot study in 6 men to determine the E2V dose needed to induce similar E2 levels in men and women and to estimate the sample size for the main experiment.

2.3. Statistical analyses

The hormone levels were analyzed as a function of Sex (F/M) and Day (days 1 and 2) in type III ANOVAs based on mixed effects models using the *lme4* and *lmerTest* packages with subject as a random factor using the *Anova* function of the *car* package in *R*. Since hormone levels were not normally distributed, median and interquartile ranges are reported. Residuals of the models were confirmed to be normally distributed for all hormone analyses using the *check_distribution* function in *R* package *performance*, as part of linear mixed model assumption checks. Post-hoc Tukey HSD tests were calculated using the *lsmeans* package. Cohen's d were calculated for the paired t-tests in the pilot study, and partial omega squared (ω_p^2) were calculated for all mixed effects models using the *effectsize* package. The significance level was set at $p < .05$. Results that approached this threshold, i.e.$5 \leq p < .1$, are mentioned as "trend towards significance" in order to minimize type II errors.

3. Results

3.1. Pilot study to estimate the pharmacokinetics of E2 valerate in men

Six men (age 28.0 y ± SD 0.6; weight = 67.8 kg ± 3.1; BMI = 21.95 ± 1.21 kg/m2) took two 12-mg doses of E2V on two consecutive days: the first dose in the evening of Day 1, the second dose 14.68 ± 1.34 h later in the morning of Day 2. Three saliva samples were collected (mean concentrations of the three samples are reported) 6.58 ± 0.17 h later. Five of the participants provided a blood sample 6.2 ± 0.2 h after the second dose.

The two 12-mg doses of E2V resulted in an increase in saliva **E2** levels from a median 2.2 (mean 2.8) pg/ml on Day 1–27.7 (10.4) pg/ml on Day 2 ($t(5) = 6.12$, $p < .005$). Serum **E2** concentrations increased from 45.5 (10.9) ng/ml on Day 1–694.3 (132.7) ng/ml on Day 2 ($t(4) = 4.8$, $p < .01$). Saliva **TST** concentrations decreased significantly from 136.6 (88.9) pg/ml on Day 1–48.7 (30.7) pg/ml on Day 2 ($t(5) = 4.5$, $p < .01$). Serum **TST** levels decreased from 3.2 (0.92) ng/ml on Day1 to 1.1 (1.0) ng/ml on Day 2 ($t(4) = 4.9$, $p < .01$). The effect sizes of the decrease in TST levels were Cohen's d_z 2.031 (saliva) and 2.38 (serum), indicating an achieved power of > 0.99.

The sample size needed to detect E2V effects on the other hormones of interest was based on effect sizes in studies on chronic E2 treatment in transwomen (Mueller et al., 2006; Wierckx et al., 2014). In one of these studies, the reduction of TST was 97 %, LH 87 %, FSH 89 %, and the increase in SHGB 172 %– all with similar variance (Mueller et al., 2006; similar effect sizes in the other study). In other words, the sizes of the effects of chronic E2 treatment on TST, LH, FSH and SBGH seem to be comparable. Therefore, we assumed that also the 24-h treatment with E2V would result in comparable effect sizes for these hormones and therefore, it would be sufficient to know the effect size of any one of the hormones such as TST, which we had from the pilot study. Because the effects of chronic oral E2 on IGF-1 seem to be about half as large (e.g., 43 % in Isotton et al., 2012), we opted for a sample size of 20 participants per group.

3.2. Main study

The analysis of saliva **E2** levels (Fig. 1A, Table 1) revealed a significant effect of Day ($F(1,36.08) = 289.56$, $p < .001$, $\omega_p^2 = .76$) but no effect of Sex or interaction between the two factors ($F(1,36.12) = 0.92$, $p = .34$, $\omega_p^2 = .00$; $F(1,36.08) = 2.23$, $p = .14$, $\omega_p^2 = .02$). Although the ANOVA did not reveal a significant effect of Sex, we conducted planned comparisons of E2 levels on Day 1 and on Day 2 between the sexes, and also changes from Day 1 to Day 2 to have an idea of how similar the levels were. These tests did not show any sex differences on Day 1 or Day 2 ($p = .30$; $p = .99$) and confirmed an increase from Day 1 to Day 2 in both sexes (p's < 0.001). Serum levels (Fig. 1A, Table 1) showed a similar pattern, i.e. E2 levels increased from Day 1 to Day 2 ($F(1,35.73) = 398.74$, $p < .001$, $\omega_p^2 = .88$), but there were no effects of Sex nor an interaction with Day ($F(1,36.10) = 1.60$, $p = .22$, $\omega_p^2 = .02$; $F(1,35.73) = 0.01$, $p = .92$, $\omega_p^2 = .02$). Again, planned comparisons confirmed that there were no sex differences on Day 1 or 2 ($p = .70$, $p = .76$, respectively) and that there was an increase in both sexes (p's < 0.001).

