Hormonal Interactions and Sex Matter: Neural and Behavioral Effects of Estradiol and Oxytocin

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List of abbreviations

rsFC	resting-state functional connectivity

1. Abstract

The sex hormone estradiol and the neuropeptide oxytocin induce a variety of behavioral and neural effects, but possible underlying interactions have not been tested in a sample involving both sexes. Thus, the current thesis aimed to elucidate sex-specific effects of estradiol, and decipher its interactions with oxytocin. Study 1 focused on the sex-specific behavioral effects of estradiol on economic decision-making and revealed that it opposingly affects the sensitivity to perceived fairness in women and men. Furthermore, the simple belief in receiving an estradiol treatment altered responses to unfair framed offers in both sexes. Therefore, future studies are warranted to control for stereotypical beliefs associated with a specific treatment, beyond the direct hormonal effects. Study 2 investigated the impact of estradiol, oxytocin, and their interaction on sex differences in emotional episodic memory. Irrespective of the emotional valence, women exhibited a better overall memory performance than men under placebo, accompanied by increased hippocampus responses to remembered items. The single treatment with estradiol or oxytocin diminished this mnemonic sex difference and reversed the sex-specific hippocampus responses. Intriguingly, the combined treatment produced no significant effect. Hence, the results suggest that the separate treatments modulate sex differences in episodic memory, while their co-administration indicates an antagonistic interaction between both hormones. To probe task-independent effects, study 3 examined the sex-specific influence of both hormones on hippocampus and amygdala resting-state functional connectivity (rsFC). In women, the single treatments enhanced rsFC between the right hippocampus and the left anterior cingulate gyrus compared to placebo. By contrast, in men, either hormone decreased rsFC between the left amygdala and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus. Interestingly, in both sexes, the observed single treatment effects were reversed following the combined treatment, further supporting the notion that both hormones might antagonistically interact. Collectively, the current thesis detected sex- and region-specific effects of estradiol and oxytocin. Additionally, their potentially antagonistic relation might have contributed to priorly reported opposing hormonal effects in women and men. In conclusion, this thesis highlights the necessity for future studies to consider sex and hormonal interactions, besides simple hormonal effects and stereotypical beliefs, as important moderating factors and to integrate them into their research designs.

2. Introduction and aims with references

If you think about sex hormones, what usually comes to mind is the development of sex organs and physical maturation during puberty. Indeed, sex hormones are critical regulators of mammalian development and physiology, but their effects are not restricted to reproduction (Hornung et al., 2020). In particular, they possess the ability to modulate functional and structural brain organization, accompanied by numerous behavioral consequences (Hornung et al., 2020; Luine, 2014). Additionally, their interactions with neurotransmitters, including dopamine and serotonin (Barth et al., 2015), and neuropeptides, like oxytocin (Engel et al., 2019) might lead to synergistic, but also antagonistic effects. The current thesis focused on estradiol, as one of the most prominent sex hormones. Since estradiol naturally fluctuates across the female menstrual cycle, prior research mainly concentrated on women. Albeit, evidence deriving from rodent and human studies revealed that solely perceiving estradiol as a “female” hormone would underestimate its complex role in biological, developmental, and cognitive processes in men (Taxier et al., 2020). Therefore, it is vital to directly compare estradiol effects in both sexes, but studies simultaneously involving men and women are scarce. Thus, the current thesis observed both sexes to detect possible sex-specific effects of estradiol on economic decision-making, memory, and resting-state functional connectivity (rsFC).

2.1 Estradiol

In humans, correlative studies revealed that estradiol impacts a variety of behaviors, including emotion processing (Gamsakhurdashvili et al., 2021), learning and memory (Taxier et al., 2020), in addition to its potential role in the development of psychiatric disorders in women (Cover et al., 2014). Furthermore, estradiol also affects bargaining behavior (Ambrase et al., 2021) and might contribute to sex differences in economic decision-making (Bos et al., 2012). In general, women are more sensitive to the context of an economic offer and show an increased tendency to behaviorally adapt with changing frames in contrast to men (Miller and Ubeda, 2012). However, whether this sensitivity varies along with differing estradiol levels needs to be clarified. First evidence suggests that higher endogenous estradiol levels are associated with increased risk-taking behavior, including a higher disposition to risk monetary punishment, besides a decreased loss aversion and willingness to cooperate (Ambrase et al., 2021; Eisenbruch and Roney,

2016). Interestingly, apart from the direct hormonal effects, folk hypotheses on the effects of sex hormones have also been shown to alter economic decision-making, as the mere belief in receiving a testosterone treatment priorly enhanced unfair bargaining behavior (Eisenegger et al., 2010). Thus, certain stereotypical beliefs associated with estradiol, such as both sexes perceiving women as more empathetic and affable in contrast to men (Hentschel et al., 2019), could affect decision-making. However, potential effects elicited by stereotypical beliefs about estradiol have not been tested yet.

Beyond estradiol's possible effects on economic decision-making, its impact on memory (Luine, 2014) and associated mnemonic brain regions, as for instance the hippocampus, has been emphasized (Taxier et al., 2020). In female rats, pre-training estradiol infusions resulted in an improved memory performance (Luine et al., 2003) and also in male rodents a beneficial effect of estradiol on memory function has been detected (Taxier et al., 2020). In contrast, previous human studies examining estradiol fluctuations along the menstrual cycle yielded heterogenous results concerning estradiol's mnemonic effects (Loprinzi and Frith, 2018). Some studies found that high estradiol levels enhanced memory performance (Hampson and Morley, 2013), while others reported null effects (Mihalj et al., 2014). This inconsistency might partially be rooted in interactive effects with other circulating hormones or different task designs, including the chosen valence of the presented material (Bayer et al., 2018).

In order to further decipher estradiol's mnemonic effects, functional magnetic resonance imaging studies provide the opportunity to examine estradiol's role on the neural level. The hippocampus and the amygdala are the most intensely studied target regions for estradiol effects, as both express a high density of estradiol receptors (Barth et al., 2015). In women, higher estradiol levels positively correlate with gray matter volume and rsFC of the hippocampus and amygdala (Engman et al., 2018; Lisofsky et al., 2015; Ossewaarde et al., 2013). Heretofore, estradiol's effects on rsFC in men have not been tested yet, but testosterone has been shown to affect amygdala rsFC in men (Votinov et al., 2020). As testosterone can be catalyzed into estradiol via the enzyme aromatase (Schulster et al., 2016), estradiol might also modulate rsFC in men. Nevertheless, similar to estradiol's impact on memory functioning, its effects on rsFC have been discussed to be affected by interactions with other circulating hormones as well. Since sex-specific effects have also

been observed for the neuropeptide oxytocin, interactions with sex hormones such as estradiol should be taken into consideration (Lieberz et al., 2020).

2.2 Oxytocin

In humans, the hypothalamic peptide oxytocin is broadly known for its role in labor induction and lactation (Gimpl and Fahrenholz, 2001). Yet, further effects have been reported for multifaceted domains including fear (Eckstein et al., 2015) and trauma processing (Scheele et al., 2019), as well as romantic attachment (Kreuder et al., 2017). Furthermore, due to its prosocial and anxiolytic effects, oxytocin has even emerged as a potential target for augmenting treatment approaches for various mental disorders (Heinrichs et al., 2009). In addition, memory performance has also been shown to be altered by oxytocin. However, reports on oxytocin's mnemonic effects are inconsistent ranging from memory enhancement (Guastella et al., 2008) to impairment (Heinrichs et al., 2004), which highlights the necessity to examine potential mechanisms contributing to this heterogeneity. For instance, inter-individual differences in attachment insecurity (Bartz et al., 2010), or task-dependent factors like stimulus valence (Bartz et al., 2011) have been discussed to moderate oxytocin's effects on memory. Further, based on previous findings, which indicated that oxytocin sex-specifically modified the perception of socio-emotional stimuli (Lieberz et al., 2020), a moderating role of sex hormones, such as estradiol, on oxytocin's mnemonic effects, is conceivable, but needs to be tested.

Apart from oxytocin's depicted role in memory formation, it additionally has been found to modulate rsFC (Brodmann et al., 2017). Oxytocin affects emotion-related networks including the amygdala (Jiang et al., 2021) and interregional coupling, i.e., between the amygdala and the hippocampus (Alaerts et al., 2019), which corresponds to the high density of oxytocin receptors in both regions (Gimpl and Fahrenholz, 2001). Moreover, first evidence suggests divergent effects of oxytocin on rsFC in women and men, thus underlining the importance to decipher the impact of fluctuating sex hormones, including estradiol, on the sex-specific effects of oxytocin on rsFC (Seeley et al., 2018).

2.3 Estradiol-Oxytocin interactions

Preliminary evidence for interactions between estradiol and oxytocin originates from rodent studies. In female rats, estradiol altered oxytocin signaling in the paraventricular

nucleus of the hypothalamus (Suzuki and Handa, 2005), while in male rats, estradiol and oxytocin modulated the synaptic plasticity in the medial nucleus of the amygdala (Frankiensztajn et al., 2018). Furthermore, studies in mice revealed that dimerized estradiol receptors possess the ability to bind to the composite hormone response element of the oxytocin promotor gene, and thereby induce the production of oxytocin (Acevedo-Rodriguez et al., 2015). Corresponding findings in female rats demonstrated that an estradiol administration sufficed to increase serum oxytocin levels (Tokui et al., 2021). Interestingly, a previous study in healthy and bulimic women reported that a single estradiol treatment also elevated endogenous oxytocin levels (Chiodera et al., 1991). Additionally, similar to estradiol, endogenous oxytocin levels have been shown to peak right before ovulation, potentially as a consequence of increased estradiol receptor functioning and estradiol availability (Engel et al., 2019). Possible interactions of oxytocin and estradiol in humans have been suggested for various domains including social anxiety (Schneider et al., 2021) and migraine attacks (Krause et al., 2021). Yet, a recent study introduces the notion, that estradiol-oxytocin interactions might also be antagonistic (Lieberz et al., 2020). While comparing the neural response patterns of women in their luteal phase, which is associated with high estradiol levels, to those of men, who naturally exhibit low estradiol levels, sex-specific effects were observed, which interestingly were diminished following an oxytocin treatment (Lieberz et al., 2020). Thus, further studies are needed to clarify possible mechanisms underlying estradiol-oxytocin interactions. To date, no study has simultaneously examined the interactive effects of oxytocin and estradiol on rsFC or the formation of episodic memories in both sexes.

2.4 Research aims

The aim of the current thesis is to investigate sex- and region-specific effects of estradiol and its interaction with oxytocin. Hence, three studies were conducted to explore estradiol's behavioral impact and associated stereotypical beliefs on economic decision-making (study 1), as well as potential neural and behavioral effects of estradiol, oxytocin, and their interaction on episodic memory (study 2) and rsFC (study 3). The following research questions were addressed in healthy free-cycling women and men:

(1) Do estradiol and its associated stereotypical beliefs differentially contribute to the sensitivity to the perceived fairness of economic offers in women and men?

(2) Do estradiol, oxytocin, and their interaction affect the neural mechanisms underlying the formation of emotional memories in a sex-dependent manner, and how are these mechanisms reflected in a subsequent memory task?

(3) Do estradiol, oxytocin, and their interaction sex-specifically modulate the rsFC of the amygdala and hippocampus?

The current thesis tried to illuminate the modulating role of sex on estradiol's effects since antecedent estradiol studies primarily examined women. The additional focus on oxytocin and its interaction with estradiol might help to explain previously observed sex differences in oxytocin research.

