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Sexual attraction to visual sexual stimuli in association with steroid hormones across menstrual cycles and fertility treatment

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ABSTRACT

Background: Steroid hormones (i.e., estradiol, progesterone, and testosterone) are considered to play a crucial role in the regulation of women's sexual desire and sexual attraction to sexual stimuli throughout the menstrual cycle. However, the literature is inconsistent, and methodologically sound studies on the relationship between steroid hormones and women's sexual attraction are rare.

Methods: This prospective longitudinal multisite study examined estradiol, progesterone, and testosterone serum levels in association with sexual attraction to visual sexual stimuli in naturally cycling women and in women undergoing fertility treatment (in vitro fertilization, IVF). Across ovarian stimulation of fertility treatment, estradiol reaches supraphysiological levels, while other ovarian hormones remain nearly stable. Ovarian stimulation hence offers a unique quasi-experimental model to study concentration-dependent effects of estradiol. Hormonal parameters and sexual attraction to visual sexual stimuli assessed with computerized visual analogue scales were collected at four time points per cycle, i.e., during the menstrual, preovulatory, mid-luteal, and premenstrual phases, across two consecutive menstrual cycles ($n = 88$ and $n = 68$ for the first and second cycle, respectively). Women undergoing fertility treatment ($n = 44$) were assessed twice, at the beginning and at the end of ovarian stimulation. Sexually explicit photographs served as visual sexual stimuli.

Results: In naturally cycling women, sexual attraction to visual sexual stimuli did not vary consistently across two consecutive menstrual cycles. While in the first menstrual cycle sexual attraction to male bodies, couples kissing, and at intercourse varied significantly with a peak in the preovulatory phase, (all $p \leq 0.001$), there was no significant variability across the second cycle. Univariable and multivariable models evaluating repeated cross-sectional relationships and intraindividual change scores revealed no consistent associations between estradiol, progesterone, and testosterone and sexual attraction to visual sexual stimuli throughout both menstrual cycles. Also, no significant association with any hormone was found when the data from both menstrual cycles were combined. In women undergoing ovarian stimulation of IVF, sexual attraction to visual sexual stimuli did not vary over time and was not associated with estradiol levels despite intraindividual changes in estradiol levels from 122.0 to 11,746.0 pmol/l with a mean (SD) of 3553.9 (2472.4) pmol/l.

Conclusions: These results imply that neither physiological levels of estradiol, progesterone, and testosterone in naturally cycling women nor supraphysiological levels of estradiol due to ovarian stimulation exert any relevant effect on women's sexual attraction to visual sexual stimuli.

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1. Introduction

As ovarian steroid hormone levels vary throughout a woman's life, in particular across the menstrual cycle, and are modified by hormonal contraception, it is of interest to elucidate how steroid hormonal changes influence women's reactions to sexual stimuli. Moreover, approximately 10% of women suffer from hypoactive sexual desire disorder (HSDD), the most prevalent female sexual dysfunction (Goldstein et al., 2017). Better understanding of hormonal influences on sexual motivation would contribute to the improvement of hormone therapies to address persistent and distressing lack of reactions to sexual stimuli.

Motivation for sexual activities in women is complex and a result of different external and internal factors, such as sexual attraction or desire but also cultural norms, desire for pregnancy or even the feeling to have to fulfil partners' needs. Sexual desire may be considered as an internally generated energy arising independent of attraction or motivated by attractions from the environment, (Freud, 1953; Both et al., 2007; Toates, 2009; reviewed in Gangestad and Dinh, 2022), with visual sexual stimuli being one of these potential attractions. A sexual response system, eventually influenced by steroid hormones could therefore modulate sexual motivation by potentiating (or de-potentiating) perceived attraction to visual sexual stimuli. Sexual motivation has been reported to be increased by steroid hormones during fertile cycle phases (Roney, 2018; reviewed in Jones et al., 2019). Women's sexual desire peaks in the periovulatory phase of the menstrual cycle (Arslan et al., 2021; Bullivant et al., 2004; Marcinkowska et al., 2022; Roney and Simmons, 2013; Stern et al., 2021; van Stern et al., 2019; reviewed in Cappelletti and Wallen, 2016, and Motta-Mena and Puts, 2017).

However, the literature about steroid hormonal effects on sexual motivation and sexual desire is mixed: Positive effects of estradiol and testosterone on sexual desire were reported by studies with peri- and postmenopausal women, which found that declining estradiol levels led to decreases in sexual desire in perimenopausal women (Dennerstein et al., 2002), that estradiol therapies increased sexual desire in postmenopausal women (reviewed in Cappelletti and Wallen, 2016), and which resulted in an evidence-based indication for testosterone therapy for postmenopausal women with HSDD (Davis et al., 2019; Islam et al., 2019). In premenopausal women, however, the effect of steroid hormones on sexual desire is to date unclear: while some studies reported a positive effect of estradiol, a negative effect of progesterone, and a null effect of testosterone on sexual desire (Jones et al., 2018; Marcinkowska et al., 2022; Roney and Simmons, 2013), others found that androgens were positively associated with sexual desire (Wåhlin-Jacobsen et al., 2015; Zheng et al., 2020), and further studies showed null associations between steroid hormones and sexual desire (Davis et al., 2005; Shirazi et al., 2019; Stern et al., 2021). The mixed findings are presumably due to differences in methods. Hence, further high-powered, methodologically sound studies are needed to come to conclusions.

As sexual desire may strongly depend on the actual situation for example partner behaviour, several studies focussed on women's perceived attraction towards visual sexual stimuli as one aspect of women's sexual reactions potentially resulting in sexual desire. In early studies, women's sexual attraction to such stimuli did not vary across the menstrual cycle (Bossio et al., 2014; Gizewski et al., 2006; Mass et al., 2009; Meuwissen and Over, 1992; Rupp et al., 2009; Slob et al., 1991; Suschinsky et al., 2014). However, in contrast to our study these studies are underpowered, as they fail to meet recommendations of the most recent power simulation of cycle studies (Gangestad et al., 2016). Recent within-subject studies found increased women's sexual attraction to visual sexual stimuli to be associated with higher conception risk and increases in sexual desire (Stern et al., 2021), but sexual attraction was not consistently associated with changes in estradiol or progesterone levels (Jünger et al., 2018; Stern et al., 2021).