Serum **LH** levels decreased in both sexes (Fig. 1B, Table 1; effect of Day: $F(1,36) = 21.44$, $p < .001$, $\omega_p^2 = .35$), but there was no effect of Sex

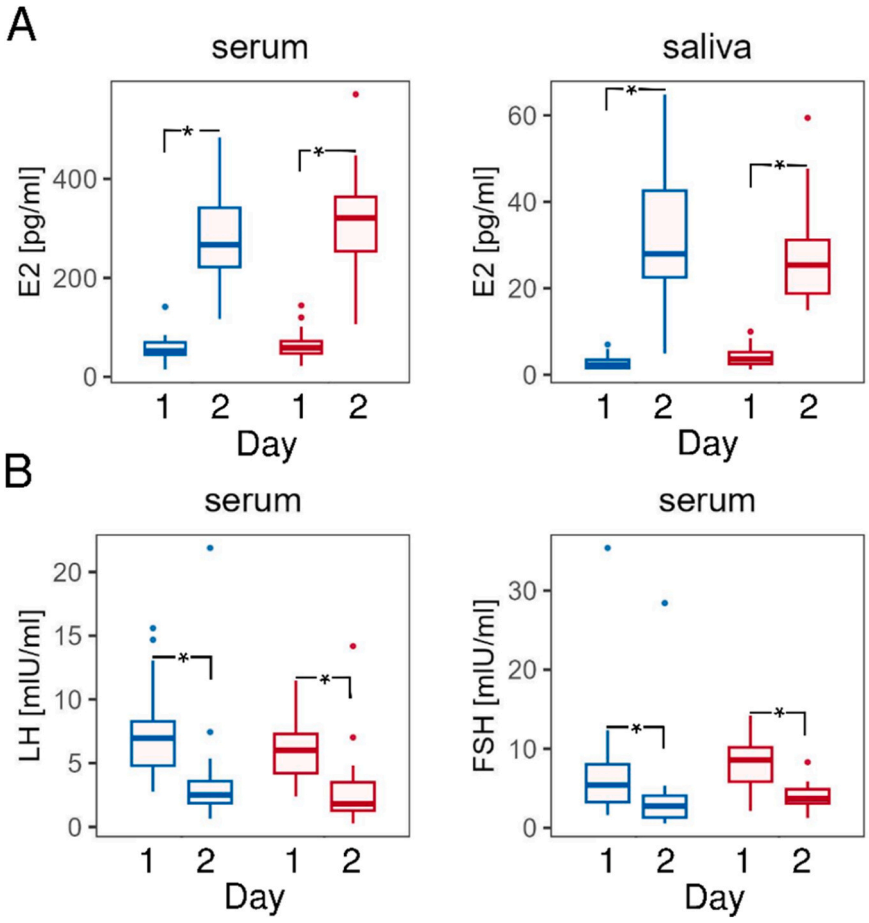


Fig. 1. Hormone concentrations in response to the E2V regimen. (A) Day 1 and 2 saliva and serum levels of estradiol (E2). (B) Day 1 and 2 serum levels of luteinizing (LH) and follicle stimulating hormones (FSH). Box plots show the median and interquartile range, * $p < .05$ post-hoc tests from Day 1 to Day 2 within Sex. Hormone levels of women in red, of men in blue.

or interaction (F(1,36) = 1.11, p = .30, ω_p^2 = .00; interaction: F(1,36) = 0.07, p = .79, ω_p^2 = .00). Post-hoc tests confirmed a decrease in women and men (p = .02; p = .01). Serum **FSH** levels showed a similar pattern (Fig. 1B, Table 1), i.e. a decrease in both sexes (F(1,36) = 91.37, p < .001, ω_p^2 = .70), but no effect of Sex or interaction (F(1,36) = 0.19, p = .67, ω_p^2 = .00; F(1,36) = 1.76, p = .19, ω_p^2 = .02). Post-hoc tests indicated that FSH levels decreased in both sexes (p's < 0.001).

The following results from the analyses of **P4** levels do not include the three female participants whose levels are usually observed in the luteal phase, though including them does not change the pattern of results. Serum levels decreased in both sexes (Fig. 2 B, Table 1; F(1,33) = 23.61, p < .0001, ω_p^2 = .39), but there was neither an effect of Sex nor interaction (F(1,13) = 2.19, p = .15, ω_p^2 = .03; F(1,33) = 0.01, p = .94, ω_p^2 = .00). Post-hoc tests confirmed an effect for Day in women (p = .02) and men (p = .004). The average serum P4 level of the three excluded women decreased from 9.98 ng/ml to 4.98 ng/ml. The decrease in saliva P4 levels showed only a trend towards significance (F(1,33) = 3.26, p = .08, ω_p^2 = .06) but no effect of Sex or interaction (F(1,33) = 0.79, p = .28, ω_p^2 = .00; F(1,33) = 1.47, p = .23, ω_p^2 = .01). None of the post-hoc tests reached significance. Saliva P4 levels of the excluded women decreased from 160 pg/ml to 92.23 pg/ml.