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3. Publications

Publication overview:

Coenjaerts M, Pape F, Santoso V, Grau F, Stoffel-Wagner B, Philipsen A, Schultz J, Hurlemann R, Scheele D. Sex differences in economic decision-making: Exogenous estradiol has opposing effects on fairness framing in women and men. Eur Neuropsychopharmacol 2021; 50: 46-54. DOI: 10.1016/j.euroneuro.2021.04.006

Coenjaerts M, Trimborn I, Adrovic B, Stoffel-Wagner B, Cahill L, Philipsen A, Hurlemann R, Scheele D. Exogenous estradiol and oxytocin modulate sex differences in hippocampal reactivity during the encoding of episodic memories. NeuroImage 2022; 264: 119689. DOI: 10.1016/j.neuroimage.2022.119689

Coenjaerts M, Adrovic B, Trimborn I, Philipsen A, Hurlemann R, Scheele D. Effects of exogenous oxytocin and estradiol on resting-state functional connectivity in women and men. Sci Rep 2023; 13(1): 3113. DOI: 10.1038/s41598-023-29754-y

3.1 Publication 1: Sex differences in economic decision-making: Exogenous estradiol has opposing effects on fairness framing in women and men

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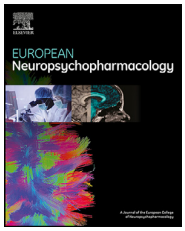

ORIGINAL RESEARCH PAPER

Sex differences in economic decision-making: Exogenous estradiol has opposing effects on fairness framing in women and men

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Context;
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Fairness frame;
Sex differences;
Ultimatum game

Abstract
Burgeoning evidence indicates that women are more sensitive to the context of an offer and show a stronger propensity to adjust their behavior with changing fairness frames. We evaluated whether the sex hormone estradiol and associated stereotypical beliefs contribute to fairness framings by administering topical estradiol (2 mg) to 108 healthy women and 104 heathy men in a randomized, double-blind, placebo-controlled between-subject study design. Participants played the role of the responder in a modified version of the Ultimatum Game (UG), in which identical offers for the division of a given amount of money were framed as either fair

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or unfair. Furthermore, participants completed an unframed UG and a delayed discounting task to probe possible effects of estradiol on altruistic preferences and delay gratification. Our results show that women were more sensitive to fairness frames than men. Intriguingly, however, estradiol had sex-specific effects on fairness sensitivity by increasing the acceptance rate of proposals with a fair frame in men and reducing it in women. Furthermore, the mere belief of receiving estradiol treatment significantly increased the acceptance of unfair-framed offers in both sexes, but estradiol did not significantly alter the response to unframed offers and impulsive decision-making. Collectively, our findings indicate that estradiol has opposing effects on the sensitivity to the perceived fairness of economic offers in women and men. The profound effects of estradiol treatment and stereotypical beliefs provide support for the notion that sex differences in fairness framing are rooted in both biological and environmental factors.

1. Introduction

Human economic decision-making is not perfectly rational, but highly susceptible to the framing of choices (Ruggeri et al., 2020; Tversky and Kahneman, 1981) and social preferences such as fairness norms (Fehr and Gachter, 2002; Henrich et al., 2006). Depending on the context and the available options, the same monetary offer can be considered fair or unfair. Accumulating evidence indicates that women are more sensitive to the context of an offer than men and show a stronger propensity to adjust their behavior with changing frames (Ellingsen et al., 2013; Espinosa and Kovarik, 2015; Miller and Ubeda, 2012). Surprisingly, however, the mechanisms mediating sex-specific framing effects are still unclear.

Current perspectives on the neurobiological substrates of framing biases in the context of risk decisions emphasize a central role of an affect heuristic, evident for example in the sensitivity of limbic brain regions to risk framing (De Martino et al., 2006). According to a dual system view, different frames evoke distinct emotional responses that require "top-down" mental efforts to resist them (Gosling and Moutier, 2019; Kahneman and Frederick, 2007). Interindividual differences in the susceptibility to framing bias have been linked to genetic variations of serotonergic and dopaminergic pathways (Gao et al., 2017; Roiser et al., 2009). Fairness-related framing effects have been probed with an adapted version of the Ultimatum Game (UG) (Güth et al., 1982), in which a proposer has two options how to split a stake (Falk et al., 2003). If the responder accepts, the deal goes ahead and if the responder rejects, neither player gets anything. Rejection rates for the same offer vary substantially depending on the proposer's alternative because the chosen offer signals either an unfair or fair intentionality. The incorporation of intentionality into decision-making follows a linear developmental trajectory across adolescence, with the relative importance of the proposer's intentions increasing with age (Guroglu et al., 2009; Sutter, 2007). The gradual emergence of intention-consideration is paralleled by enhanced activation in the temporoparietal junction and the dorsolateral prefrontal cortex during rejection of unintentional unfair offers, which may reflect increased perspective taking (Guroglu et al., 2011).

Various lines of research indicate that women are more sensitive to the context of an offer and its associated social cues than men (Ellingsen et al., 2013; Espinosa and Kovarik, 2015; Miller and Ubeda, 2012). For instance, procedural fairness in the UG is more important for determining subsequent behavior in women than men (Hack and Lammers, 2009). It is clear that sex differences in social-cognitive domains may result from interactions of numerous environmental and biological factors including stereotypical beliefs as well as hormonal and genetic variables (Cahill, 2006; Kret and De Gelder, 2012). Gonadal steroids are likely to contribute to sex-specific behaviors. While several previous studies examined the impact of the primary male sex hormone testosterone on human social-emotional behavior (Bos et al., 2012; McCall and Singer, 2012), very little is known about the modulatory role of the female sex hormone estradiol. Studies exploring natural variations of endogenous estradiol in women found menstrual cycle effects on reward-based decision-making. Specifically, higher estradiol levels are positively related to increased risk-taking behavior and reduced loss aversion (Ambrase et al., 2021). In addition, elevations in estradiol levels during the reproductive cycle were associated with a reduced immediate reward selection bias in intertemporal decision-making (Smith et al., 2014), as well as higher proposer demands in the UG, which suggests a reduced willingness to cooperate and an increased disposition to risk a monetary punishment (Eisenbruch and Roney, 2016). Furthermore, the administration of exogeneous estradiol enhanced the ability to recall extinction memory in women (Graham and Milad, 2013) and increased vicarious emotional reactivity in men (Olsson et al., 2016), but as yet no study probed the effects of estradiol administration on decision-making in both women and men. Interestingly, stereotypical beliefs about gonadal steroids seem to be influential beyond the hormonal effects. The folk hypothesis on the effects of testosterone implies an increased antisocial, egoistic and aggressive behavior. The mere belief in receiving testosterone, led to an increased unfair bargaining behavior in healthy women, although against stereotypical beliefs, the actual treatment with testosterone promoted fair bargaining behavior in participants (Eisenegger et al., 2010). By contrast, the folk hypothesis on estradiol predicts that men and women view females as being more affable and empathetic as well as more concerned about others than males (Hentschel et al., 2019).

Consequently, estradiol as a typical female hormone, might be associated with a distinctive prosocial behavior.

Previous research on estradiol mostly focused on women's risk behavior during the different menstrual cycle phases. However, natural hormonal fluctuations in the menstrual cycle hinder a hormone-specific interpretation of these results (e.g. behavioral changes may result from changes in estradiol levels but could also be related to changes in progesterone levels). The goal of our study is to specifically investigate the modulatory role of the sex hormone estradiol on sex differences in fairness framing via a selective exogenous hormone administration.

We hypothesized that if women are more sensitive to fairness frames than men, estradiol may contribute to these sex differences and administration of the hormone would increase the fairness sensitivity of women and men (Ambrase et al., 2021; Eisenbruch and Roney, 2016; Smith et al., 2014). In accordance with the folk hypothesis on estradiol we expected that stereotypical beliefs about estradiol would be associated with increased acceptance of unfair-framed offers (Eisenegger et al., 2010; Hentschel et al., 2019).

2. Experimental procedures

2.1. Participants

A total of 212 healthy adults (108 females; mean age $\pm$ SD $= 23.55 \pm 3.75$ years; cf. Table S1) participated in the study after giving written, informed consent. The study was part of a larger project (cf. SI) and was approved by the institutional review board of the Medical Faculty of the University of Bonn and carried out in compliance with the latest revision of the Declaration of Helsinki. Screenings of the participants were conducted prior to the test sessions. Subjects were free of current and past physical or psychiatric illness, as assessed by medical history and the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998) prior to enrollment. In addition, they were naive to prescription-strength psychoactive medication, and had not taken any over-the-counter psychoactive medication in the past 4 weeks. The participants were asked to maintain their regular bed and waking times and to abstain from caffeine and alcohol intake on the day of the experiment. None of the women used hormonal contraceptives or were pregnant during the study. All women were tested in their early follicular phase of their menstrual cycle (days 1–6) as validated by blood assays obtained on the testing day (see Table S2).

2.2. Experimental design and procedures

We conducted a randomized, double-blind, placebo-controlled, parallel-group design study. The estradiol gel (Estramon 2 mg estradiol, Hexal AG, Holzkirchen, Germany) or the placebo gel (ultrasonic gel) was transdermally applied to the participants' back prior to the experiment. The dose was chosen in accordance with a recent pharmacokinetic study (Eisenegger et al., 2013) to minimize side effects and negative feedback loops in the neuroendocrine system. The estradiol treatment was balanced within the male subsample (estradiol $n = 53$, placebo $n = 51$) and the female subsample (estradiol $n = 54$, placebo $n = 54$). In accordance with our pharmacokinetic pre-study (cf. SI), the decision-making tasks commenced 2.5 h after the gel administration. Blood samples were collected before and 3.5 h after the gel administration. At the end of the experiment, participants were asked to estimate their received treatment. Out of the 105 participants in the estradiol group with available treatment estimates (two data points missing), 34 (32.4%; 22 men) believed that they had received estradiol, while 28 subject (26.9%; 16 men) in the placebo group (with $n = 104$) believed that they had received the verum treatment ($X_{(1)} = 0.75$, $p = 0.39$).

2.3. Tasks

2.3.1. Ultimatum game

In the UG, a proposer suggests a way to divide a fixed sum of money and if the responder accepts, the deal goes ahead. If the responder rejects, neither player gets anything. Each trial started with the presentation of a fixation cross for a random time interval between 1 and 2 s. Then a picture of the proposer was displayed for 1 s, after which the proposer's offer was shown. Subjects could accept or reject an offer by pressing one of two buttons. They were instructed to decide as fast as possible.

Participants played three different versions of the UG (framed, unframed and computer). In the framed version, the proposer had to decide between two given options of monetary splits. Thus, the chosen offer could be framed as fair or unfair depending on the alternative offer. For instance, an offer of 3€ can be perceived as fair, if the alternative option is 2€, but as unfair if the other option is 4€. In the framed version of the UG, there were 36 trials, half with a fair framing (4€ vs. 3€, 3€ vs. 2€, 2€ vs. 1€ and 1€ vs. 0€) and half with an unfair framing (4€ vs. 5€, 3€ vs. 4€, 2€ vs. 3€ and 1€ vs. 2€). The two potential offers were displayed for 6 s and the selected option was marked with a black box (cf. Figure S2).

In the unframed UG, the proposer could freely decide how to split 10€. There were 24 trials in the unframed UG, each with a different proposer. The offers systematically varied between 0 and 5€, each proposal was repeated 4 times. In addition, participants completed 24 trials of a computer version of the unframed UG, in which the word "computer" was shown instead of a picture of the proposer. The orders of the UG version and the proposers' offers were randomized across participants.

As a cover story, participants were told that they would play against real partners, who had taken part in previous experiments. However, the proposals and stimuli were predetermined and equally divided into offers made by female and male proposers with common names in Germany. Pictures of the proposers were selected from the Center for Vital Longevity (Park Aging Lab, PAL) database (Minear and Park, 2004). Subjects were told that they were randomly assigned to either the responder or proposer group, although it was predetermined that all subjects acted as responders. It was emphasized that there are no repeated interactions (i.e. they encountered every player only once; "one-shot" trials).

The UG was implemented in Presentation 20 (Neurobehavioral Systems, Albany, CA). After completing the experiment, one decision was randomly selected and participants were paid according to their choice (i.e. they either received no payment if they rejected the offer or obtained the amount they accepted).

2.3.2. Delayed discounting task

We used a delayed discounting task to assess the ability to control impulsive preferences (i.e. to suppress the impulsive choice of smaller, but sooner incentives over long-term greater benefits). In 36 trials participants had to choose between rewards, which were either smaller and paid sooner or larger and paid later. The amounts were pseudo-randomly drawn from a normal distribution with a mean of 45€ and a standard deviation of 12€. The larger-later rewards were 0.5–75% larger than the smaller-sooner rewards. The order of the trials was randomized with half of the trials including an immediate reward as the smaller-sooner option and the larger-later reward being delayed for two or four weeks. In the other half of the trials, the smaller-sooner option was paid in two weeks and

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the larger-later alternative in four or six weeks. The proportion of patient choices (i.e. larger-later rewards) was used as dependent variable.