In light of the need for further sufficiently powered, methodologically sound studies to elucidate the relationship between menstrual cycle phases and women's sexual attraction to visual sexual stimuli on the one hand, and between steroid hormones and women's sexual attraction to visual sexual stimuli on the other hand, we applied a stringent prospective longitudinal study design across two consecutive menstrual cycles, which went beyond methods that were recently recommended as best practice for menstrual cycle research (Blake et al., 2016; Gangestad et al., 2016). In addition to the naturally cycling cohort, women undergoing in vitro fertilization (IVF) were included. Across the ovarian stimulation of IVF treatment, estradiol reaches much higher levels than in a natural menstrual cycle due to the simultaneous growth of the whole cohort of follicles, while other ovarian hormones remain nearly stable. Thus, ovarian stimulation offers a quasi-experimental model to examine the isolated effect of endogenous estradiol at supraphysiological levels on sexual attraction to visual sexual stimuli.

With this background, the present study aimed to examine (i) whether women's sexual attraction to visual sexual stimuli varies across the menstrual cycle and ovarian stimulation of fertility treatment (IVF), (ii) whether estradiol, progesterone, and testosterone levels are associated with sexual attraction to visual sexual stimuli in naturally cycling women, and (iii) whether supraphysiological estradiol levels at the end of ovarian stimulation exert an effect on women's sexual attraction to visual sexual stimuli.

2. Material and methods

2.1. Participants and design

A prospective longitudinal multisite study was conducted to investigate associations between steroid hormone levels (i.e., estradiol, progesterone, and testosterone) and sexual attraction to visual sexual stimuli in naturally cycling women and in women undergoing ovarian stimulation for fertility treatment (IVF). The present study is part of a project designed to model women's hormonal changes in association with neuropsychological functions (Hengartner et al., 2017; Leeners et al., 2017, 2019, 2021).

88 naturally cycling women were assessed four times per cycle in the menstrual, preovulatory, midluteal, and premenstrual phase; 68 of them were re-assessed in a second, consecutive menstrual cycle, in an attempt to replicate findings to minimize the probability of false-positive chance findings. 44 women undergoing IVF were assessed twice: at the beginning and at the end of ovarian stimulation. At each measurement, participants rated their sexual attraction to visual sexual stimuli, and blood samples to quantify steroid hormones were collected. For the naturally cycling women, estradiol, progesterone, and testosterone serum levels were measured; for women undergoing ovarian stimulation, only estradiol serum levels were measured.

2.1.1. Cohort of naturally cycling women

For the cohort of naturally cycling women, a baseline visit served to verify inclusion and exclusion criteria, to collect information on the cycle length of the preceding six menstrual cycles, and to perform a physical examination. The exclusion criteria were as follows: use of oral contraceptives, pregnancy or breastfeeding within the past six months, medication or surgery interfering with endocrine parameters, severe psychiatric or general diseases, working irregular shifts, menstrual or ovulation disorders except those investigated in the study (i.e., endometriosis, PCOS and hyperprolactinemia), and additional abnormal hormonal parameters (LH, FSH, estradiol, progesterone, testosterone, prolactin, fasting glucose, fasting insulin, TSH, and anti-Müllerian hormone) measured at cycle day 4 following the baseline examination. Further, a transvaginal ultrasound was conducted at cycle day 4 to exclude any cysts interfering with the menstrual cycle.

For each woman with a cycle length of 28 $\pm$ 4 days, blood samples to quantify hormonal parameters were collected eight times per menstrual cycle: i.e., for a 28-day cycle at cycle days 4, 7, 9 or 10, 12, 13, 17, 21,

and 28; with adjustment in case of known shorter or longer cycles. Besides measuring the hormones of interest of the present study (i.e., estradiol, progesterone, and testosterone), further, LH and FSH were measured as part of the big study project (Hengartner et al., 2017; Leeners et al., 2017). At four out of these eight hormone measurement occasions, participants completed sexual attraction ratings of visual sexual stimuli in the menstrual, preovulatory, mid-luteal, and premenstrual phase, i.e., for a 28-day cycle at cycle days 4, 13, 21, and 28, respectively. For visualisation of measurement occasions, see Fig. 1. Hormone measurements in between sexual attraction ratings, marked grey in Fig. 1, served to adjust test sessions to varying cycle length. A second ultrasound was conducted around cycle day 11, preponed in case of known shorter cycles, to determine follicular development and to place the preovulatory measurement occasion precisely. A follicle of 18 – 19 mm diameter in combination with a rise in LH was considered the ideal time point for the preovulatory measurement. When no dominant follicle was seen, additional ultrasounds were conducted every 4–5 days, until follicular development was confirmed or cycle day 30 was reached. To detect ovulation, ovulation tests based on urine LH measurements were applied starting either five days prior to the earliest expected ovulation based on the previous six cycles or when a 14 mm follicle was seen in transvaginal ultrasound (Evial Ovulationstest Midstream, Inopharm GmbH, Muri, Switzerland, and Clearblue digital Ovulationstest, SPD Swiss Precision Diagnostics GmbH, Geneva, Switzerland). As presented in Fig. 1 the mid-luteal measurement was conducted 7 days after ovulation and the and premenstrual measurement 13/14 days after ovulation. A mid-luteral progesterone measurements served to confirm ovulation.

In total, 88 naturally cycling women with a mean age of 30.2 ± 5.5 years (range 20–40 years) were evaluated, of whom 58 had no endocrinological pathology, 13 were diagnosed with endometriosis, 16 with PCOS, and one with hyperprolactinemia. 12 women were obese (BMI > 30.0); the mean BMI was 25.0 ± 5.4 (range 17.7–45.7). 31 women were married, 27 had children, and 27 had a university degree. 50 women were recruited at the Department of Psychiatry, Social Psychiatry and Psychotherapy, Medical School Hannover, Germany; 38 women were recruited at the Department of Reproductive Endocrinology, University Hospital Zurich, Switzerland. All women with endocrinological pathologies were recruited in Zurich. Out of the 88 participants, 68 women were re-assessed during a second, consecutive menstrual cycle (Hannover: 47 women; Zurich: 21 women). Participants were recruited through word of mouth, direct invitation during consultations at the Department of Reproductive Endocrinology, University Hospital Zurich, through referrals by gynaecological endocrinologists, and by advertisement on the hospital and university boards.