Saliva **TST** levels (Fig. 2B, Table 1) showed main and interaction effects (effect of Sex: F(1,36) = 35.20, p < .001, ω_p^2 = .47; effect of Day: F(1,36) = 30.23, p < .001, ω_p^2 = .43, interaction: F(1,36) = 18.19, p < .001, ω_p^2 = .12). Post-hoc tests showed sex differences on Day 1 (p < .001), a decrease only in men (p < .001), and a trend for sex differences on Day 2 (p = .07). Serum levels (Fig. 2B) showed a similar pattern (effect of Sex: F(1,36) = 96.04, p < .001, ω_p^2 = .71; effect of Day:

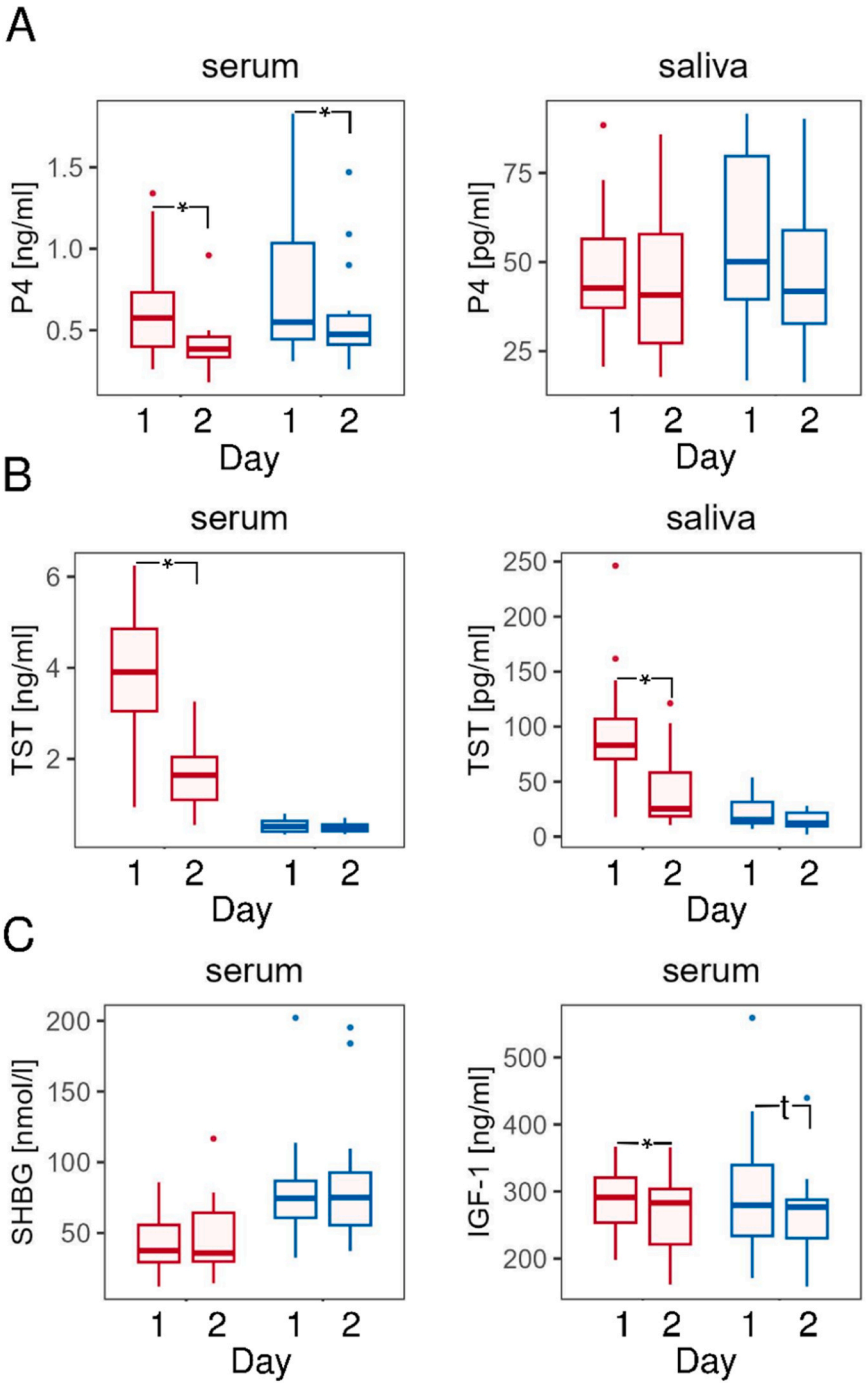


Fig. 2. Hormone concentrations in response to the E2V regimen. (A) Day 1 and 2 saliva and serum levels of progesterone (P4; three women were excluded from this plot and the P4 analyses because their P4 levels suggested they were in the luteal phase) and (B) of testosterone (TST). (C) Day 1 and 2 serum levels of sex hormone binding globulin (SHBG) and immune growth factors 1 (IGF-1). Box plots show the median and interquartile range, * p < .05 post-hoc tests from Day 1 to Day 2 within Sex, ^{t} p = .05. Hormone levels of women in red, of men in blue. Mean levels of IGF-1 (F, Day 1 301 ng/ml; F, Day 2 267 ng/ml; M, Day 1 289 ng/ml; M, Day: 2 267 ng/ml) showed a stronger decrease than median levels, and mixed models confirmed a significant decrease in women and a trend towards significance in men.

$F(1,36) = 86.80$, $p < .001$, $\omega_p^2 = .69$; interaction: $F(1,36) = 80.98$, $p < .001$, $\omega_p^2 = .68$). Post-hoc tests indicated that there were sex differences on both Day 1 and 2 (p's < .001), and there was a decrease in men (p <[0.001.