2.4. Hormonal assessments

In line with the manufacturer's instructions (Siemens Healthineers, Eschborn, Germany) and based on the LOCI™ technology on a Dimension Vista™ System, serum estradiol and serum testosterone were determined by fully automated homogeneous sandwich chemiluminescent immunoassays. For estradiol, the detection limit of the assay was 5 pg/ml and the coefficients of variation for intra-assay and inter-assay precision were 5.5% and 5.9%. Testosterone was tested with a detection limit of 0.025 ng/ml and the intra-assay and inter-assay precision variation coefficients were 4.7% and 6.7%. By applying a fully automated solid-phase competitive chemiluminescent enzyme immunoassay on an Immulite™ 2000xpi System according to the manufacturerś instructions (Siemens Healthineers) the serum progesterone was analyzed with a detection limit of 0.1 ng/ml. For the intra-assay and the inter-assay precision, the coefficients varied between 4.2% and 5.5%. There was a minimal cross-reactivity of all assays with other related compounds.

2.5. Statistical analysis

The behavioral, demographical and neuropsychological data were processed using standard procedures in SPSS 24 (IBM, New York, NY, USA). The quantitative behavioral data were analyzed with mixed-design analysis of variance (ANOVA) and for correlation analyses Pearson's product-moment correlations (r) were used. The acceptance rates in percent and the response time of these decisions served as dependent variables. Independent factors were framing (fair vs. unfair), proposal magnitude (1,2,3 and 4 €), sex (female vs. male) and treatment (estradiol vs. placebo). Furthermore, the effects of the believed treatment were assessed in ANOVAs with the additional independent factor believed treatment (believed estradiol vs. believed placebo). To control for the varying increases in estradiol levels, we computed an analysis of covariance (ANCOVA). The difference score of the baseline and post-treatment estradiol levels for each participant served as a covariate in our main analysis. We used the acceptance rate of fair framed offers as our dependent variable and treatment (placebo vs. estradiol) and sex (male vs. female) as between-subject factors. The assumption of sphericity was assessed with Mauchly's test, and Greenhouse-Geisser's correction was applied for significant violations. The *P*-values are two tailed and considered as significant at a level of $P < 0.05$. Post-hoc *t*-tests were Bonferroni-corrected (P_{cor}) to account for multiple comparisons.

3. Results

3.1. Effects of proposal magnitude, fairness frames and estradiol treatment

The acceptance rate in the framed version of the UG significantly increased with the magnitude of the proposal ($F_{(1,206)} = 356.73$, $p < 0.01$, $\eta_p^2 = 0.63$) and was higher for fair-framed than unfair-framed offers ($F_{(1,206)} = 214.81$, $p < 0.01$, $\eta_p^2 = 0.51$). Importantly, the treatment effect differed significantly between the sexes and framings (treatment x framing x sex interaction: $F_{(1,206)} = 10.34$, $p < 0.01$, $\eta_p^2 = 0.05$; cf. Figure 1). Under placebo, the framing effect in the framed UG was more pronounced in women than men ($F_{(1,101)} = 16.10$, $p < 0.001$, $\eta_p^2 = 0.14$), with women accepting significantly more fair-framed offers than men ($t_{(82.84)} = 2.65$, $p_{cor} = 0.02$, $d = 0.53$). After estradiol treatment, the pattern was reversed ($t_{(105)} = -2.50$, $p_{cor} = 0.03$, $d = 0.49$). Thus, estradiol selectively decreased the acceptance rate of fair-framed offers in women ($t_{(97.63)} = -2.79$, $p_{cor} = 0.01$, $d = -0.54$) and had the opposite effect in men ($t_{(91.86)} = 2.43$, $p_{cor} = 0.03$, $d = 0.48$). The treatment effect was not moderated by the magnitude of the offer (all *p*s > 0.05). Furthermore, women accepted significantly fewer unfair-framed offers than men ($F_{(1,206)} = 4.68$, $p = 0.03$, $\eta_p^2 = 0.02$), but there was no significant main or interaction effect of treatment for unfair-framed offers (all *p*s > 0.05).

In general, participants needed more time for their decisions in the framed UG if the offer was framed unfair (mean ± SD = 1.87 ± 0.70 s) compared to a fair framing (1.77 ± 0.63 s; $F_{(1,206)} = 13.80$, $p < 0.001$, $\eta_p^2 = 0.06$). Additionally, they were faster in responding to smaller (1.75 ± 0.64 s) than larger offers (1.88 ± 0.73 s; $F_{(2.86,588.41)} = 7.63$, $p < 0.001$, $\eta_p^2 = 0.04$). After the estradiol treatment women had a faster reaction time (1.70 ± 0.47 s) compared to the placebo group (1.90 ± 0.67 s), in contrast to men, who decided more slowly after receiving estradiol (placebo: 1.75 ± 0.46 s; estradiol: 1.94 ± 0.84 s; interaction between sex and treatment, $F_{(1,206)} = 5.20$, $p = 0.02$, $\eta_p^2 = 0.03$). However, post-hoc comparisons showed no significant treatment effects on the reaction times in the male and female subsample (all *p*s > 0.05).

3.2. Hormonal assessments

At baseline, women had significantly higher estradiol concentrations than men ($t_{(187.4)} = 2.44$, $p_{cor} = 0.03$, $d = 0.34$), but lower progesterone ($t_{(102.74)} = -5.85$, $p_{cor} < 0.001$, $d = -0.82$) and testosterone ($t_{(102.36)} = -27.28$, $p_{cor} < 0.001$, $d = -3.82$). Importantly, baseline levels of all three hormones were comparable between treatment groups in women (all *p*s > 0.05) and men (all *p*s > 0.05). Estradiol administration significantly increased blood estradiol levels in women (time x treatment: $F_{(1,105)} = 187.20$, $p < 0.001$, $\eta_p^2 = 0.64$) and men (time x treatment: $F_{(1,100)} = 111.55$, $p < 0.001$, $\eta_p^2 = 0.53$), but had no significant effect on testosterone and progesterone concentrations (cf. Table S2). However, the treatment-induced increase in estradiol was significantly higher in women than men ($F_{(1,207)} = 26.84$, $p < 0.001$, $\eta_p^2 = 0.12$). Importantly, the treatment x sex interaction for fair framed offers remained significant in an ANCOVA after including the increase in blood estradiol levels as a covariate ($F_{(1,202)} = 7.402$, $p < 0.01$, $\eta_p^2 = 0.035$). Furthermore, the increase in estradiol was not significantly related to the acceptance rate of fair framed offers ($F_{(1,202)} = 0.97$, $p = 0.326$, $\eta_p^2 = 0.05$). Likewise, controlling for individual baseline estradiol levels did not change the significant treatment x sex interaction for fair-framed offers ($F_{(1,203)} = 14.65$, $p < 0.01$, $\eta_p^2 = 0.067$).

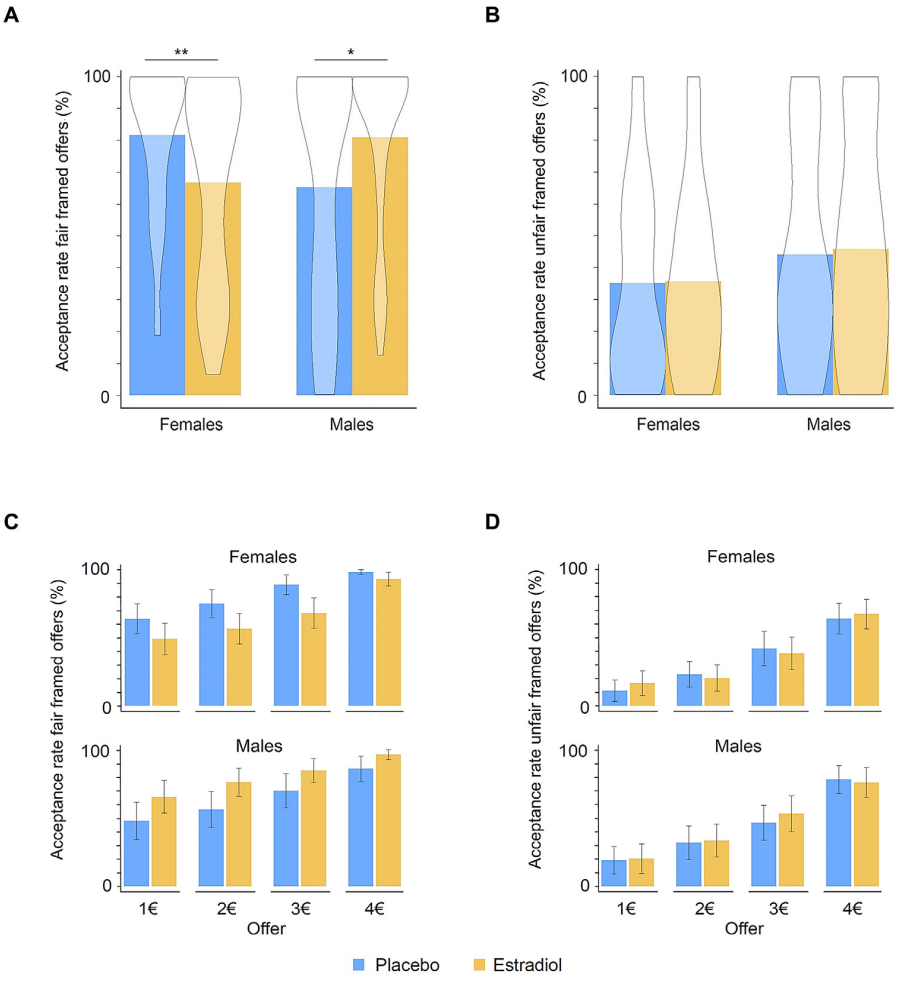

Figure 1 The acceptance rate was significantly lower for unfair-framed offers and women accepted significantly more fair-framed offers than men. Estradiol significantly increased the acceptance rate of fair-framed offers in men and had the opposite effect in women (**A**). The treatment had no significant effect on unfair-framed offers (**B**). The estradiol effect was independent of the offer size (1-4€) (**C, D**). Violin plots are kernel density plots which are comparable to histograms with infinitely small bin sizes. Error bars indicate the 95%-confidence intervals. $^{*}p < 0.05$; $^{**}p < 0.01$.

3.3. Estradiol does not affect sensitivity to offer magnitude or delayed discounting

As expected from the literature, acceptance rates increased with the magnitude of the offer in both, the unframed ($F_{(3.21,663.44)} = 507.74$, $p < 0.001$, $\eta_p^2 = 0.71$) and the computer versions of the UG ($F_{(2.85,586.25)} = 370.28$, $p < 0.001$, $\eta_p^2 = 0.64$). However, there were no significant main or interaction effects of the estradiol treatment in the unframed version of the UG or the computer UG. A significant sex x offer size interaction in the unframed UG ($F_{(3.21,663.44)} = 4.05$, $p < 0.01$, $\eta_p^2 = 0.02$) and computer UG ($F_{(2.85,586.25)} = 7.95$, $p < 0.01$, $\eta_p^2 = 0.04$) showed that men accepted more lower offers than women, while this effect was reversed for higher offers.

In the delayed discounting task, participants chose the later-larger option more often when there was a greater relative difference in sooner-smaller/later-larger magnitudes (i.e. main effect of relative difference; $F_{(3.43,710.82)} = 384.16$, $p < 0.01$, $\eta_p^2 = 0.65$), but there were no significant sex or treatment effects (all ps > 0.05). Thus, the treatment effect in the framed version of the UG is probably driven by framing sensitivity rather than global changes in economic decision-making or altered intertemporal decision making.