2.1.2. Cohort of women undergoing ovarian stimulation for IVF

Preceding ovarian stimulation of fertility treatment (IVF), a downregulation treatment with either a GnRH-analogue or progestin was administered. For ovarian stimulation, daily injections of FSH or FSH plus LH were administered for 9–13 days with regular estradiol and ultrasound measurements to time ovulation induction with HCG or a GnRH-analogue. Across ovarian stimulation, measurements of sexual attraction ratings and serum estradiol levels were taken twice: at the beginning of ovarian stimulation after downregulation, when estradiol values are lowest, and at the end of ovarian stimulation at the day of ovulation induction, when estradiol values are highest. Ovarian stimulation takes between 9 and 13 days, i.e., measurement occasions were at least 9 and at maximum 13 days apart (Fig. 1).

All women included in this cohort sought medical support because of failure to conceive spontaneously. They underwent standard investigations of fertility disorders at the Departement of Reproductive Endocrinology, University Hospital Zurich, Switzerland. A gynecological examination including transvaginal ultrasound was conducted to determine antral follicle count and uterine or adnexal abnormalities. To evaluate endocrinological disorders, the following hormonal parameters were measured in serum samples at cycle days 2–5: estradiol, 17-OH progesterone, testosterone, anti-Müllerian hormone, LH, FSH, TSH, and prolactin. Depending on the male partner's semen analysis, hydrosonography of the uterine cavity, hydro-contrast-sonography or hysterosalpingography were performed to evaluate uterine and/or tubal pathology. Chlamydia, HIV, Hepatitis B and C infections were

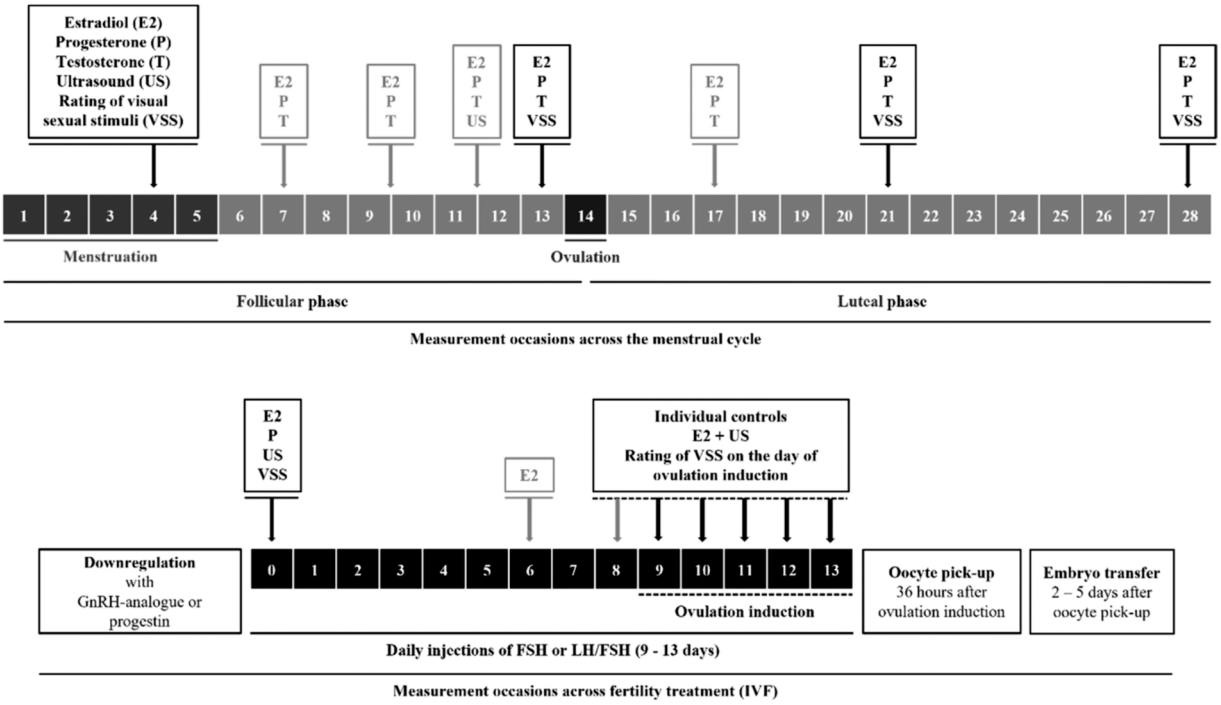

Fig. 1. Measurement occasions across the menstrual cycle and ovarian stimulation of fertility treatment (IVF) are displayed. Hormonal measurements at days of sexual attraction rating tasks (VSS) were analyzed in the present study (marked in black). Additional hormonal measurements and ultrasounds (marked in grey) served to schedule the measurement occasions according to varying cycle length and duration of ovarian stimulation.

investigated in both partners. Exclusion criteria were premenstrual syndrome and medical conditions related to cognitive performance (i.e., psychiatric diseases).

Data were collected from 44 women receiving in vitro fertilization at the Departement of Reproductive Endocrinology, University Hospital Zurich, Switzerland, with a mean age of 36.7 ± 3.5 years (range 29–45 years), 26 of whom received their first treatment and 18 their second treatment. None of the women had received any hormonal treatment in the three months prior to the fertility treatment. Indications for fertility treatment were mechanical problems (n = 13), endometriosis (n = 14), PCOS (n = 10), idiopathic sterility (n = 6), and male factors only or reduced sperm quality in addition to the female indications (n = 34). Some of the couples had several causes of infertility. Baseline estradiol levels did not differ between women with different indications for fertility treatment and the cohort of naturally cycling women.

2.2. Hormone measurements and assays

Blood samples were collected between 7:00 and 10:00 am. In Zurich blood samples were sent immediately to the laboratory, while they were frozen at − 30 °C and then stored at − 80 °C and later sent to Zurich in Hannover. To avoid bias due to different measurement methods and laboratory procedures, samples were all analysed by the Institute of Clinical Chemistry, University Hospital Zurich. External quality controls were conducted at regular intervals by the Society for Promoting Quality Assurance in Medical Laboratories (INSTAND, Duesseldorf, Germany) and the Reference Institute for Bioanalytics (RfB, Bonn, Germany).