Serum **DHT** levels showed a similar pattern to TST (effect of Sex: $F(1,36) = 10.63$, $p < .001$, $\omega_p^2 = .20$; effect of Day: $F(1,36) = 87.11$, $p < .001$, $\omega_p^2 = .69$; interaction: $F(1,36) = 34.21$, $p < .001$, $\omega_p^2 = .47$). Post-hoc tests indicate a sex difference on Day 1 ($p < .001$), a decrease in men ($p < .001$), a trend towards a significant increase in women ($p = .09$) but no evidence for a sex difference on Day 2 ($p = .37$).

SHBG levels (Fig. 2C, Table 1) were higher in women than in men ($F(1,36.56) = 13.68$, $p < .001$, $\omega_p^2 = .25$) but did not change on Day 2 ($F(1,35.61) = 0.33$, $p = .57$, $\omega_p^2 = .00$) and showed no interaction effect ($F(1,35,61) = 1.30$, $p = .26$, $\omega_p^2 = .00$). Post-hoc tests showed higher levels in women on Day 1 and 2 (p's < .01).

IGF-1 (Fig. 2C, Table 1) showed an effect of Day ($F(1,36) = 21.33$, p <[0.001 $\omega_p^2 = .36$) but not of Sex nor an interaction ($F(1,36) = 0.09$, $p = .76$, $\omega_p^2 = .00$; and $F(1,36) = 0.88$, $p = .35$, $\omega_p^2 = .00$, respectively). Planned tests were conducted for both sexes although the interaction had not reached significance to confirm that levels significantly decreased also in men, which to our knowledge has not been shown before. They indicated a decrease in women ($p = .003$) and a strong trend towards significant decrease in men ($p = .05$).

hGH levels (Table 1) in most samples were below the sensitivity of the assay (0.04 ng/ml). and therefore only analyzed in an exploratory fashion for the female participants using a Kruskal-Wallis rank sum test. No change was found ($\chi^2(1) = .32$, p =[0.57.

3.2.1. Mood and physical side effects

On the calmness dimension of the Multidimensional Mood Questionnaire (calm-nervous, Table 1), there was a trend towards significance of men being calmer than women in general ($F(1,37) = 2.94$, $p = .09$). On the valence dimension (good-bad mood), there were effects of Sex ($F(1,37) = 5.27$, $p < .05$) and an interaction ($F(1,37) = 8.23$, $p < .01$), with mood worsening in men in general. There were no differences on the energetic arousal dimension (awake-tired) (ps >.12). From the side effect questionnaire, there was only a Sex effect with women generally reporting more abdominal pain ($F(1,37.29,= 10.2$, p <[0.005, breast soreness ($F(1,37,10) = 5.9$, $p < .05$), nausea ($F(1,37.26) = 6.6$, $p < .05$) and dizziness ($F(1,36.17) = 4.5$, $p < .05$), but no difference in headaches and back pain.

4. Discussion

A double dose of 8 mg of E2V over two consecutive days for women in their early follicular phase resulted in similar saliva and serum E2 levels as in men given a double dose of 12 mg of E2V. A lower dose is needed to bring E2 levels in women to the same levels in men because one of the main pathways leading to E2 release requires enzymes mainly produced by the ovaries. Orally administered E2 is rapidly transformed in the intestinal tract and liver (first-pass effect) in both sexes to estrone and its sulfate, which circulate and serve as reservoirs from which E2 can be reconverted (Kuhl, 2005). As the enzyme required for E2-to-estrone metabolism, 17b-hydroxysteroid-dehydrogenase type 1, is mainly localized in the ovaries (Pelletier et al., 2004), this re-conversion occurs more frequently in women than in men.

The lower E2 levels observed in the main study, compared to our previous studies (e.g., Bayer et al., 2018) and the pilot experiment, are most likely due to the difference in sample collection times. Whereas saliva samples in the other studies were collected 6.5 h after the second E2V dose, they were collected later in the main study: 8.5 h after the second E2V dose, effectively after the t_{max} of 3–6 h reported for E2V. Peak E2 levels would have already been reached and were on the decline when the samples were collected. The peak E2 levels induced by the current treatment were therefore probably slightly higher than what was actually measured. By extension, 8 mg per day over two consecutive days resulted in women having peripheral E2 levels above the menstrual cycle maximum, i.e. to levels often termed 'supraphysiological' (but see discussion below). The dosage required for lower E2 levels in both sexes can be derived from our previous study where we administered 2, 4, 6, and 12 mg E2V to women (Bayer et al., 2018) and the current data indicating that men need about 1.5 times the dose to reach similar E2 levels.

As E2 synthesis in the ovaries is synchronized with hippocampal E2 synthesis (via GnRH), the E2V administration in the current study most likely down-regulated not only peripheral E2 synthesis in the ovaries but also local E2 synthesis in the hippocampus (Fester and Rune, 2021). The administered E2 easily crosses the blood-brain barrier, resulting in central E2 levels that are likely to be similar to the peripheral levels. However, because endogenous hippocampal E2 levels are much higher than peripheral levels in both sexes (Hojo and Kawato, 2018), the high peripheral E2 levels reached by the current treatment, which are above the cycle maximum in females, are not necessarily supraphysiological in the hippocampus.