3.4. Effects of the believed treatment

An independent sample of 133 subjects (85 women) described estradiol with the attributes caring, empathetic, loving and friendly, but also weak and anxious (cf. SI). Thus, the mere belief of receiving a treatment could alter the acceptance rate of fair and unfair offers. In line with this prediction, the believed treatment had a significant effect that varied as a function of the actual treatment and fram-

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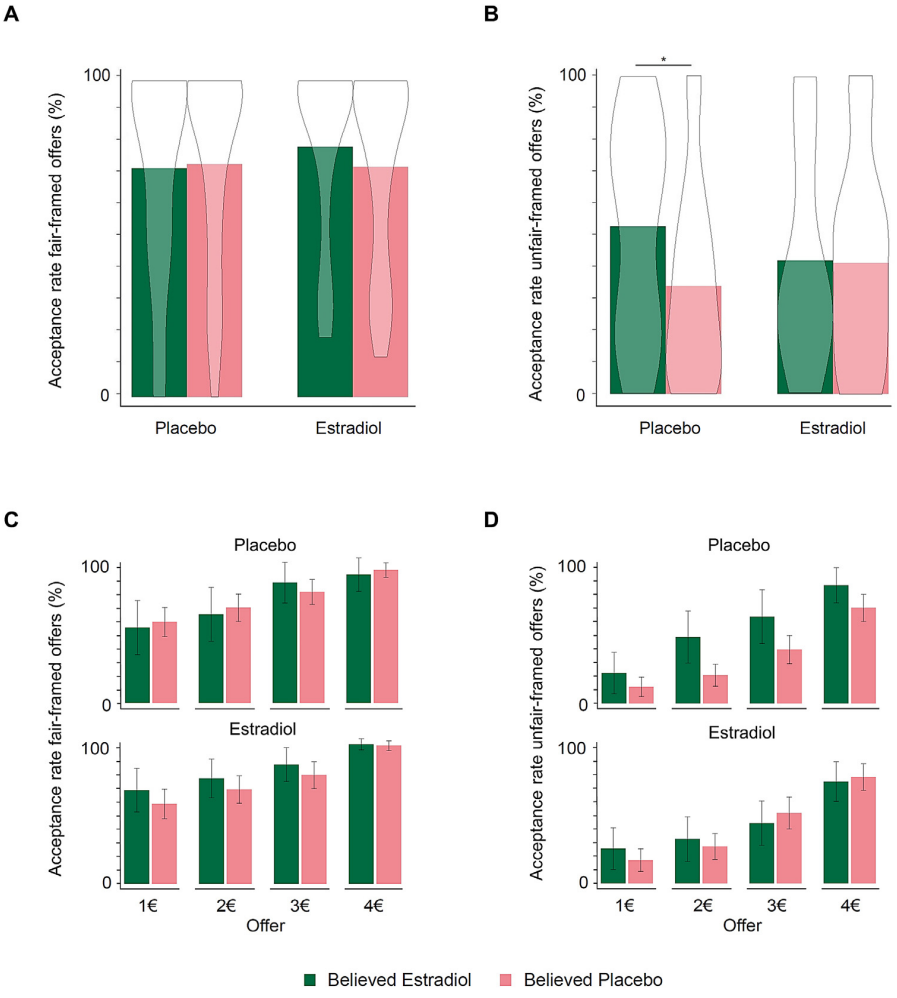

Figure 2 There were no significant belief effects for mean fair-framed offers (**A**). However, participants in the placebo group who believed that they had received estradiol accepted significantly more unfair offers compared to those who believed they had received a placebo treatment (**B**). This effect was not evident in the estradiol group **.** The belief effect was independent of the offer size (1-4€) (**C, D**). Violin plots are the kernel density plots which are comparable to histograms with infinitely small bin sizes. Error bars indicate the 95%-confidence intervals. $^{*}p < 0.05$.

ing ($F_{(1,199)} = 4.89$, $p = 0.03$, $\eta_p{}^2 = 0.02$, cf. Figure 2). In the placebo group, subjects who believed that they had received estradiol accepted significantly more unfair-framed offers than subjects who believed that they had received placebo ($t_{(100)} = 2.68$, $p_{cor} = 0.02$, $d = 0.61$). This belief effect was not evident for fair-framed offers or estradiol-treated subjects (all $ps > 0.05$). Notably, the believed treatment was not significantly associated with the actual treatment in women and men (all $ps > 0.05$).

Furthermore, the believed treatment had significant effects on acceptance rates in the unframed UG and the computer UG. In the unframed UG, there was a significant three-way interaction of the offer size, the treatment and the believed treatment ($F_{(3.27,653.03)} = 2.68$, $p < 0.05$, $\eta_p{}^2 = 0.013$). Likewise, in the computer UG, participants with believed estradiol treatment accepted more lower offers and this effect was significantly stronger in men than in women (i.e. significant three-way-interaction of sex, offer size and believed treatment; $F_{(2.86,568.38)} = 2.76$, $p < 0.05$, $\eta_p{}^2 = 0.014$). No significant belief effects were evident in the delayed discounting task.

4. Discussion

The aim of the current study was to investigate the impact of exogenous estradiol and the associated stereotypical beliefs on fairness framing in women and men. Our results provide evidence for strong sex differences in the impact of fairness frames on the acceptance of ultimatum offers, with women demonstrating a stronger fairness sensitivity than men. This observation is consistent with previous research indicating a stronger propensity of women to adjust their behavior with changing frames (Espinosa and Kovarik, 2015;

Fehr and Gachter, 2002; Miller and Ubeda, 2012). Importantly, in contrast to our hypothesis, this sex-specific effect was reversed after estradiol treatment, with the sex hormone increasing the acceptance rate of proposals with a fair frame in men and reducing it in women. Furthermore, we found that stereotypical beliefs about estradiol modulated the acceptance rate of unfair-framed offers in both sexes under placebo. Thus, our results support the notion that both biological and environmental factors contribute to framing effects on ultimatum bargaining.

Sex differences in emotion recognition are less pronounced during periods of high estradiol levels in women (Derntl et al., 2008) and estradiol-treated women in our study showed a fairness framing effect comparable to men under placebo. It has been proposed that low estradiol enhances attentional vigilance for emotional information (Albert and Newhouse, 2019) as the memory for emotional content is improved during the menstrual phase when estradiol is low (Ertman et al., 2011). We found a selective effect of exogenous estradiol on the acceptance rate of fair-framed offers, but no significant effect in the unframed UG. Given that the intentionality of the proposer differentiates the two versions of the UG (Radke et al., 2012), our data indicate that the sensitivity for the intentionality of bargaining offers is also increased when exogenous estradiol levels are lower in women. The absence of an estradiol effect in the unframed UG corresponds to a previous study, in which no significant effect of a long-term estradiol treatment on decision-making in the unframed UG was observed in postmenopausal women (Zethraeus et al., 2009). Unfair-framed offers seem to be less volatile than fair-framed offers because a further decrease in the acceptance rate may be hindered by bottom effects and an increase would require the participants to overcome the prepotent preference to reject unfair intentions. Of note, millions of women around the world use steroid-based hormonal contraception as an effective way of birth control (Alkema et al., 2013). While previous studies have yielded inconsistent results about the impact of hormonal contraception on altruistic preferences and financial risk taking (Buser, 2012; Chen et al., 2013; Ranehill et al., 2018; Wozniak et al., 2014), our findings introduce the question whether long-term hormone treatments may influence framing effects in economic decision-making.

In men, exogenous estradiol increased the impact of fairness framing on ultimatum bargaining similarly to what is observed in women under placebo. In contrast to the effect in women, the estradiol administration resulted in supra-physiological levels of the hormone in men, but our control analysis did not indicate a significantly different direction of effects in participants with lower estradiol increase. Studies exploring the effects of estradiol administration in men are scarce, but it was recently found that estradiol treatment made motivational choices (i.e. the preference of cocaine over food reinforcement) in male rats comparable to that of female rats (Bagley et al., 2019). There were no significant a-priori sex differences in the cognitive control of prepotent impulses during delayed discounting and estradiol did not significantly alter choice preferences. Thus, it would appear that estradiol-induced changes in the susceptibility to fairness framing effects are unlikely to result from altered intertemporal decision making. Instead, estradiol may enable men to more strongly incorporate the proposer's intentionality into their decision-making by facilitating perspective taking (Guroglu et al., 2011). Sex-specific effects of estradiol were also evident in response times, with men making slower decisions in the framed UG after estradiol treatment and women becoming faster. It has been suggested that the acceleration of the deterioration of processing speed following menopause is associated with a lack of gonadal hormones, indicating that estradiol may have pro-cognitive functions in women (Halbreich et al., 1995). Effects of endogenous estradiol on working memory function crucially depend on baseline fluctuations in cortical dopamine indexed by the catechol-O-methyltransferase (COMT) Val(158)Met-genotype (Jacobs and D'Esposito, 2011). Given sex-specific effects of COMT on inhibitory brain activation (White et al., 2014), it is conceivable that the observed sex-specific effects of estradiol on fairness framing result from dopamine-estradiol interactions. Interestingly, similar sex-specific effects have been observed for testosterone. Importantly, exogenous testosterone administration caused women to make higher offers in the role of UG proposer (Eisenegger et al., 2010), but it produced the opposite effect in men (Zak et al., 2009). However, it is still not clear why testosterone has different behavioral effects in women and men (Stanton, 2017). Sex-specific effects of testosterone are also evident in other domains. A recent study examined genetic determinants of testosterone levels and found that higher testosterone is harmful for metabolic diseases in women but beneficial in men (Ruth et al., 2020). Clearly, the apparently sex-divergent effects of estradiol would have been obfuscated by an aggregated analysis. Our findings thus underscore the importance of including both women and men in the same experimental protocol and conducting sex-specific analyses.

Participants who believed that they received estradiol may have wanted to respond in accordance with their stereotypical beliefs and show concern for the proposer by accepting significantly more unfair-framed offers. Belief effects were also evident in the unframed and computer UG, indicating that stereotypical beliefs can have a broad impact on economic decision-making. In contrast to the stereotypical beliefs, estradiol had no significant effect on unfair-framed offers and it even reduced the acceptance rate of fair-framed offers in women. Importantly, selective belief effects in the placebo group speak against the idea that estradiol mediates these stereotypical behavioral changes.

Our study has some limitations that need to be addressed in future research. First, by testing women in their early follicular phase, we ensured low estradiol and progesterone levels and thus comparable baseline conditions to the male sample. The treatment had a specific effect on the estradiol levels, but future studies are warranted to further test possible interactions with other gonadal steroids or neurotransmitters (Ambrase et al., 2021). Second, sex differences in framing effects are moderated by task domain (Huang and Wang, 2010). We observed a significant effect of exogenous estradiol on fairness framing of monetary decisions, but these results cannot directly be extrapolated to other contexts such as risky-choice frames of life-death decisions.

Collectively, our findings provide support for the notion that sex differences in fairness framing are modulated by the sex hormone estradiol. Furthermore, the believed treatment affected the acceptance of unfair-framed offers, illustrating that stereotypical beliefs about hormones can influence decision-making beyond direct hormonal effects. Therefore, integrating sex and gender analysis into research designs (Tannenbaum et al., 2019) may help deciphering the interactions of environmental and neurobiological factors that mediate framing effects in humans.

Data availability

The data that support the findings of the present study are openly available in the repository of the Open Science Foundation at https://www.osf.io/3tmpr/ (doi: 10.17605/OSF.IO/3TMPR).

Contributors

M.C. and D.S. designed the experiment; F.P., V.S. and F.G. conducted the experiments; B.S.-W. contributed new reagents/analytic tools; M.C., F.P. and D.S. analyzed the data. All authors wrote the manuscript. All authors read and approved the manuscript in its current version.

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Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2021.04.006.

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3.2 Publication 2: Exogenous estradiol and oxytocin modulate sex differences in hippocampal reactivity during the encoding of episodic memories

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Exogenous estradiol and oxytocin modulate sex differences in hippocampal reactivity during the encoding of episodic memories

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ABSTRACT

Considerable evidence supports sex differences in episodic memory. The hormones estradiol and oxytocin both affect episodic memory and may contribute to these sex differences, but possible underlying hormonal interactions have not been tested in a sample involving both sexes. To this end, we conducted a randomized, placebo-controlled, parallel-group functional magnetic resonance imaging (fMRI) study including healthy free-cycling women (n = 111) and men (n = 115). The fMRI session was conducted under four experimental conditions: 1. transdermal estradiol (2 mg) and intranasal oxytocin (24 IU), 2. transdermal placebo and intranasal oxytocin, 3. transdermal estradiol and intranasal placebo, 4. transdermal placebo and intranasal placebo. Participants were scanned during the encoding of positive, neutral, and negative scenes. Recognition memory was tested three days following the scanning sessions without additional treatments. Under placebo, women showed a significantly better recognition memory and increased hippocampal responses to subsequently remembered items independent of the emotional valence compared to men. The separate treatments with either hormone significantly diminished this mnemonic sex difference and reversed the hippocampal activation pattern. However, the combined treatments produced no significant effect. Collectively, the results suggest that both hormones play a crucial role in modulating sex differences in episodic memory. Furthermore, possible antagonistic interactions between estradiol and oxytocin could explain previously observed opposing hormonal effects in women and men.