Estradiol was measured using electrochemiluminescence immunoassays ECLIA (Elecsys® Estradiol II) based on polyclonal antibody (Roche Diagnostics GmbH, Penzberg, Germany) with a functional assay sensitivity of 44 pmol/l and a coefficient of variation (CV%) of < 7.7%. From January 15th, 2015, the ECLIA (Elecsys® Estradiol III) based on monoclonal antibody (Roche Diagnostics GmbH, Penzberg, Germany) with a functional assay sensitivity of 91.8 pmol/l (25 pg/ml) and CV% of < 3.36% was applied. Progesterone and testosterone were measured using electrochemiluminescence immunoassays (ECLIA) applied on Cobas e-602 immunoassay autoanalyzer (Roche Diagnostics GmbH, Penzberg, Germany) with functional assay sensitivities of 0.48 nmol/l and 0.416 nmol/l for progesterone and testosterone, respectively. Total imprecision expressed as coefficient of variation (CV%) for progesterone and testosterone was 5.1% and 3.9%, respectively.

2.3. Measures of women's sexual attraction to visual sexual stimuli

Sexually explicit photographs depicting the following four categories were presented to the participants as visual sexual stimuli: male faces, male bodies, heterosexual couples kissing, and heterosexual couples having sexual intercourse. As visual sexual stimuli perceived as erotic are known to be different between men and women (Rupp and Wallen, 2008), photographs were selected in a prestudy. In this prestudy, 50 women other than the study participants rated their level of sexual attraction to a selection of eight photographs per category. The four photographs rated highest per category were selected for the present study. To ensure standardized conditions, the same set and order of visual sexual stimuli was applied at all test sessions and to all participants.

Sexual attraction to visual sexual stimuli was quantified with a computerized visual analogue scale (VAS) from 0 to 100; 0 referring to "not at all sexually attractive" and 100 meaning "extremely sexually attractive". Participants were instructed to rate their level of sexual attraction to the visual sexual stimuli as quickly and as precisely as possible upon presentation. The test was performed on a touch screen computer; the same model was used in Hannover and Zurich. Participants were placed in a quiet room to complete the test, with a trained study staff member present to explain the test and answer any questions that arose. During the test, the study staff member turned their back to the participants to ensure undisturbed rating of the sexual stimuli. The rating of sexual attraction to visual sexual stimuli was part of a series of tests on neurocognitive functioning, which were assessed with a standardized computer-assisted test system (CANDIT: Computer Assisted Neuropsychological Diagnostics and Therapy, Candit.com). Other neuropsychological parameters in association with women's hormonal changes have been previously reported (Hengartner et al., 2017; Leeners et al., 2017, 2019, 2021).

2.4. Ethics

This study followed the guidelines of the World Medical Association Declaration of Helsinki 1964, updated in October 2013, and was conducted after approval by the Ethics Committees of Zurich and Hannover for investigations involving human subjects. All participants provided written informed consent and were compensated for their expenditures. The study has been registered in clin.trial.gov (NCT02098668).

2.5. Statistical analysis

The repeated measures of sexual stimuli were estimated using generalized estimating equations (GEE). These statistical models were introduced to fit regression analyses that account for within-subject correlation, which is an inherent part of longitudinal studies that rely on repeated outcome measures (Zeger et al., 1988). GEE are considered state of the art for longitudinal data analysis and superior to repeated measures ANOVA due to their psychometric properties (Ballinger, 2004; Gibbons et al., 2010). GEE use all available data and impute missing values under the assumption of missing completely at random (MCAR). Repeated measures of visual sexual stimuli scores were successively entered as the outcome variables. The time slope was included both as a within-subject effect and as a main effect (covariate) in all models. Because the outcome measures were approximately normally distributed, all models were fitted with normal distribution and applied the identity link-function. The within-subject covariance was specified with the "unstructured" correlation type to avoid having any constraints on the covariance structure; a robust sandwich estimator was used to reduce the effects of outliers and influential observations. Importantly, these GEE models align closely with mixed regression models (Gibbons et al., 2010; Twisk, 2003).

To analyse changes in preferences for sexual stimuli over the phases of the menstrual cycle, we included only time as a predictor variable. Next, we computed models where we additionally entered the hormone measures separately as predictor variables to test their associations with the preferences for sexual stimuli. Because these models merely provide a pooled within-person estimate for the repeated cross-sectional associations between differential hormone levels and visual sexual stimuli (e.g., attraction to people kissing being higher at cycle phases when estradiol levels are higher), we additionally computed longitudinal intraindividual change models (Twisk, 2003). In such models, relative change values between consecutive measurements of both the outcome variable and the predictor variable are examined instead of absolute values for each time point (e.g., attraction to people kissing increases over time when estradiol levels increase over consecutive cycle phases). Following Twisk (2003), in these change models the covariance structure was specified as "independent". These models were again fitted with normal distribution and the identity link-function. Hormone levels were tested both separately, that is, in consecutive univariable models, and simultaneously in a multivariable model. Due to multiple testing (four categories of sexual stimuli were regressed on each hormone), the level of statistical significance was set at Bonferroni-corrected $\alpha = 0.0125$ for each hormone. Extreme outliers of hormone levels in naturally cycling women, i.e., values occurring 3 times above the 75th percentile, were considered likely measurement artefacts. These were excluded from the statistical analysis. For each hormone measured, this affected 1 or 2 women.

For women undergoing fertility treatment, only one change score

was available, and thus no repeated-measure analysis was feasible. Instead, a linear regression analysis was employed, regressing change in sexual stimuli ratings on changes in estradiol levels.

Standardized hormone measures (z-transformation) were entered in all regression models to facilitate the comparison among estradiol, progesterone, and testosterone. All analyses were performed with SPSS version 28 for Windows.

3. Results

In the naturally cycling cohort, ovulation was confirmed in 84 and 65 women in the first and second cycle, respectively. 4 and 3 women had an anovulatory cycle in the first and second menstrual cycle, respectively. These women were included in the study, since the exclusion did not alter the results relevantly, and since correlations between hormone levels and sexual attraction ratings were of particular interest.