The observed decrease in LH and FSH levels is consistent with the known negative feedback E2 has on LH and FSH secretion during the early follicular phase in females and implies that exogenous E2 administration down-regulates endogenous E2 synthesis in the ovaries (Skorupskaite et al., 2014). LH and FSH levels also decreased in men, indicating that E2 down-regulates the male secretion of these two hormones, i.e. induced negative feedback on the male HPG axis (Chimento et al., 2014; Tilbrook and Clarke, 2001). Because TST and DHT levels in men decreased in response to the E2V treatment, this down-regulation of the HPG axis can only be due to E2′s action on the hypothalamus. So far, such effects of exogenous E2 have only been shown for long-term E2 treatments in postmenopausal and transwomen (Ligniers et al., 1986; Mueller et al., 2006; Wierckx et al., 2014; Yahyaoui et al., 2008). Our data show that in young women in their low-hormone phase and in men, E2 down-regulates the HPG axis to the same degree.

The down-regulation of LH and FSH also explains the lower serum P4 levels on Day 2 observed in both sexes and the lower TST (and DHT) levels on Day 2 in men since LH and FSH stimulate endogenous P4 and TST synthesis in the gonads (Skorupskaite et al., 2014; Tilbrook and Clarke, 2001). Reduced P4 levels in women have been observed after chronic E2 treatment in postmenopausal women (Edlefsen et al., 2010). P4 is also a male hormone, and its production in the testes is regulated via LH and FSH, which explains the decrease in male P4 serum levels here (El-Hefnawy and Huhtaniemi, 1999). The decrease in saliva P4 levels only trended towards significance. Because salivary levels reflect the concentration of unbound serum steroids (Bellem et al., 2011), the difference between the decrease in saliva and serum P4 levels might indicate that the fraction of free P4 increases in response to the exogenous E2 increase, perhaps because P4 competes with E2 to bind to SHBG. P4 can have both impairing and beneficial effects on memory and its neural correlates depending on dose and time of administration (Barros et al., 2015). Moreover, its effects on affective processing and amygdala function have been reported (van Wingen et al., 2011). However, in these studies, P4 levels were substantially pharmacologically increased, in contrast to the slight concentration decrease in response to the current E2V treatment. Therefore, the effect of the reduced P4 levels on cognition and its neural substrates probably will not interfere with the effects of the substantially enhanced E2.

The robust decrease of TST only in men is consistent with the downregulation of TST synthesis in the testes by the low LH and FSH levels and confirms that the negative feedback on the HPG axis in men is mediated by E2 and not TST (Tilbrook and Clarke, 2001). We did not observe a decrease in TST levels in women although its ovarian synthesis is also stimulated by LH and FSH. Stable TST levels have been reported after long-term treatment of postmenopausal women with E2 and might be explained by TST secretion in the adrenal gland and its production from circulating androstenedione (Burger, 2002; Mathur et al., 1985). It has also been shown that E2 phosphorylates aromatase (Fester et al.,

2016). Phosphorylation inactivates aromatase, which, in turn, elevates testosterone levels. We have preliminary data pointing to stronger phosphorylation in male animals even under control conditions that would not be greatly changed with the additional phosphorylation caused by elevated E2 levels. In women, however, increased aromatase phosphorylation might counteract the decreased TST synthesis in the ovaries due to down-regulated FSH and LH, therefore evening out TST levels in women.

Taken together, the 24-h E2V treatment here resulted in changes in LH, FSH, TST and P4 levels that were similar to that reported for long-term treatment in postmenopausal and transwomen. However, we did not observe an increase in SHBG levels as in these studies, which might be due to the greater binding competition between the sex steroids, as discussed above for P4. SHGB is usually found – as in our study – in concentrations that are twice as high in women as in men (Dunn et al., 1981).

The higher TST and DHT levels on Day 1 are an inevitable confound when studying how E2 affects men and in comparisons to women, even when women are in their low hormone phase. For this reason, treatment effects in men cannot be unequivocally attributed to increased E2 – we need to also take into account studies on TST to determine whether an observed effect of the current E2V treatment might be due to an increase in E2 or to a decrease in TST (and DHT). In general, TST effects appear to be weaker (Tobiansky et al., 2018).

The decrease in mean IGF-1 levels observed is also of basic science interest as it has been described only in women under chronic E2 treatment so far (Davis et al., 2008). In particular, only oral but not transdermal E2 treatment lowers circulating IGF-1, which has been attributed to E2′s first pass effects (Leung et al., 2004). Chronic oral E2 treatment results, on the other hand, in an increase in hGH levels. However, we were able to measure hGH in too small a sample to make any conclusions. The decrease in IGF-1 might counteract effects of E2 given that IGF-1 likely mediates some of E2′s effects in the animal brain (Nelson et al., 2016; Pristerà et al., 2019). However, the subtlety of this decrease makes it unlikely that the lower IGF-1 confounds the results of the massive increase in E2.

We did not observe any mood changes or physical side effects of the E2V treatment, as the only effect of Day found was worsened mood in men, which was probably due to the demanding fMRI tasks they completed just prior to answering questionnaires. Participants in the larger sample including placebo participants could not correctly guess whether they were administered E2V (Joue et al., 2021). Therefore, a double-blind administration of these E2V doses is possible without the risk that participants are de-blinded based on experienced symptoms.