1. Introduction

Sex differences in memory have been reported repeatedly (Andreano and Cahill, 2009; Cahill, 2003; Loprinzi and Frith, 2018). Metanalytical evidence indicates that women tend to outperform men in episodic memory functions (Andreano and Cahill, 2009; Asperholm et al., 2019; Herlitz et al., 1997; Herlitz and Yonker, 2002; Loprinzi and Frith, 2018), including autobiographical and recognition memory (Fuentes and Desrocher, 2012; Heisz et al., 2013). The magnitude of this sex difference is moderated by the type of task and stimulus material, with a female superiority found for tasks based on verbal abilities and a male advantage observed for tasks in which spatial processing is required (Asperholm et al., 2019; Loprinzi and Frith, 2018). The female advantage in verbal episodic memory appears to be stronger in recall than recognition tasks (Hirnstein et al., 2022). However, women also outperform men in some non-verbal tasks such as the recognition of faces, which may be related to increased scanning behavior (i.e. more fixations) at encoding in females (Heisz et al., 2013). Moreover, women have been found to remember more highly arousing negative pictures than men (Canli et al., 2002), although it has been proposed that sex differences in emotional memory may be related to feminine and masculine traits rather than actual sex per se (Cahill et al., 2004). Emotional hypermnesia is partially mediated through activation of the amygdala (Aikins et al., 2010; Hamann et al., 1999) and imaging studies revealed a sex-related hemispheric lateralization of the amygdala in response to emotional stimuli: right amygdala activation while viewing emotional stimuli is more significantly related to subsequent memory for the images in men than women, whereas the reverse sex difference is evident for the left amygdala (Cahill, 2006; Cahill et al., 2001; Canli et al., 2002, 2000).

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Potential biological mechanisms explaining these sex differences include the effects of sex hormones on memory function and neuroplasticity (Andreano and Cahill, 2009; Cahill, 2006; Cover et al., 2014; Duarte-Guterman et al., 2015; Herlitz and Yonker, 2002; Loprinzi and Frith, 2018). In particular, the role of estradiol (EST) as the most potent and prevalent circulating estrogen is discussed. As EST elicits effects on the memory consolidation in fear extinction learning, the regulating function of mnemonic brain regions, such as the hippocampus, has been highlighted (Taxier et al., 2020). Previous studies in rodents and humans have shown that hippocampal function is sensitive to changes in estrogens that occur across the reproductive cycle, pregnancy, or aging (Daniel, 2013; Duarte-Guterman et al., 2015; Pawluski et al., 2009). EST affects the dendritic spine density in the hippocampus (Loprinzi and Frith, 2018; Taxier et al., 2020) and enhances neuronal growth by promoting the formation of new synaptic connections (Cooke and Woolley, 2005). Several studies in female rodents have shown that the infusion of EST before or immediately after a training improves memory performance (Boulware et al., 2013; Fernandez et al., 2008; Luine et al., 2003; Pereira et al., 2014; Tuscher et al., 2019), but accumulating evidence indicates that EST also enhances cognition in male rodents (Taxier et al., 2020). In humans, correlative studies of natural hormonal fluctuations during the menstrual cycle found that estrogen levels are associated with greater episodic memory performance, but estrogen exposure has not been consistently shown to correlate with memory parameters in all studies (Loprinzi and Frith, 2018). For instance, some studies observed that higher levels of circulating EST correlate with better working memory performance (Hampson and Morley, 2013), whereas other studies reported changes in neither working memory nor delayed recall across the menstrual cycle (Mihalj et al., 2014). The mnemonic effects of EST might be task-dependent and vary with emotional valence and dose (Bayer et al., 2018; Luine, 2014). Despite the established sex differences in memory, there is little work directly comparing EST effects in women and men (Loprinzi and Frith, 2018).

Sex-specific behavioural and limbic effects have also been observed for the hypothalamic peptide oxytocin (OXT) (Gao et al., 2016; Luo et al., 2017; Rilling et al., 2014; Scheele et al., 2014). For instance, a dose of 24 IU intranasal OXT reduces amygdala responses to fearful faces in men (Spengler et al., 2017), but the same dose of the peptide increases amygdala activation in women (Lieberz et al., 2020). Likewise, OXT has been found to improve empathic accuracy in more socially proficient men but had no significant effect in women regardless of the social proficiency (Bartz et al., 2019). Considering the peptide's prosocial and anxiolytic effects, together with its high tolerability, OXT has evolved as a potential candidate compound for treating various mental disorders (Heinrichs et al., 2009; Herpertz and Bertsch, 2015; Meyer-Lindenberg et al., 2011). In male rodents, OXT plays a critical role in mediating social memory, with OXT receptors in the hippocampus (Raam et al., 2017), and amygdala (Ferguson et al., 2000) being necessary for social recognition. However, findings regarding OXT effects on human memory encoding have been inconsistent, with reports ranging from memory impairment (Bate et al., 2015; Heinrichs et al., 2004) to enhancement (Acevedo-Rodriguez et al., 2015; Guastella et al., 2008; Rimmele et al., 2009; Savaskan et al., 2008) via modulation of insula activity (Striepens et al., 2012). Of note, OXT effects may be moderated by inter-individual variables such as attachment insecurity (Bartz et al., 2010) or contextual factors including valence of the stimulus material (Bartz et al., 2011; Gao et al., 2016; Heinrichs et al., 2004; Wagner and Echterhoff, 2018). In addition, it has been discussed whether OXT primarily facilitates the processing of social cues (Guastella and MacLeod, 2012), but it is more and more recognized that OXT can modulate social and non-social behavior (Quintana and Guastella, 2020).

Inconsistent and sometimes opposing effects of OXT in women and men may result from interactive effects with EST. Evidence for interactions between OXT and EST derives from animal studies, showing extensive coexpression of EST receptor ß in OXT neurons of the paraventricular nucleus of the hypothalamus (Suzuki and Handa, 2005) and combinatorial modulation of synaptic plasticity in the medial nucleus of the amygdala in male rats (Frankiensztajn et al., 2018). The EST receptor additionally binds in a dimerized form to the composite hormone response element of the OXT promotor gene and may thus induce the production of OXT (Acevedo-Rodriguez et al., 2015; McCarthy et al., 1996; Young et al., 1998). In fact, orally administered EST induced an increase in OXT plasma levels in bulimic and healthy women (Chiodera et al., 1991). Importantly, in humans, possible OXT-EST interactions have been proposed for various domains including migraine attacks (Krause et al., 2021) and social anxiety (Schneider et al., 2021). An intriguing notion is that both hormones may antagonistically interact in a way that yields opposing effects on limbic reactivity in women and men (Lieberz et al., 2020).

To date, no study has simultaneously probed the modulatory mnemonic effects of both hormones and possible interactions in women and men. Therefore, we conducted a randomized, placebo-controlled, parallel-group functional magnetic resonance imaging (fMRI) study to test the effects of EST, OXT, and their interaction on emotional memory and to elucidate the neural mechanisms involved in mnestic sex differences (see Fig. 1). Healthy men and free-cycling women were scanned under four experimental conditions: 1. transdermal placebo gel and intranasal placebo (PLC_{tra} & PLC_{int}), 2. transdermal placebo and intranasal OXT (24 IU) (PLC_{tra} & OXT_{int}), 3. transdermal EST (2 mg) and intranasal placebo (EST_{tra} & PLC_{int}), and 4. transdermal EST and intranasal OXT (EST_{tra} & OXT_{int}). As we were primarily interested in the effects of sex, EST, and OXT and their interaction on memory encoding, the participants received their treatment prior to being scanned. During fMRI, participants viewed positive, neutral, and negative scenes. A surprise recognition task three days later was used to classify encoding trials as remembered or forgotten.

Our first hypothesis was that the EST_{tra} administration would trigger an increase in the endogenous OXT levels (Acevedo-Rodriguez et al., 2015). Due to the previously observed correlation between higher EST and enhanced memory performance, we hypothesized that an EST_{tra} treatment prior to encoding would increase recognition memory of the encoded emotional material and encoding activity in the hippocampus and amygdala, in both sexes (Loprinzi and Frith, 2018; Taxier et al., 2020). Based on previous findings about valence- and sex-specific effects of OXT (Lieberz et al., 2020; Striepens et al., 2012), we expected that the pre-encoding OXT_{int} treatment would increase recognition memory of negative stimuli and insula activity to subsequently remembered negative items in men and that it would produce the opposite effect in women (Rilling et al., 2014). Additionally, the potential antagonistic relation between EST and OXT in women (Schneider et al., 2021) and the observed opposing effects of OXT on limbic reactivity in women and men (Lieberz et al., 2020; Schneider et al., 2021), led to the hypothesis that the effects of OXT_{int} in the combined treatment group would be reduced or even inverted in women and more pronounced in men. In additional explorative analyses, we examined possible sex-related hemispheric lateralization effects of the amygdala in response to remembered emotional stimuli (Cahill, 2006; Cahill et al., 2001; Canli et al., 2002, 2000) and probed whether OXT differentially modulated the encoding of subsequently remembered social and non-social stimuli (Guastella and MacLeod, 2012; Quintana et al., 2019).

2. Material and methods

2.1. Ethics and enrolment

The study was part of a larger project and was approved by the institutional review board of the Medical Faculty of the University of Bonn and carried out in accordance with the latest revision of the Declaration of Helsinki. The study was registered in the ClinicalTrials.gov database (Identifier: NCT04330677) and the data analyses were preregistered (https://osf.io/hvknp/). The current paper focuses on the effects

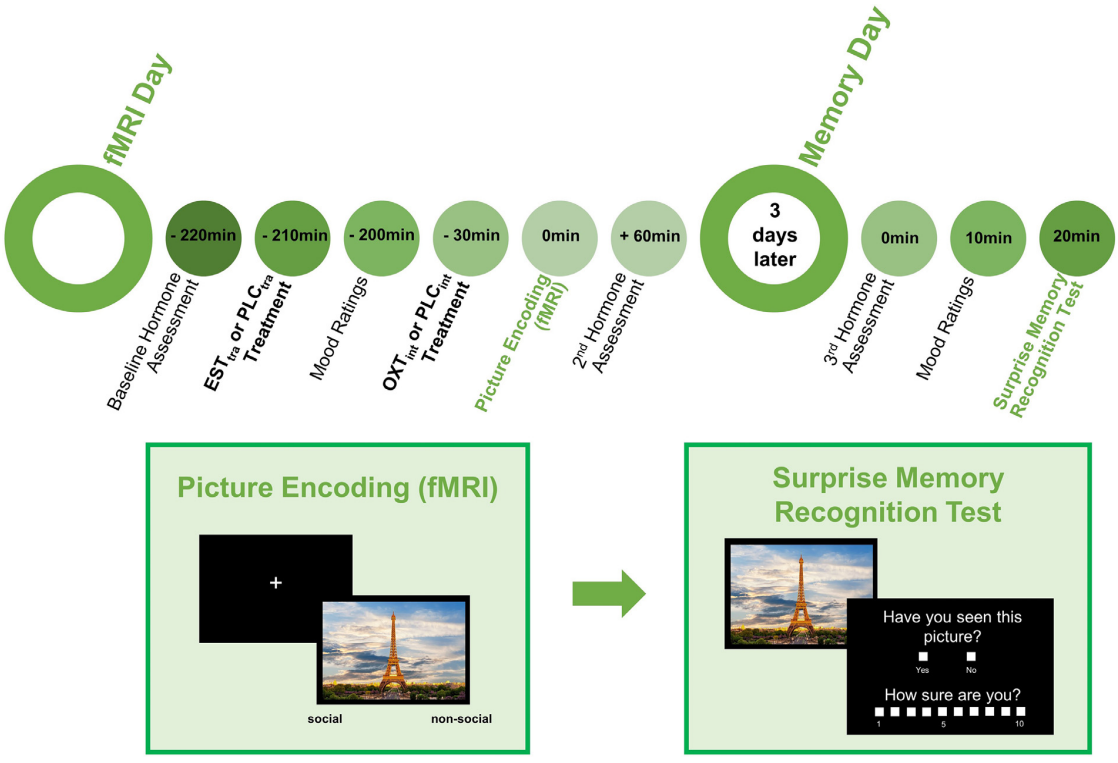

Fig. 1. *Study Design.* The functional magnetic resonance imaging (fMRI) day commenced with gel administration. Nasal sprays were administered 3 h after the gel treatment and a high-resolution structural MRI scan was conducted 30 min after the nasal spray administration. Functional imaging data collection commenced 45 min after nasal spray treatment and included a picture encoding task. Participants viewed emotional and nonemotional scenes in a randomized order and had to press a button to indicate whether the picture's context was social (i.e., if a human person was shown). Blood samples were collected at baseline and immediately after the fMRI testing session (approx. 4.5 h after the gel administration). Three days later a surprise memory recognition task was administered in a second testing session and a third blood sample was collected. The recognition task included the 120 pictures shown in the scanner and 60 new distractor pictures. Participants had to rate whether they had seen the picture in the MRI task (yes/no) and their level of confidence on a 10-point Likert scale.

of EST_{tra} and OXT_{int} on the encoding of subsequently remembered or forgotten stimuli (i.e. hypotheses 4a-h of the preregistration). The results of additional tasks and hypotheses will be reported elsewhere. The participants were enrolled in the study after giving written informed consent, and they received a monetary reimbursement of 100€ after completing the study.