3.1. Sexual attraction to visual sexual stimuli across the menstrual cycle and ovarian stimulation

Ratings of women's sexual attraction to visual sexual stimuli and steroid hormone levels across menstrual cycles and ovarian stimulation in fertility treatment are shown in Table 1. Sexual attraction to male bodies, couples kissing and couples at intercourse varied significantly across the first menstrual cycle (all $p \leq 0.001$), peaking in the preovulatory phase. Across the second menstrual cycle, sexual attraction to visual sexual stimuli did not vary significantly over time. For all steroid hormones, changes in mean levels over time were statistically significant (all $p < 0.001$) across both menstrual cycles with estradiol and testosterone peaking in the preovulatory phase and progesterone in the mid-luteal phase.

Sexual attraction to visual sexual stimuli did not vary significantly across ovarian stimulation of IVF (all $p > 0.6$), but as expected, estradiol levels increased dramatically from the beginning to the end of the ovarian stimulation ($p < 0.001$). Intraindividual changes in estradiol levels ranged from 122.0 to 11,746.0 pmol/l with a mean (SD) of 3553.9 (2472.4) pmol/l. Hence, estradiol levels increased substantially in all women across ovarian stimulation, although the amount of intra-individual increase varied considerably.

3.2. Repeated within-person cross-sectional associations between steroid hormone levels and sexual attraction ratings

Univariable associations between women's sexual attraction to visual sexual stimuli and (a) steroid hormone levels across menstrual cycles and (b) ovarian stimulation of fertility treatment are shown in Table 2. Across the first menstrual cycle, progesterone related positively to sexual attraction ratings of male faces ($B = 2.22$, $p = 0.017$). Across the second menstrual cycle, progesterone related negatively to sexual attraction ratings of couples kissing ($B = -2.75$, $p = 0.014$). These associations remained significant after adjusting for age, obesity, endometriosis, and PCOS. However, neither of the two associations replicated in the other menstrual cycle. Further, no single effect reached statistical significance at Bonferroni corrected $\alpha = 0.0125$. When the data from both menstrual cycles were combined, resulting in 8 repeated measures, and thus increased statistical power, not one significant association with any hormone was found (all $p > 0.05$).

The multivariable models where all three hormone levels were tested simultaneously confirmed a positive association between progesterone and sexual attraction ratings of male faces during the first cycle ($B = 2.64$; 95%-CI $= 0.54 - 4.74$, $p = 0.014$). Testosterone was negatively associated with sexual attraction ratings of male faces ($B = -3.30$; 95%-CI $= -6.59$ to 0.02, $p = 0.049$). However, both associations failed to meet strict criteria for Bonferroni corrected $\alpha = 0.0125$ and the results could not be replicated in the second menstrual cycle (for progesterone: $B = -1.36$; 95%-CI $= -4.99$ to 2.27, $p = 0.463$; for testosterone: $B = 3.40$; 95%-CI $= -2.44$ to 9.24, $p = 0.254$). By contrast, in the second menstrual cycle we found significant associations that where not observed in the first cycle, specifically, sexual attraction ratings of couples kissing were associated with higher estradiol ($B = 3.70$; 95%-CI $= 0.64 - 6.75$, $p = 0.018$) and lower progesterone ($B = -5.08$; 95%-CI $= -7.87$ to 2.30, $p < 0.001$), and sexual attraction ratings of couples at

Table 1
Ratings of women's sexual attraction to visual sexual stimuli and steroid hormone levels across menstrual cycles and ovarian stimulation of fertility treatment (IVF).

First menstrual cycle					
Measures	Measurement occasion				Main effect of time
	Menstrual phase	Preovulatory phase	Mid-luteal phase	Premenstrual phase	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P
Male faces	48.9 (43.6 – 54.1)	48.2 (43.2 – 53.1)	46.0 (40.5 – 51.5)	44.7 (39.4 – 50.0)	0.167
Male bodies	36.8 (33.1 – 40.5)	38.1 (34.4 – 41.7)	35.8 (32.2 – 39.5)	33.4 (29.8 – 37.0)	0.001
Couples kissing	56.3 (52.1 – 60.5)	63.2 (59.4 – 67.1)	57.1 (53.3 – 60.9)	60.2 (56.2 – 64.1)	< 0.001
Couples at intercourse	61.6 (57.4 – 65.7)	67.9 (64.0 – 71.8)	63.6 (59.6 – 67.6)	65.5 (61.4 – 69.5)	< 0.001
Estradiol (pmol/l)	173.4 (155.5 – 191.3)	750.9 (653.8 – 848.1)	570.6 (520.5 – 620.7)	361.0 (307.7 – 414.3)	< 0.001
Progesterone (nmol/l)	1.95 (1.75 – 2.14)	2.43 (2.09 – 2.76)	39.97 (34.94 – 44.99)	16.41 (12.80 – 20.03)	< 0.001
Testosterone (nmol/l)	1.03 (0.91 – 1.16)	1.27 (1.13 – 1.42)	0.98 (0.86 – 1.11)	1.01 (0.88 – 1.14)	< 0.001
Second menstrual cycle					
Measures	Measurement occasion				Main effect of time
	Menstrual phase	Preovulatory phase	Mid-luteal phase	Premenstrual phase	
	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	P
Male faces	45.4 (39.4 – 51.4)	46.0 (39.5 – 52.5)	44.5 (37.9 – 51.0)	42.2 (35.7 – 48.6)	0.382
Male bodies	33.2 (29.3 – 37.0)	33.0 (29.2 – 36.7)	31.4 (27.4 – 35.4)	31.7 (27.8 – 35.6)	0.296
Couples kissing	59.3 (54.8 – 63.8)	59.0 (54.2 – 63.8)	58.6 (54.2 – 63.0)	59.3 (54.4 – 64.3)	0.940
Couples at intercourse	62.9 (58.2 – 67.6)	65.5 (60.6 – 70.4)	64.4 (59.8 – 69.0)	63.7 (58.7 – 68.7)	0.510
Estradiol (pmol/l)	187.4 (166.5 – 208.3)	800.5 (675.8 – 925.2)	570.5 (509.4 – 631.6)	306.3 (247.0 – 365.5)	< 0.001
Progesterone (nmol/l)	1.88 (1.65 – 2.10)	2.42 (2.09 – 2.75)	41.04 (35.28 – 46.79)	12.22 (8.75 – 15.69)	< 0.001
Testosterone (nmol/l)	0.95 (0.82 – 1.07)	1.16 (1.02 – 1.30)	0.92 (0.79 – 1.06)	0.91 (0.78 – 1.04)	< 0.001
Ovarian stimulation of fertility treatment (IVF)					
Measures	Measurement occasion				Main effect
	Beginning of ovarian stimulation		End of ovarian stimulation		
	Mean (95% CI)		Mean (95% CI)		P
Male faces	53.18 (46.51 – 59.85)		53.03 (45.58 – 60.49)		0.951
Male bodies	37.29 (32.56 – 42.02)		37.43 (32.37 – 42.49)		0.937
Couples kissing	53.69 (49.10 – 58.27)		54.56 (49.57 – 59.55)		0.634
Couples at intercourse	64.17 (59.08 – 69.26)		63.29 (57.01 – 69.57)		0.708
Estradiol (pmol/l)	54.54 (40.02 – 74.33)		3624.45 (2959.25 – 4439.19)		< 0.001