We have used the pharmacological regimen of E2V over two consecutive days in three larger projects (Bayer et al., 2020, 2018; Joue et al., 2022; Nouri et al., 2022; Sommer et al., 2018). We had observed an increase in memory-related hippocampal activity (Bayer et al., 2018), contrary to a study using transdermal administration of E2, which instead reported a decrease (Bayer et al., 2018; Coenjaerts et al., 2022). Similarly, we observed opposite effects of E2 on the learning rate in a reinforcement learning task in men (Joue et al., 2022) to what was reported for a transdermal dose over a shorter period of time (Veselic et al., 2021). These study differences are likely due to the different effects of rapid and genomic E2 actions on the hippocampus and the dopaminergic system. They highlight the value of the current pharmacological treatment in order to translate genomic effects of E2 from animal to human experiments.

One limitation of the current study is that we did not assess E2 levels often enough to describe pharmacokinetics. However, this limitation seems less critical as E2 levels were elevated for a relatively long time period compared to transdermal application, and thus the resulting changes on the cellular level are relatively stable.

To summarize, the pharmacological regimen presented here resulted in increased E2 levels for more than 24 h, hence permitting both rapid and genomic effects to take place without causing side effects or physical signs of having been administered E2, which would have confounded double-blind pharmacological studies. The exogenous increase in E2 levels down-regulated the HPG axis and resulted in a similar decrease in LH and FSH levels in both sexes. The levels of the gonadal hormone P4 decreased in both sexes but only in serum (bound fraction) and not saliva (bioavailable fraction), whereas TST and its derivate DHT decreased only in men. SHBG levels were not affected. We also observed lower IGF-1 levels in both sexes. Therefore, the consequences of the exogenous increase of E2 levels for 24 h on the other investigated hormones are similar to those of longer term E2 treatments, except for SHBG levels which did not increase. IGF-1, P4, TST and DHT are neuroactive hormones that, like E2, can affect neural plasticity and morphology as well as behavior. However, as suggested by previous studies, only the changes in TST and DHT levels in the current study seem large enough to potentially confound the effects of E2, and therefore need to be considered when interpreting the results of the presented pharmacological regimen on brain and behavior.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tobias Sommer reports financial support was provided by German Research Foundation.

Acknowledgement

We thank H. Kuhl, A. O. Mück, and J. Höchel for pharmacokinetic advice. The project was supported by a grant of the German Research Foundation (DFG SO 952/8–1, DFG RU 436/7–1).

References

Barker, J.M., Galea, L.A., 2009. Sex and regional differences in estradiol content in the prefrontal cortex, amygdala and hippocampus of adult male and female rats. Gen. Comp. Endocrinol. 164, 77–84.

Barros, L.A., Tufik, S., Andersen, M.L., 2015. The role of progesterone in memory: an overview of three decades. Neurosci. Biobehav. Rev. 49, 193–204. https://doi.org/10.1016/j.neubiorev.2014.11.015.

Bayer, J., Gläscher, J., Finsterbusch, J., Schulte, L.H., Sommer, T., 2018. Linear and inverted U-shaped dose-response functions describe estrogen effects on hippocampal activity in young women. Nat. Commun. 9, 1220. https://doi.org/10.1038/s41467-018-03679-x.

Bayer, J., Rusch, T., Zhang, L., Gläscher, J., Sommer, T., 2020. Dose-dependent effects of estrogen on prediction error related neural activity in the nucleus accumbens of healthy young women. Psychopharmacology 237, 745–755. https://doi.org/10.1007/s00213-019-05409-7.

Bellem, A., Meiyappan, S., Romans, S., Einstein, G., 2011. Measuring estrogens and progestagens in humans: an overview of methods. Gend. Med. 8, 283–299. https://doi.org/10.1016/j.genm.2011.07.001.

Brandt, N., Rune, G.M., 2020. Sex-dependency of oestrogen-induced structural synaptic plasticity: inhibition of aromatase versus application of estradiol in rodents. Eur. J. Neurosci. 52, 2548–2559. https://doi.org/10.1111/ejn.14541.

Burger, H.G., 2002. Androgen production in women. Fertil. Steril. 77 (Suppl 4) https://doi.org/10.1016/s0015-0282(02)02985-0.

Chimento, A., Sirianni, R., Casaburi, I., Pezzi, V., 2014. Role of estrogen receptors and G protein-coupled estrogen receptor in regulation of hypothalamus–pituitary–testis axis and spermatogenesis. Front. Endocrinol. 5 https://doi.org/10.3389/fendo.2014.00001.

Coenjaerts, M., Trimborn, I., Adrovic, B., Stoffel-Wagner, B., Cahill, L., Philipsen, A., Hurlemann, R., Scheele, D., 2022. Exogenous estradiol and oxytocin modulate sex differences in hippocampal reactivity during the encoding of episodic memories. NeuroImage, 119689. https://doi.org/10.1016/j.neuroimage.2022.119689.

Davis, S.R., Stuckey, B.G.A., Norman, R.J., Papalia, M.-A., Drillich, A., Bell, R.J., 2008. Effects of the route of estrogen administration on insulinlike growth factor-I, IGF binding protein-3, and insulin resistance in healthy postmenopausal women: results from a randomized, controlled study. Menopause 15, 1065–1069. https://doi.org/10.1097/gme.0b013e318174f16e.

Dunn, J.F., Nisula, B.C., Rodbard, D., 1981. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. J. Clin. Endocrinol. Metab 53, 58–68. https://doi.org/10.1210/jcem-53-1-58.