2.2. Power analysis

We used G*Power 3 (Faul et al., 2007) to conduct an a-priori power analysis for the project. As we started planning the project in 2016, we were not aware of any study testing the effects of exogenous EST on memory in women and men. Therefore, we based the power analysis on the effect size obtained in our OXT dose-response study (Spengler et al., 2017). Regarding the effect of intranasal oxytocin (24 IU at a latency of 45 min) on the amygdala response to high intensity fearful faces we observed an effect size of $dz = 0.56$ in a within-subject design. To detect an oxytocin effect of this size (with $\alpha = 0.05$ and power = 0.75), we needed to test at least 48 participants in a between-subject design (i.e. 24 male participants in the placebo group and 24 male participants in the OXT group). Thus, we planned to include at least 24 participants in each treatment group (1. PLC & PLC; 2. PLC & OXT; 3. EST & PLC; 4. EST & OXT) separately for both sexes (1. female; 2. male). In total, 122 healthy women and 124 healthy men were included in the study to control for drop-outs and exclusions. The final sample included 44 participants (25 women) in the PLC_{tra} and PLC_{int} group, 56 participants (29 women) in the PLC_{tra} and OXT_{int} group, 54 participants (25 women) in the EST_{tra} and PLC_{int} group, and 48 participants (24 women) in the EST_{tra} and OXT_{int} group.

2.3. Participants

In total, 295 participants (160 women) were invited to a screening session prior to the testing session. The 246 participants (122 women) who met the inclusion criteria (see below) were tested. The participants were randomly assigned to one of four experimental conditions: (1. PLC_{tra} & PLC_{int}; 2. PLC_{tra} & OXT_{int}; 3. EST_{tra} & PLC_{int}; 4. EST_{tra} & OXT_{int}). We had to exclude 44 participants from all analyses. The data of 11 participants were not completely recorded due to technical errors: the logfiles of the fMRI task were not completely saved for 4 participants and the synchronization of the MRI scanner and the computer used to display the stimuli failed in 7 participants. Furthermore, 3 participants did not finish the study, because they did not return to the last testing day due to scheduling issues. Additional 6 participants were excluded due to hormonal ($n = 4$) or anatomical ($n = 2$) abnormalities, resulting in a sample of 226 participants (PLC_{tra} & PLC_{int}: 25 men, 26 women; PLC_{tra} & OXT_{int}: 33 men, 31 women; EST_{tra} & PLC_{int}: 32 men, 27 women; EST_{tra} & OXT_{int}: 25 men, 27 women). In accordance with our preregistered analysis of the data, we had to exclude additional 24 participants from the neural and behavioral analyses, who remembered all or none of the stimuli in at least one valence category. Thus, our final sample for the neural and behavioural analyses included 202 participants (PLC_{tra} & $PLC_{int:}$ 19 men, 25 women; PLC_{tra} & OXT_{int}: 27 men, 29 women; EST_{tra} & PLC_{int}: 29 men, 25 women; EST_{tra} & OXT_{int}: 24 men,

24 women). For demographic and psychometric baseline characteristics see **Supplementary Table S7**.

2.4. Screening session and exclusion criteria

Screenings of the participants were conducted prior to the test sessions. Participants were free of current or past physical or psychiatric illnesses assessed by self-report and the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998). In addition, participants were naive to prescription-strength psychoactive medication, and had not taken any over-the-counter psychoactive medications in the 4 weeks prior to the study. The participants were right-handed, nonsmoking and between 18 and 40 years old. Furthermore, the participants were asked to maintain their regular bed and waking times and to abstain from alcohol intake on the day of the experiment. Additional exclusion criteria were current pregnancy, MRI contraindications and the use of hormonal contraceptives. All women completed the fMRI testing session simultaneously with the onset of their menstruation (days 1–6). Thus, the women were tested in their early follicular phase of their menstrual cycle. The onset of their menstruation was measured via self-report, which was further validated by blood assays obtained on the testing day. Female participants (n = 4) showing estradiol pre-treatment values larger than 300 pg/ml were excluded, because it can be assumed that they were not in the follicular phase of their menstrual cycle (Kratz et al., 2004).

2.5. Experimental design

We used a randomized, double-blind, placebo-controlled parallel-group study design (see Fig. 1). After a screening session, the participants completed the fMRI testing session. Three days later a surprise memory recognition task was administered in a second testing session. The fMRI day commenced with the gel administration. In accordance with our pharmacokinetic pre-study (see **Supplementary Information [SI]**), the OXT_{int} or placebo spray was administered 3 h after gel administration. Functional imaging data collection commenced 45 min after nasal spray administration, because it was found to be the most effective dose-test interval for OXT_{int} (Spengler et al., 2017). The imaging data collection included a high-resolution structural MRI scan and an emotional subsequent memory task. To validate the cycle phase and control for baseline differences in gonadal hormone levels, blood samples were collected at baseline, immediately after the fMRI testing session (approx. 4.5 h after gel administration), and three days following the treatment. In addition, questionnaires assessing mood (Positive and Negative Affect Schedule [PANAS] (Watson et al., 1988)) were administered twice, first immediately following the EST_{tra} or PLC_{tra} treatment at the beginning of the testing session and after the fMRI session (see **SI**).

2.6. Treatments

2.6.1. Estradiol / placebo gel treatment

On the fMRI testing day, the participants received either EST_{tra} gel (Estramon, 2 mg EST, Hexal AG, Holzkirchen, Germany) or placebo gel (2 mg ultrasonic gel), which was transdermally applied to the participants' backs. In line with a pharmacokinetic study (Eisenegger et al., 2013), a 2 mg dose was chosen to reduce the possibility of side effects. The same dose has also been found to increase emotional vicarious reactivity in men when watching a distressed other (Olsson et al., 2016).

2.6.2. Intranasal oxytocin / placebo treatment

Via a nasal spray, the participants self-administered 24 International Units (IU) of synthetic OXT_{int} (Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Rome, Italy) or placebo prior to the fMRI scanning in line with the standardization guidelines (Guastella et al., 2013) and under supervision of a trained research assistant. There is compelling evidence that OXT_{int} bypasses the blood-brain barrier, elevates OXT concentrations in the cerebrospinal fluid (Lee et al., 2018; Striepens et al., 2013) and brain (Lee et al., 2020), and thus is a valid approach to target the brain's oxytocin system (Martins et al., 2022). The placebo was equivalent to the OXT_{int} solution without the peptide itself. The amount of administered substance was weighed and supplemented until 24 IU were reached. The puffs were balanced between the nostrils to allow the solution to be absorbed by the nasal epithelium and an interpuff interval of approx. 45 s was chosen.

2.7. Emotional subsequent memory task

2.7.1. fMRI task

During the fMRI, participants viewed a picture set (see **SI**) of negative (n = 40), neutral (n = 40), and positive (n = 40) scenes in a randomized order. Each picture was presented for 4 s with a randomized inter-stimulus-interval of minimal 4 to maximal 6 s. The content of the pictures was either social (defined as the presence of a depicted human) or non-social, which were equally distributed across valence categories. The selection of the stimuli was based on a pilot study and stimuli were chosen such that negative and positive stimuli produced comparable arousal ratings and sex differences were absent for all ratings (for further details see **SI**). The final picture set consisted of 20 pictures in each of the following categories: social negative (e.g. crying humans), nonsocial negative (e.g. trash or unwashed dishes), social neutral (e.g. humans with a neutral facial expression), non-social neutral (e.g. neutral objects like a cup), social positive (e.g. happy and laughing humans) and non-social positive (e.g. beautiful landscapes). Examples for each valence category can be found in the SI (see **Figure S1**). During fMRI, as an attention control, the participants had to press a button, if the picture's context was social (i.e., a human person was shown) or non-social (i.e., no human person was shown). The participants could choose their responses using an MRI-compatible response grip system (NordicNeuroLab AS, Bergen, Norway). The paradigm was written in Presentation code (Neurobehavioral Systems, Albany, CA) and the stimuli were presented on a 32-inch MRI-compatible TFT LCD monitor (NordicNeuroLab, Bergen, Norway) placed at the rear of the magnet bore.

2.7.2. Surprise recognition task

The participants performed a surprise memory recognition task three days after the MRI scan to classify pictures as remembered and nonremembered. Based on our pharmacokinetic pre-study, the delay of three days was chosen to ensure that the neuroendocrine parameters return to baseline levels before the surprise recognition task. The recognition task included the 120 pictures shown in the scanner and 60 new distractor pictures (matched for valence and arousal ratings, see **SI**). The participants had to rate whether they had seen the picture in the MRI task (yes/no) and their level of confidence on a 10-point Likert scale.

2.8. Data analysis

2.8.1. Memory performance

Stimuli were classified as remembered if the picture was included in the emotional subsequent memory task and correctly identified in the recognition task (see **SI** for further information). In accordance with our preregistration, the participants had to rate their confidence for an item as ≥ 2 on the 10-point Likert scale to be classified as remembered. The participants who remembered all or none of the stimuli in at least one valence category were excluded from the analysis (n = 24), because a minimum of one trial was required in every valence category for the neural and behavioral model estimation. For the behavioral model, we calculated d prime (d') by subtracting the z-standardized false alarm rate from the z-standardized hit rate. The hit rate was the mean of the correctly identified stimuli used in the fMRI paradigm. The false alarm rate was the mean of the distractors in our recognition paradigm, which were incorrectly identified as seen by the participant, although

they were not included in the fMRI task. When the hit rate or false alarm rate equals zero or one, the corresponding z-score would be -/+∞. Thus, we adjusted *d'* according to the loglinear method (Stanislaw and Todorov, 1999) by adding 0.5 to both the number of hits and the number of false alarms and adding 1 to both the number of signal trials and noise trials, before calculating the hit and false alarm rates (Hautus, 1995). A high *d'* indicated that the signal was easily detected (Haatveit et al., 2010).

2.8.2. fMRI data acquisition and analysis

All fMRI data were acquired using a 3T Siemens TRIO MRI system (Siemens AG, Erlangen, Germany) with a Siemens 32-channel head coil. fMRI data were preprocessed and analysed using standard procedures in SPM12 software (Wellcome Trust Center for Neuroimaging; http://www.fil.ion.ucl.ac.uk/spm) implemented in MATLAB (MathWorks).

In a first model, twelve conditions (valence (negative, neutral, positive) x memory (remembered, forgotten) x sociality (social, non-social)) were modeled by a stick function convolved with a hemodynamic response function. In a second model, the factor "sociality" was aggregated, resulting in six conditions (valence (3) x memory (2)). Button presses were included as regressors of no interest. On the first level, task-specific effects were modelled (details see **SI**). On the second level, a full factorial design with the between-subjects variables "OXT_{int} treatment", "EST_{tra} treatment", and "sex" was conducted. Based on previous findings (Aikins et al., 2010; Hamann et al., 1999; Striepens et al., 2012; Taxier et al., 2020), analyses were conducted focusing on the anatomically defined amygdala, hippocampus, and insula cortex based on the WFU PickAtlas as regions of interest (ROIs). The significance threshold for the ROI analyses was set to $p < 0.05$, familywise error-corrected for multiple comparisons based on the size of the ROI. In addition, an exploratory whole-brain analysis was performed (cluster defining threshold $p < 0.001$; significance threshold $p_{FWE} < 0.05$ at peak level). Parameter estimates of significant contrasts were extracted using MarsBaR (https://www.nitrc.org/projects/marsbar, RRID: SCR_009605) and further analysed in SPSS 25 (IBM Corp., Armonk, NY). For further analyses and details see SI.