Table 2
Univariable associations between steroid hormone levels and sexual attraction ratings of visual sexual stimuli across menstrual cycles and ovarian stimulation of fertility treatment (IVF).

First menstrual cycle						
Measures	Estradiol		Progesterone		Testosterone	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Male faces	-1.49 (−3.86 to 0.89)	0.220	2.22 (0.40 – 4.05)	0.017	-2.73 (−6.49 to 1.03)	0.155
Male bodies	-1.15 (−2.80 to 0.51)	0.176	0.60 (−0.42 to 1.61)	0.250	0.37 (−2.25 to 2.98)	0.783
Couples kissing	-0.19 (−1.21 to 0.83)	0.711	0.52 (−0.46 to 1.51)	0.298	1.03 (−1.39 to 3.44)	0.406
Couples at intercourse	-0.27 (−1.81 to 1.28)	0.735	0.10 (−1.77 to 1.98)	0.915	1.93 (−1.75 to 5.61)	0.305
Second menstrual cycle						
Measures	Estradiol		Progesterone		Testosterone	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Male faces	0.83 (−1.21 to 2.87)	0.425	-0.74 (−4.03 to 2.55)	0.659	2.54 (−0.83 to 5.91)	0.140
Male bodies	0.08 (−0.96 to 1.10)	0.887	-0.68 (−1.42 to 0.06)	0.071	-2.19 (−8.05 to 3.67)	0.463
Couples kissing	0.23 (−2.27 to 2.73)	0.857	-2.75 (−4.94 to −0.56)	0.014	-1.49 (−8.38 to 5.41)	0.673
Couples at intercourse	0.66 (−1.23 to 2.56)	0.493	-0.73 (−2.57 to 1.11)	0.439	-2.82 (−9.04 to 3.41)	0.375
Ovarian stimulation of fertility treatment (IVF)						
Measures	Estradiol					
	B (95% CI)	P				
Male faces	0.99 (−2.92 to 4.9)	0.620				
Male bodies	-1.44 (−3.62 to 0.74)	0.194				
Couples kissing	-0.48 (−2.99 to 2.03)	0.708				
Couples at intercourse	-2.51 (−6.06 to 1.04)	0.165				

intercourse were also associated with higher estradiol ($B = 2.88$; 95%-CI $= 0.32 – 5.43$, $p = 0.027$) and lower progesterone ($B = -2.65$; 95%-CI $= -5.10$ to 0.19, $p = 0.035$), but note that the latter three effects all failed to reach statistical significance at Bonferroni-corrected $\alpha = 0.0125$. As in the univariable models detailed above, when both menstrual cycles were combined, not one significant association emerged (all $p > 0.05$), confirming that there is no robust association between hormone levels and sexual attraction to visual sexual stimuli. Standardized regression coefficients beta are reported in supplementary Table S1. The beta coefficients show that all associations were weak (all $\beta < 0.14$).

Across ovarian stimulation in women undergoing IVF, estradiol levels did not associate significantly with sexual attraction ratings of visual sexual stimuli.

3.3. Intraindividual change score associations between steroid hormone levels and sexual attraction ratings

Univariable associations between intraindividual changes in steroid hormone levels and sexual attraction ratings across menstrual cycles and ovarian stimulation are indicated in Table 3. No single effect reached statistical significance at $\alpha = 0.05$. Adjusting for age, obesity, endometriosis, and PCOS did not significantly alter the results. The multivariable models likewise showed no significant effects (all $p > 0.05$) in the first and second menstrual cycle. In women undergoing fertility treatment, we found likewise no association between change in ratings of sexual stimuli and estradiol levels (all $p \geq 0.1$; see Table 3). Scatter plots showed very weak, close to zero linear relationships with multiple outliers (i.e., $R^2 = 0.004$, −0.004, −0.004, and −0.07 for ratings of faces, bodies, kissing, and intercourse, respectively) between intraindividual changes in estradiol levels and sexual attraction ratings across ovarian stimulation (Suppl. Fig. 1). Standardized regression coefficients beta are shown in the supplementary Table S2; all associations were weak (all $\beta < 0.14$) except a moderate albeit statistically non-significant association between change in ratings of couples at intercourse and change in estradiol levels in women undergoing fertility treatment ($\beta = -0.264$, $p = 0.100$).

4. Discussion

4.1. Sexual attraction to visual sexual stimuli across the menstrual cycle

In the present study, women's sexual attraction to visual sexual

Table 3
Univariable associations between intraindividual changes in steroid hormone levels and sexual attraction ratings of visual sexual stimuli across menstrual cycles and ovarian stimulation of fertility treatment (IVF).