Edlefsen, K.L., Jackson, R.D., Prentice, R.L., Janssen, I., Rajkovic, A., O'Sullivan, M.J., Anderson, G., 2010. The effects of postmenopausal hormone therapy on serum estrogen, progesterone, and sex hormone-binding globulin levels in healthy

postmenopausal women. Menopause 17, 622–629. https://doi.org/10.1097/gme.0b013e3181cb49e9.
Eisenegger, C., von Eckardstein, A., Fehr, E., von Eckardstein, S., 2013. Pharmacokinetics of testosterone and estradiol gel preparations in healthy young men. Psychoneuroendocrinology 38, 171–178. https://doi.org/10.1016/j.psyneuen.2012.05.018.
El-Hefnawy, T., Huhtaniemi, I.T., 1999. Progesterone and testicular function. Aging Male 2, 240–245. https://doi.org/10.3109/13685539909042351.
Fernandez, A.M., Torres-Alemán, I., 2012. The many faces of insulin-like peptide signalling in the brain. Nat. Rev. Neurosci. 13, 225–239. https://doi.org/10.1038/nrn3209.
Fester, L., Rune, G.M., 2015. Sexual neurosteroids and synaptic plasticity in the hippocampus. Brain Res 1621, 162–169. https://doi.org/10.1016/j.brainres.2014.10.033.
Fester, L., Brandt, N., Windhorst, S., Pröls, F., Bläute, C., Rune, G.M., 2016. Control of aromatase in hippocampal neurons. J. Steroid Biochem. Mol. Biol. 160, 9–14. https://doi.org/10.1016/j.jsbmb.2015.10.009.
Finkelstein, J.S., O'Dea, L.St.L., Whitcomb, R.W., Crowley, W.F., 1991. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men*. J. Clin. Endocrinol. Metab. 73, 621–628. https://doi.org/10.1210/jcem-73-3-621.
Frick, K.M., Kim, J., Koss, W.A., 2018. Estradiol and hippocampal memory in female and male rodents. Curr. Opin. Behav. Sci. 23, 65–74. https://doi.org/10.1016/j.cobeha.2018.03.011.
Graves, N.S., Hayes, H., Fan, L., Curtis, K.S., 2011. Time course of behavioral, physiological, and morphological changes after estradiol treatment of ovariectomized rats. Physiol. Behav 103, 261–267. https://doi.org/10.1016/j.physbeh.2011.02.017.
Heany, S.J., van Honk, J., Stein, D.J., Brooks, S.J., 2016. A quantitative and qualitative review of the effects of testosterone on the function and structure of the human social-emotional brain. Metab. Brain Dis. 31, 157–167. https://doi.org/10.1007/s11011-015-9692-y.
Hojo, Y., Kawato, S., 2018. Neurosteroids in adult hippocampus of male and female rodents: biosynthesis and actions of sex steroids. Front. Endocrinol. 9, 183. https://doi.org/10.3389/fendo.2018.00183.
Isotton, A.L., Wender, M.C.O., Casagrande, A., Rollin, G., Czepielewski, M.A., 2012. Effects of oral and transdermal estrogen on IGF1, IGFBP3, IGFBP1, serum lipids, and glucose in patients with hypopituitarism during GH treatment: a randomized study. Eur. J. Endocrinol. 166, 207–213. https://doi.org/10.1530/EJE-11-0560.
Joue, G., Chakroun, K., Bayer, J., Gläscher, J., Zhang, L., Fuss, J., Hennies, N., Sommer, T., 2022. Sex differences and exogenous estrogen influence learning and brain responses to prediction errors. Cereb. Cortex N. Y 1991 (32), 2022–2036. https://doi.org/10.1093/cercor/bhab334.
Kuhl, H., 2005. Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric 8, 3–63.
Larsson-Cohn, U., Johansson, E.D.B., Kagedal, B., Wallentin, L., 1978. Serum FSH, LH and oestrone levels in postmenopausal patients on oestrogen therapy. BJOG Int. J. Obstet. Gynaecol. 85, 367–372. https://doi.org/10.1111/j.1471-0528.1978.tb14896.x.
Leung, K.-C., Johannsson, G., Leong, G.M., Ho, K.K.Y., 2004. Estrogen regulation of growth hormone action. Endocr. Rev. 25, 693–721. https://doi.org/10.1210/er.2003-0035.
Ligniers, B.D., Basdevant, A., Thomas, G., Thalabard, J.-C., Mercier-Bodard, C., Conard, J., Guyene, T.-T., Mairon, N., Corvol, P., Guy-Grand, B., Mauvais-Jarvis, P., Sitruk-Ware, R., 1986. Biological effects of estradiol-17β in postmenopausal women: oral versus percutaneous administration. J. Clin. Endocrinol. Metab. 62, 536–541. https://doi.org/10.1210/jcem-62-3-536.
Luine, V.N., 2014. Estradiol and cognitive function: past, present and future. Horm. Behav. 66, 602–618. https://doi.org/10.1016/j.yhbeh.2014.08.011.
Mathur, R.S., Landgrebe, S.C., Moody, L.O., Semmens, J.P., Williamson, H.O., 1985. The effect of estrogen treatment on plasma concentrations of steroid hormones, gonadotropins, prolactin and sex hormone-binding globulin in post-menopausal women. Maturitas 7, 129–133. https://doi.org/10.1016/0378-5122(85)90018-0.