2.9. Statistical analyses

Behavioural, neuroendocrine, and demographic data were analysed in SPSS 25 using standard procedures including analyses of variances (ANOVAs) and post-hoc *t*-tests. Based on the view that main and interaction effects cannot be interpreted separately if the interaction effect is significant (Schnuck and Nisic, 2020; Mayerl and Urban, 2019), we report the significant main effects in the results section, but we abstain from interpreting them. Post hoc *t*-tests were Bonferroni-corrected (p_{cor}). In general, two-tailed *p* values < 0.05 were considered significant, except for directional hypotheses, in which case one-tailed t-tests were calculated. If the assumption of sphericity was significantly violated, a Greenhouse-Geisser correction was applied. As measures of effect sizes, partial eta-squared and Cohen's d were calculated. Furthermore, frequentist inference was complemented by computing Bayes factors (BFs) with default priors via JASP (version 0.14.1.0). For Bayesian *t*-tests, BF_{10} is reported. A BF_{10} larger than 1 can be interpreted as evidence in favor of the alternative hypothesis given the current data. For Bayesian ANOVAs, BF_{incl} (with fixed seed = 1) was calculated, which compares the performance of all models that include the effect to the performance of all the models that do not include the effect. Mixed-design ANOVAs with between-subjects variables "OXT_{int} treatment" (OXT_{int}, placebo nasal spray), "EST_{tra} treatment" (EST_{tra}, placebo gel) and "sex" (women, men) and the within-subject factors "valence" (negative, neutral, positive) and "sociality" (social, non-social) were conducted for the outcome "memory" (*d'*). In a second and third model, either the factor "sociality" or the factor "valence" was aggregated. In a fourth model, both factors "valence" and "sociality" were aggregated to provide an overall *d'* that represented the general memory performance of the participants irrespective of valence and sociality. Changes in hormone concentrations were examined with mixed-design ANOVAs with the between-subject factors "OXT_{int} treatment", "EST_{tra} treatment", and "sex" and the within-subject variable "time" (baseline, after treatment, and three days after treatment; for OXT changes: baseline vs. after treatment). Furthermore, to explore the potential moderating effects of treatment-induced hormonal changes, the magnitude of the increases in hormone concentrations (levels of EST, OXT, testosterone, and progesterone after the fMRI session minus baseline) were considered covariates in the main analyses with significant behavioural (*d'*) and neural outcomes (i.e., parameter estimates of significant contrasts of interests).

3. Results

3.1. Neuroendocrine parameters

Two sample t-tests showed that women at baseline had significantly higher EST concentrations ($t_{(117.24)} = -5.70$, $p < 0.001$, $d = -0.80$; $BF_{10} = 206{,}050.22$), but lower testosterone ($t_{(98.31)} = 27.09$, $p < 0.001$, $d = 3.89$; $BF_{10} = 9.96 \times 10^{64}$) and OXT levels ($t_{(196)} = 2.75$, $p < 0.01$, $d = 0.39$; $BF_{10} = 5.04$) than men. The progesterone baseline concentrations were comparable between the two sexes ($t_{(98.72)} = -1.76$, $p = 0.08$, $d = -0.25$; $BF_{10} = 0.65$). Importantly, all baseline levels were comparable between treatment groups in women and men (all *p*s > 0.05; $BF_{10} < 1$).

The EST_{tra} administration significantly increased blood EST levels in both sexes (see Fig. 2 **and Supplementary Table S4**; mixed-design ANOVA: time * EST_{tra} treatment: $F_{(1.00, 182.64)} = 265.92$, $p < 0.001$, $\eta_p^2 = 0.60$; $BF_{incl} = 1.14 * 10^{79}$), with women exhibiting a significantly larger increase than men (time * sex * EST_{tra} treatment: $F_{(1.01, 182.645)} = 16.30$, $p < 0.001$, $\eta_p^2 = 0.08$; $BF_{incl} = 742{,}418.03$). There were no significant main or interaction effects of the OXT_{int} treatment on EST levels (all *p*s > 0.05; $BF_{10} < 0.2$), indicating that the OXT_{int} treatment did not modulate the EST increase.

OXT_{int} administration significantly increased blood OXT levels in both sexes (see Fig. 2 **and Supplementary Table S5**; mixed-design ANOVA: time * OXT_{int} treatment: $F_{(1.00,190)} = 215.77$, $p < 0.001$, $\eta_p^2 = 0.53$; $BF_{incl} = 2.43 * 10^{31}$). There were no significant main or interaction effects of the EST_{tra} treatment on the OXT levels (all *p*s > 0.05; $BF_{10} < 0.4$), indicating that the EST_{tra} treatment did not modulate the OXT increase. To specifically test our first hypothesis that the EST_{tra} administration would trigger an increase in the endogenous OXT levels, we computed an additional mixed-design ANOVA with the between-subject factor treatment (EST_{tra} & PLC_{int} vs. PLC_{tra} & PLC_{int}), the within-subject factor time (pre vs. post), and the OXT level as dependent variable. In contrast to our first hypothesis, we did not find a significant main ($p = 0.58$; $BF_{incl} = 0.39$) or interaction effect ($p = 0.44$, $BF_{incl} = 0.28$) of the EST_{tra} treatment on blood OXT levels, indicating that the EST_{tra} treatment did not induce a significant OXT increase. The Bayes factors provide moderate evidence that the EST_{tra} administration had no effect on OXT levels.

To examine whether the changes in OXT_{int} and EST_{tra} levels moderated behavioural and neural treatment effects, we included the hormonal changes (after treatment minus baseline) as separate covariates in the analyses and all sex * treatment interactions remained significant. Furthermore, the treatments did not significantly alter the participants' mood (see **SI** and **Supplementary Table S6**).

3.2. Results for hypothesized valence-specific effects

Recognition memory was significantly better for emotional than neutral items (main effect valence: $F_{(2.00,388)} = 13.18$, $p < 0.001$, $\eta_p^2 = 0.06$; $BF_{incl} = 3054.02$) and for social than non-social stimuli (main effect of sociality: $F_{(1.00,194)} = 7.59$, $p < 0.01$, $\eta_p^2 = 0.04$; $BF_{incl} = 5.29$). However,

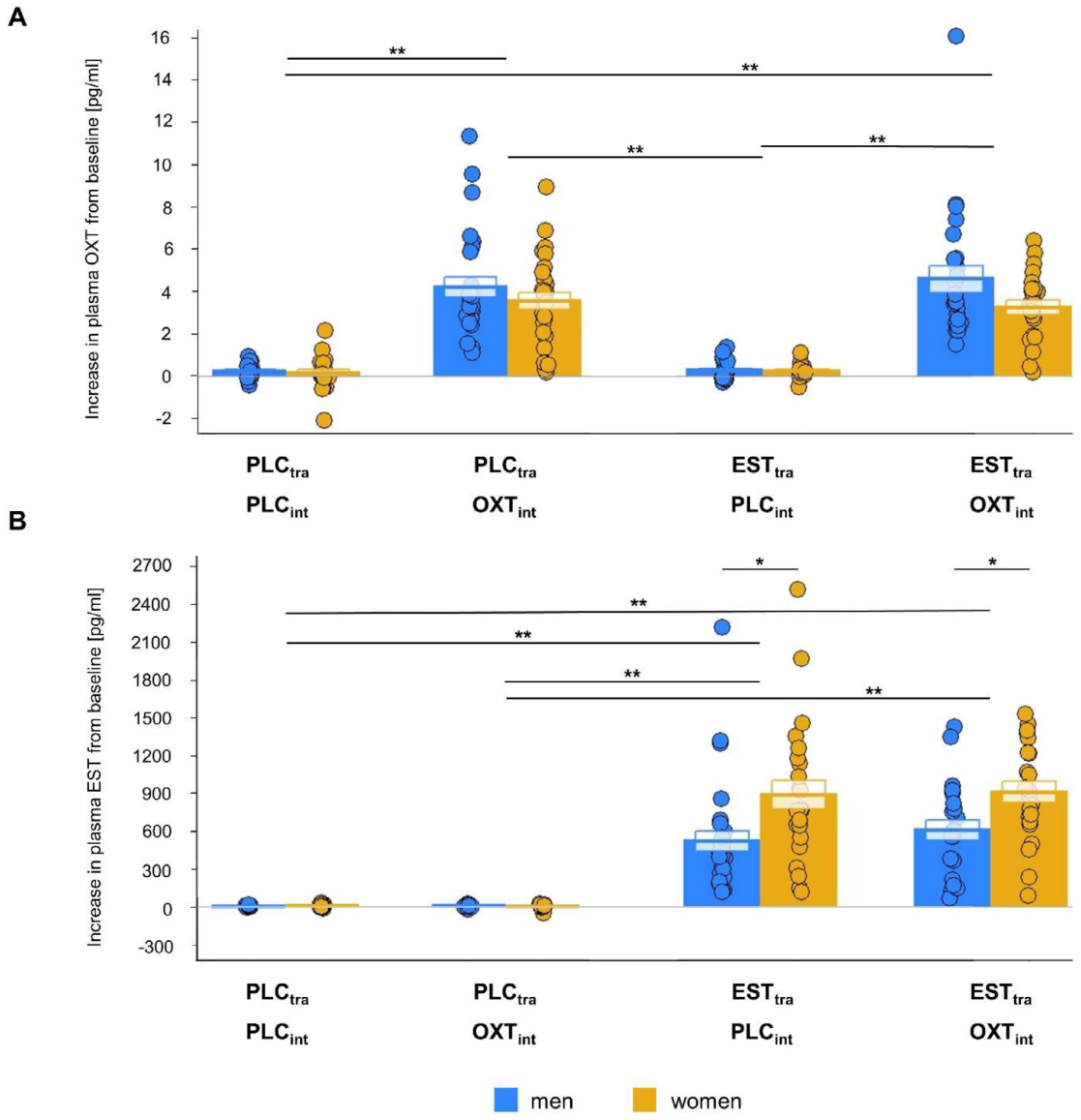

Fig. 2. *Treatment-induced changes (immediately after fMRI session minus baseline) in oxytocin and estradiol plasma levels.* **A** The administration of 24 international units (IU) of intranasal OXT_{int} induced a significant increase in blood oxytocin levels in both sexes. There was no significant interaction with the estradiol treatment. **B** The transdermal administration of 2 mg estradiol (EST_{tra}) significantly elevated blood estradiol levels in both sexes. EST_{tra} treatment induced a significantly stronger increase in women than in men, but there was no significant interaction with oxytocin treatment. PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol. $^{*}p < 0.05$, $^{**}p < 0.01$.

in contrast to our hypothesized effects of EST_{tra} or OXT_{int} on emotional memory, there were no significant interactions between these factors (valence and sociality) and sex or treatments ($BF_{incl} < 0.4$). We further hypothesized that the OXT_{int} treatment would increase the recognition memory of negative stimuli in men with placebo gel, whereas we expected a decrease in women. Furthermore, we expected that OXT_{int} would increase the activity in the insula cortex during the processing of subsequently remembered negative stimuli compared to forgotten items (i.e. [Negative $_{Remembered}$ > Negative $_{Forgotten}$]) in men with placebo gel and the opposite effect in women. We calculated two-sample *t*-tests to compare *d'* for negative stimuli and the insula responses to negative remembered stimuli compared to negative forgotten stimuli between the PLC_{tra} & PLC_{int} and PLC_{tra} & OXT_{int} groups separately for women and men. No significant group differences were detected (all *ps* > 0.05). Further mixed-design ANOVAs with EST_{tra} treatment and OXT_{int} treatment as between-subject factors and either *d'* for negative stimuli or the insula responses to negative remembered stimuli compared to negative forgotten stimuli as dependent variables separately for both sexes did not reveal any significant main or interaction effects of the treatment types (all *ps* > 0.05). Thus, in contrast to our hypotheses, we did not observe selective treatment effects for negative items.