First menstrual cycle						
Measures	Estradiol		Progesterone		Testosterone	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Male faces	-1.20 (−3.56 to 1.16)	0.318	1.33 (−1.46 to 4.12)	0.350	-2.02 (−4.34 to 0.31)	0.089
Male bodies	0.03 (−1.18 to 1.25)	0.959	1.11 (−0.29 to 2.50)	0.121	-0.05 (−1.77 to 1.66)	0.951
Couples kissing	-0.70 (−2.12 to 0.72)	0.334	1.38 (−0.27 to 3.03)	0.101	0.64 (−1.01 to 2.29)	0.449
Couples at intercourse	0.20 (−1.58 to 1.97)	0.828	0.94 (−0.76 to 2.63)	0.278	1.09 (−0.94 to 3.13)	0.293
Second menstrual cycle						
Measures	Estradiol		Progesterone		Testosterone	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Male faces	2.12 (−0.40 to 4.64)	0.099	-0.50 (−3.60 to 2.61)	0.754	0.04 (−2.25 to 2.32)	0.976
Male bodies	0.55 (−0.55 to 1.65)	0.328	-0.37 (−1.56 to 0.82)	0.541	0.15 (−1.07 to 1.37)	0.814
Couples kissing	-1.19 (−3.13 to 0.74)	0.227	-1.41 (−3.43 to 0.62)	0.173	0.37 (−1.40 to 2.14)	0.684
Couples at intercourse	0.74 (−1.26 to 2.74)	0.469	-0.08 (−1.69 to 1.53)	0.919	0.01 (−1.63 to 1.65)	0.992
Ovarian stimulation of fertility treatment (IVF)						
Measures	Estradiol					
	B (95% CI)	P				
Male faces	0.98 (−4.18 to 6.14)	0.702				
Male bodies	-0.72 (−4.42 to 2.98)	0.696				
Couples kissing	-0.78 (−4.65 to 3.09)	0.685				
Couples at intercourse	-4.05 (−8.90 to 0.81)	0.100				

stimuli varied across the first menstrual cycle with a weak increase in the preovulatory phase, which is in line with previous research, but showed no variability across the second cycle. As the cycle effect was miniscule and did not replicate across the second cycle, we suggest that the weak variability across the first menstrual cycle likely reflects random fluctuations or measurement artefacts.

The presentation of the same set and order of visual sexual stimuli at all test sessions was chosen for better comparability and higher reliability, but might have had an impact on the results from the second cycle. Specifically, the habituation of women's sexual response through repeated exposure to the same stimuli may be a reason for failed replication of the cycle effect (Both et al., 2011; Dawson et al., 2013; Kelley and Musialowski, 1986; Meuwissen and Over, 1992; reviewed in Ventura-Aquino et al., 2018). However, in these studies, time intervals between repeated exposure were much shorter (i.e., daily exposure or multiple re-presentation within a day), which does not compare to four test sessions per cycle as in the present study. Although there is evidence against habituation to sexual stimuli (Laan and Everaerd, 1995) a counterbalanced study design would likely have been beneficial to avoid confounding by sequential effects. Also, it has to be considered, that statistical power was lower in the second cycle, which might have added to the lack of associations.

Other sufficiently powered within-subject studies reported increased sexual attraction to male bodies in the preovulatory phase of the menstrual cycle, but standardized effect sizes were close to zero (Jünger et al., 2018; Stern et al., 2021). Due to the very small cycle effect found in those studies and the lack of consistent cycle effect in the present study, there is to date no compelling evidence for a relevant effect of menstrual cycle phase on women's sexual attraction to visual sexual stimuli. In contrast, female sexual desire has been found to be increased in the preovulatory phase of the menstrual cycle, which has been discussed to be a result from hormonal changes especially in testosterone levels (Arslan et al., 2021; Bullivant et al., 2004; Marcinkowska et al., 2022; Roney and Simmons, 2013; van Stern et al., 2019; reviewed in Cappelletti and Wallen, 2016, and Motta-Mena and Puts, 2017, Wåhlin-Jacobsen et al., 2015). This is particularly relevant as sexual attraction is positively associated with sexual desire (Stern et al., 2021). Visual sexual stimuli (i.e., sexually explicit photographs) may not be the optimal modality to evaluate reactions to sexual stimuli, as audio-visual sexual stimuli (i.e., sexually explicit videos) induce stronger subjective sexual arousal than images, fantasy, or auditory narratives (Kukkonen, 2015). As methodologically sound within-subject studies investigating sexual attraction to audio-visual sexual stimuli across the menstrual cycle are missing, future studies should not only implement different sexual stimuli but also test sexual attraction and sexual desire, to better understand the role and interaction of different factors determining womens sexual motivation (Both et al., 2007; Toates, 2009; reviewed in Gangestad and Dinh, 2022).

4.2. Associations between steroid hormone levels and sexual attraction to visual sexual stimuli

In naturally cycling women, univariable and multivariable models evaluating repeated cross-sectional relationships and intraindividual change scores revealed no consistent associations between estradiol, progesterone, and testosterone and sexual attraction to visual sexual stimuli throughout both menstrual cycles. Also, no significant association with any hormone was found when the data from both menstrual cycles were combined. Estradiol's predominant null effects on sexual attraction in cycling women was confirmed by our findings of the fertility treatment cohort. Despite the dramatic rise in estradiol across ovarian stimulation, sexual attraction ratings did not vary and did not associate with estradiol levels (including between- and within-subject effects). Hence, supraphysiological estradiol levels far beyond the cyclic maximum did not exert any effect on women's sexual attraction to visual sexual stimuli. Moreover, as other ovarian hormones remained nearly constant across ovarian stimulation, the null association cannot be due to confounding effects of other steroid hormones. However, sexual attraction ratings could be confounded by the psychological burden associated with infertility (Pasch et al., 2016; Rockliff et al., 2014), as psychosocial determinants associate with women's sexual dysfunctions (Basson, 2021; Zheng et al., 2020). On the other hand, the fertility treatment itself is experienced as less stressful than the period before treatment initiation, and the stress level is comparable to naturally cycling women (Leeners et al., 2019). However, these findings do not permit to draw final conclusions on causal effects as hormonal changes especially during a menstrual cycle are very complex and our study design does not allow to get an insight into the multivarious interactions of different signals from the hormonal system, sexual variables, and potential further factors. Also, it cannot be excluded that different levels of estrogens may induce different effects, for example regarding the up- or down regulation of receptors, so that (patho) physiological mechanisms may depend on estradiol levels.

Assuming that sexual attraction is related to sexual motivation, the lack of a coherent association between estradiol, progesterone, and testosterone and women's sexual attraction to visual sexual stimuli found in the present study do not support a steroid hormonal regulation of sexual motivation in premenopausal women as postulated in the "motivational priorities theory" (Roney, 2018; reviewed in Jones et al., 2019).