Mueller, A., Binder, H., Cupisti, S., Hoffmann, I., Beckmann, M., Dittrich, R., 2006. Effects on the male endocrine system of long-term treatment with gonadotropin-releasing hormone agonists and estrogens in male-to-female transsexuals. Horm. Metab. Res. 38, 183–187. https://doi.org/10.1055/s-2006-925198.
Nelson, B.S., Black, K.L., Daniel, J.M., 2016. Circulating estradiol regulates brain-derived estradiol via actions at GnRH receptors to impact memory in ovariectomized rats. eneuro 3. https://doi.org/10.1523/ENEURO.0321-16.2016.
Nouri, S., Biedermann, S.V., Joue, G., Auer, M.K., Sommer, T., Fuss, J., 2022. Effects of circulating estradiol on physiological, behavioural, and subjective correlates of anxiety: a double-blind, randomized, placebo-controlled trial. Psychoneuroendocrinology 138, 105682. https://doi.org/10.1016/j.psyneuen.2022.105682.
Nyberg, F., Hallberg, M., 2013. Growth hormone and cognitive function. Nat. Rev. Endocrinol. 9, 357–365. https://doi.org/10.1038/nrendo.2013.78.
Pelletier, G., Luu-The, V., Li, S., Ren, L., Labrie, F., 2004. Localization of 17β-hydroxysteroid dehydrogenase type 1 mRNA in mouse tissues. J. Mol. Endocrinol. 33, 459–465. https://doi.org/10.1677/jme.1.01567.
Pristerà, A., Blomeley, C., Lopes, E., Threlfell, S., Merlini, E., Burdakov, D., Cragg, S., Guillemot, F., Ang, S.-L., 2019. Dopamine neuron-derived IGF-1 controls dopamine neuron firing, skill learning, and exploration. Proc. Natl. Acad. Sci. USA 116, 3817–3826. https://doi.org/10.1073/pnas.1806820116.
Skorupskaite, K., George, J.T., Anderson, R.A., 2014. The kisspeptin-GnRH pathway in human reproductive health and disease. Hum. Reprod. Update 20, 485–500. https://doi.org/10.1093/humupd/dmu009.
Sommer, T., Richter, K., Singer, F., Derntl, B., Rune, G.M., Diekhof, E., Bayer, J., 2018. Effects of the experimental administration of oral estrogen on prefrontal functions in healthy young women. Psychopharmacol. (Berl.) 235, 3465–3477. https://doi.org/10.1007/s00213-018-5061-y.
Steyer, R., Schwenkmezger, P., Notz, P., Eid, M., 1997. Der Mehrdimensionale Befindlichkeitsfragebogen (MDBF). Handanweisung. Hogrefe, Göttingen.
Taxier, L.R., Gross, K.S., Frick, K.M., 2020. Oestradiol as a neuromodulator of learning and memory. Nat. Rev. Neurosci. 21, 535–550. https://doi.org/10.1038/s41583-020-0362-7.
Tilbrook, A.J., Clarke, I.J., 2001. Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. Biol. Reprod. 64, 735–742. https://doi.org/10.1095/biolreprod64.3.735.
Tobiansky, D.J., Wallin-Miller, K.G., Floresco, S.B., Wood, R.I., Soma, K.K., 2018. Androgen regulation of the mesocorticolimbic system and executive function. Front. Endocrinol. 9, 279. https://doi.org/10.3389/fendo.2018.00279.
Veselic, S., Jocham, G., Gausterer, C., Wagner, B., Ernhoefer-Reßler, M., Lanzenberger, R., Eisenegger, C., Lamm, C., Losecaat Vermeer, A., 2021. A causal role of estradiol in human reinforcement learning. Horm. Behav. 134, 105022 https://doi.org/10.1016/j.yhbeh.2021.105022.
Wierckx, K., Van Caenegem, E., Schreiner, T., Haraldsen, I., Fisher, A., Toye, K., Kaufman, J.M., T'Sjoen, G., 2014. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: results from the European network for the investigation of gender incongruence. J. Sex. Med. 11, 1999–2011. https://doi.org/10.1111/jsm.12571.
van Wingen, G.A., Ossewaarde, L., Backstrom, T., Hermans, E.J., Fernandez, G., 2011. Gonadal hormone regulation of the emotion circuitry in humans. Neuroscience 191, 38–45.
Yagi, S., Galea, L.A.M., 2019. Sex differences in hippocampal cognition and neurogenesis. Neuropsychopharmacology 44, 200–213. https://doi.org/10.1038/s41386-018-0208-4.
Yahyaoui, R., Esteva, I., Haro-Mora, J.J., Almaraz, M.C., Morcillo, S., Rojo-Martínez, G., Martínez, J., Gómez-Zumaquero, J.M., González, I., Hernando, V., Soriguer, F., 2008. Effect of long-term administration of cross-sex hormone therapy on serum and urinary uric acid in transsexual persons. J. Clin. Endocrinol. Metab. 93, 2230–2233. https://doi.org/10.1210/jc.2007-2467.
Yoest, K.E., Quigley, J.A., Becker, J.B., 2018. Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. Horm. Behav 104, 119–129. https://doi.org/10.1016/j.yhbeh.2018.04.002.