Altogether, there is few data on the association between steroid hormones on sexual attraction, but several studies have investigated sexual desire in relation to steroid hormones. While there is evidence that estradiol positively and progesterone negatively predict sexual desire in naturally cycling women (Jones et al., 2018; Marcinkowska et al., 2022; Roney and Simmons, 2013), other research did not find that estradiol and progesterone were associated with sexual desire (Shirazi et al., 2019; Stern et al., 2021) nor sexual attraction to visual sexual stimuli (Stern et al., 2021).

With respect to androgens in naturally cycling women, one cross-sectional study found that sexual desire associates positively with testosterone and androstenedione (Wåhlin-Jacobsen et al., 2015), and another one reported a positive association with dehydroepiandrosterone and androstenedione but not testosterone (Zheng et al., 2020). However, these findings are limited, as associations were small and cross-sectional studies do not assess within-person change across the menstrual cycle. Intraindividual change models with repeated measures are the more stringent test of causal pathways and require much lower sample sizes to achieve sufficient power. Unfortunately, due to lack of data, we cannot compare our findings on sexual attraction by visual sexual stimuli and testosterone levels with other study results. However, null associations between testosterone and sexual desire, another aspect of sexual motivation, in naturally cycling women have been reported by longitudinal studies (Jones et al., 2018; Roney and Simmons, 2013; Shirazi et al., 2019) and cross-sectional studies (Davis et al., 2005). Finally, the "Global Consensus Position Statement on the Use of Testosterone Therapy for Women" and a review concluded that associations between endogenous androgen concentrations and sexual function remain unclear and that there are no cut-off serum levels for androgens to distinguish between women with and without sexual dysfunction (Basson, 2021; Davis et al., 2019, 2005).

While there is an evidence-based indication for testosterone therapy for postmenopausal women with HSDD (Davis et al., 2019; Islam et al., 2019) and there is evidence that estradiol therapies increase sexual desire in postmenopausal women (reviewed in Cappelletti and Wallen, 2016), there is to date no compelling evidence for hormonal differences between women with HSDD and age-matched peers without sexual dysfunctions (Basson, 2021; Davis et al., 2019; Goldstein et al., 2017; Pettigrew and Novick, 2021). Hence, measuring steroid hormone levels offers no diagnostic use in the assessment of HSDD. Instead, psychosocial factors and potentially other biological factors besides sex steroids appear to be more relevant determinants of women's sexual functioning,

as psychosocial determinants associate robustly with women's sexual dysfunctions (Basson, 2021; Zheng et al., 2020).

4.3. Strengths and limitations

When null results are found, it is always up for discussion whether they might be due to invalid measures or power failure. However, the null results reported in the present study are not a consequence of power failure as our study design greatly exceeded recommendations of the most recent power simulation on cycle studies (Gangestad et al., 2016): (a) our sample size was greater than the suggested minimum sample size of n = 48 in both cycles; (b) the methods we applied produced higher validity, with four measurement occasions across the menstrual cycle; (c) the attempt to replicate findings in a second cycle; (d) the precise placement of the preovulatory measurement with transvaginal ultrasound; (e) the confirmation of ovulation with urinary LH tests; and (f) blood sampling to measure steroid hormone levels in serum. To the best of our knowledge, such rigorous testing has not yet been applied to studies on women's sexual attraction to visual sexual stimuli in association to steroid hormone levels.

Moreover, the additional analysis of women undergoing ovarian stimulation of fertility treatment (IVF) represents a great strength of the present study, as it allowed to evaluate estradiol's effect on women's sexual attraction at supraphysiological levels, while any other steroid hormone remained nearly constant. To the best of our knowledge, no study has ever assessed associations between estradiol levels and sexual attraction to visual sexual stimuli in women undergoing fertility treatment.

Finally, we acknowledge the following limitations: First, though this study is sufficiently powered for effect sizes of interest, i.e., medium effect sizes, even bigger sample sizes would be preferable, because hormone levels varied substantially at given measurement occasions across the menstrual cycle and ovarian stimulation. Second, audiovisual sexual stimuli would have been the preferable modality, as they elicit stronger sexual responses than visual sexual stimuli (Kukkonen, 2015). Third, a randomized order of stimuli between test sessions would have been beneficial to avoid confounding by sequential effects. Fourth, we did not incorporate a counterbalanced study design, which would have been valuable to minimize confounding by habituation effects when re-presenting the same sexual stimuli. Fifth, investigating sexual desire in addition to sexual attraction would have been beneficial. Sixth, 30 of the naturally cycling women (34.1%) were diagnosed with endocrinological disorders, and hence their hormone levels may deviate from healthy women. However, statistical control for endocrinological disorders did not alter the results.

5. Conclusions

In this prospective longitudinal multisite study, women's sexual attraction to visual sexual stimuli did not vary consistently across two consecutive menstrual cycles and ovarian stimulation of fertility treatment (IVF). Further, no evidence for a steroid hormonal influence was found, as there were no robust associations between steroid hormone levels (i.e., estradiol, progesterone, and testosterone) and sexual attraction ratings in naturally cycling women across two consecutive cycles and in women presenting even stronger differences in estradiol levels during ovarian stimulation of fertility treatment.

Although, the present study does not allow to draw conclusions on causal effects, it provides no support for increased women's sexual motivation in fertile phases regulated by steroid hormones. In conclusion, a relevant effect of steroid hormones on women's sexual attraction to visual sexual stimuli is unlikely.

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CRediT authorship contribution statement

BL, MPH, VS: Conceptualization; MPH, FI: Data curation; MPH, BL, VS: Formal analysis; BL, TK, ET, TM, FI, SR: Funding acquisition; BL, TK, FI, ET, TM, SR: Investigation; BL, MPH, VS: Methodology; BL, ET, FI: Project administration; BL, TK, ET, TM, FI, SR: Resources; MPH, FI: Software; BL, TK: Supervision; MPH: Validation; VS, BL: Visualization; VS, BL, MPH: Writing – original draft; BL, MPH, TK, FI, ET, TM, SR: Writing – review & editing.

Conflicts of interest

In the manuscript "Sexual attraction to visual sexual stimuli in association to steroid hormones in premenopausal women" none of the authors has any conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106060.

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