3.3. Further behavioural results

Due to the missing significant interactions between valence and sociality and sex or treatments, the following exploratory analyses are

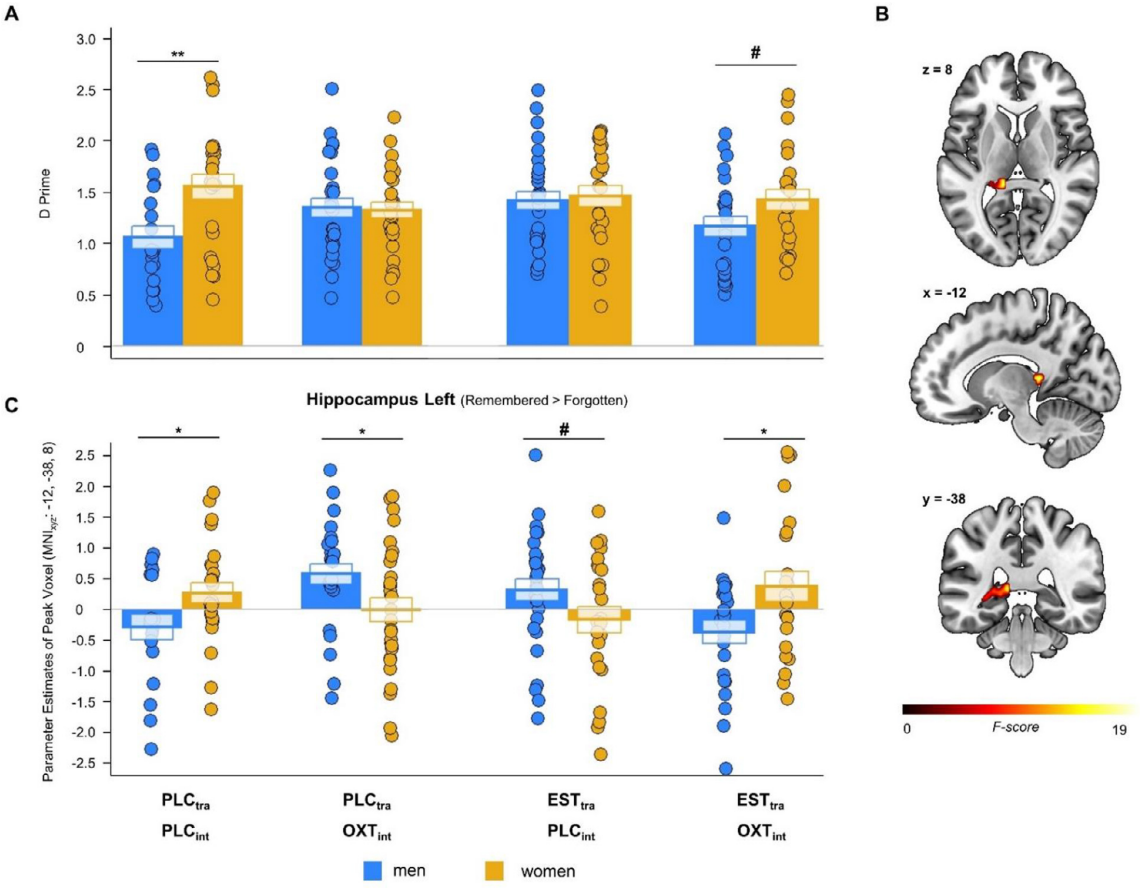

Fig. 3. *Treatment effects on mnemonic and hippocampal sex differences.* **A** Following placebo administration, women showed a significantly better memory performance (*d'*) than men, but there were no significant sex differences after single trandermal estradiol (EST_{tra}) or intranasal oxytocin (OXT_{int}) treatment. After the combined treatment, a trend similar to that observed in the placebo group was evident. **B** We observed a significant three-way interaction of sex, OXT_{int}, and EST_{tra} treatment in the left hippocampus responses to remembered compared to forgotten stimuli (MNI peak coordinates [x, y, z]: −12, −38, 8). **C** Further analyses of the parameter estimates for the left hippocampal responses revealed a similar pattern as that observed with memory performance. Women exhibited stronger left hippocampal responses to remembered items compared to forgotten items than men under placebo. This sex difference was reversed after either EST_{tra} or OXT treatment. Intriguingly, the same pattern as that observed in the placebo group was again evident in the combined treatment group. PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol. $^{\#}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$.

based on *d'* averaged across valences and sociality. There were no significant treatment effects on the confidence ratings (for further details see SI).

3.3.1. Behavioural results: treatment effects

A mixed-design ANOVA with *d'* as the dependent variable and sex, EST_{tra}, and OXT_{int} as between-subject factors revealed that sex differences in recognition memory were significantly altered by both EST_{tra} and OXT_{int} treatments (significant three-way interaction: sex * EST_{tra} treatment * OXT_{int} treatment: $F_{(1.00,194)} = 6.96$, $p < 0.01$, $\eta_p^2 = 0.04$; $BF_{incl} = 5.55$; see Fig. 3 **and Supplementary Table S1**). To disentangle the observed three-way interaction, we examined the treatment effects separately for both sexes. Therefore, we calculated separate ANOVAs for both sexes with *d'* as dependent variable and the two treatment types as between-subject factors. We found a significant interaction between the OXT_{int} and EST_{tra} treatments in men ($F_{(1.00,95)} = 7.84$, $p < 0.01$ [$p_{cor} = 0.01$], $\eta_p^2 = 0.08$; $BF_{incl} = 6.94$), but not in women ($F_{(1.00,99)} = 0.93$, $p = 0.34$, $\eta_p^2 = 0.01$; $BF_{incl} = 0.40$). In men, OXT_{int} nonsignificantly improved recognition memory after PLC_{tra} treatment (PLC_{tra} & OXT_{int} vs. PLC_{tra} & PLC_{int}: $t_{(44)} = -2.01$, $p = 0.03$ [$p_{cor} = 0.1$], $d = -0.6$; $BF_{10} = 1.45$; one-tailed) and reduced performance after EST_{tra} treatment (EST_{tra} & OXT_{int} vs. EST_{tra} & PLC_{int}: $t_{(51)} = 1.95$, $p = 0.06$ [$p_{cor} = 0.23$], $d = 0.54$; $BF_{10} = 1.30$). However, in men, EST_{tra} treatment produced significantly better memory in participants who received PLC_{int} (EST_{tra} & PLC_{int} vs. PLC_{tra} & PLC_{int}: $t_{(46)} = -2.55$, $p < 0.01$ [$p_{cor} = 0.03$], $d = -0.75$; $BF_{10} = 3.75$; one-tailed), but the EST_{tra} effect was diminished in individuals who received OXT_{int} (EST_{tra} & OXT_{int} vs. PLC_{tra} & OXT_{int}: $t_{(49)} = 1.37$, $p = 0.18$, $d = 0.38$; $BF_{10} = 0.60$). Together, the Bayes factors indicate moderate evidence for an EST_{tra} * OXT_{int} interaction in men and moderate evidence for an EST_{tra}-induced memory improvement in men who had received PLC_{int}.

3.3.2. Behavioural results: sex differences

To further disentangle the observed three-way interaction, we examined sex differences within the treatment groups by applying post-hoc Bonferroni-corrected two-sample t-tests. Under placebo (PLC_{tra} & PLC_{int}), women showed significantly better memory performance than men ($t_{(42)} = -2.96$, $p < 0.01$ [$p_{cor} = 0.02$], $d = -0.90$; $BF_{10} = 8.31$), but there were no significant sex differences in the EST_{tra} & PLC_{int} group ($t_{(52)} = -0.32$, $p = 0.75$, $d = -0.09$; $BF_{10} = 0.29$) or the PLC_{tra} & OXT_{int} group ($t_{(54)} = 0.21$, $p = 0.83$, $d = 0.06$; $BF_{10} = 0.28$). The combined treatment (EST_{tra} & OXT_{int}) produced no significant effects, but the direction

of sex differences was comparable to that of the placebo group (women > men; $t_{(46)} = -1.87$, $p = 0.07$ [$p_{cor} = 0.28$], $d = -0.54$; $BF_{10} = 1.16$). Thus, the Bayes factors indicated moderate evidence for sex differences under placebo, moderate evidence for the absence of sex differences after single treatments and an inconclusive sex effect in the combined group.

3.4. Neural results

3.4.1. Whole-brain task effects

Across treatments and sexes, the remembered items compared to forgotten items induced activations in a wide network of brain areas (see **Supplementary Table S2**), including the bilateral hippocampus (left: Montreal Neurological Institute [MNI] peak coordinates [x, y, z]: −32, −18, −14, $F_{(1.00,194)} = 28.81$, on peak level $p_{FWE} < 0.02$; right: MNI peak coordinates [x, y, z]: 20, −6, −14, $F_{(1.00,194)} = 105.12$, on peak level $p_{FWE} < 0.001$). Furthermore, an emotional memory effect (i.e., [Emotional $_{Remembered>Forgotten}$ > Neutral $_{Remembered>Forgotten}$]) was evident in the left and right amygdala (left: MNI peak coordinates [x, y, z]: −20, −6, −14, $F_{(1.00,194)} = 18.12$, on peak level $p_{FWE} < 0.01$; right: MNI peak coordinates [x, y, z]: 22, −6, −12, $F_{(1.00,194)} = 13.96$, on peak level $p_{FWE} = 0.02$), as well as the left hippocampus (MNI peak coordinates [x, y, z]: −18, −6, −14, $F_{(1.00,194)} = 18.55$, on peak level $p_{FWE} < 0.01$) and the right insula (MNI peak coordinates [x, y, z]: 26, 22, −16, $F_{(1.00,194)} = 18.85$, on peak level $p_{FWE} = 0.01$; for additional activations, see **Supplementary Table S3**).

3.4.2. ROI analysis: treatment effects

We found a significant three-way interaction of sex, OXT_{int}, and EST_{tra} treatment in the left hippocampus responses to remembered stimuli compared to forgotten stimuli (MNI peak coordinates [x, y, z]: −12, −38, 8, $F_{(1.00,194)} = 17.80$, on peak level $p_{FWE} = 0.012$; see Fig. 3), but we did not observe a significant three-way interaction for this contrast for the insula or amygdala responses. To disentangle the observed three-way interaction in the left hippocampus, we examined the parameter estimates for left hippocampal responses to remembered items compared to forgotten items separately for both sexes. In a mixed-design ANOVA with the left hippocampal responses as dependent variable and OXT_{int} and EST_{tra} treatments as between-subject factors, we found a significant interaction between the OXT_{int} and EST_{tra} treatments in men ($F_{(1.00,95)} = 17.30$, $p < 0.001$ [$p_{cor} < 0.001$], $\eta_p^2 = 0.15$; $BF_{incl} = 316.43$), but not in women ($F_{(1.00,99)} = 3.92$, $p = 0.05$ [$p_{cor} = 0.10$], $\eta_p^2 = 0.04$; $BF_{incl} = 1.38$). Post-hoc two-sample t-tests revealed that OXT_{int} significantly increased the hippocampal response to remembered stimuli compared to forgotten stimuli in men who had received PLC_{tra} (PLC_{tra} & OXT_{int} vs. PLC_{tra} & PLC_{int}: $t_{(44)} = -3.30$, $p < 0.01$ [$p_{cor} < 0.01$], $d = -0.99$; $BF_{10} = 18.29$). Importantly, in accordance with our hypothesis, we found evidence for an interaction of OXT_{int} and EST_{tra}, but not in the expected direction. OXT_{int} had the opposite effect in men after EST_{tra} treatment and significantly decreased hippocampal responses (EST_{tra} & OXT_{int} vs. EST_{tra} & PLC_{int}: $t_{(51)} = 2.61$, $p = 0.01$ [$p_{cor} = 0.04$], $d = 0.72$; $BF_{10} = 4.24$). Likewise, EST_{tra} did not significantly affect hippocampal activation in men after PLC_{int} (EST_{tra} & PLC_{int} vs. PLC_{tra} & PLC_{int}: $t_{(46)} = -2.17$, $p = 0.02$ [$p_{cor} = 0.07$], $d = -0.64$; $BF_{10} = 1.88$; one-tailed), but significantly decreased hippocampal activation when combined with OXT_{int} treatment (EST_{tra} & OXT_{int} vs. PLC_{tra} & OXT_{int}: $t_{(49)} = 3.80$, $p < 0.001$ [$p_{cor} < 0.001$], $d = 1.07$; $BF_{10} = 67.05$). Thus, again, the Bayes factors indicated strong evidence for an EST_{tra} * OXT_{int} interaction in men. Furthermore, there was moderate-to-strong evidence for opposing effects of OXT_{int} on hippocampal activation depending on EST_{tra} pretreatment and strong evidence for an EST_{tra}-induced decrease when combined with OXT_{int}. Nevertheless, in contrast to our hypothesis, we did not detect a significant effect of EST_{tra} on encoding activity for all stimuli in the hippocampus and amygdala in women.

Additionally, we did not observe a significant three-way interaction for the social memory effect in the hippocampus, insula or amygdala (i.e., [Social $_{Remembered>Forgotten}$ > Non-social $_{Remembered>Forgotten}$]). However, we found a significant two-way interaction of sex and OXT_{int} treatment in the left and right amygdala responses (left: MNI peak coordinates [x, y, z]: −24, −4, −16, $F_{(1.00,194)} = 11.31$, on peak level $p_{FWE} = 0.048$; right: MNI peak coordinates [x, y, z]: 20, −4, −16, $F_{(1.00,194)} = 18.98$, on peak level $p_{FWE} = 0.002$). To disentangle the treatment effects of OXT_{int}, two-sample *t*-tests were calculated to compare the amygdala responses across the EST_{tra} treatments in the PLC_{int} (PLC_{tra} & PLC_{int} and EST_{tra} & PLC_{int}) and OXT_{int} (PLC_{tra} & OXT_{int} and EST_{tra} & OXT_{int}) groups separately for women and men. There were no significant OXT_{int} treatment effects in men. However, in women OXT_{int} significantly decreased bilateral amygdala responses compared to PLC_{int} (OXT_{int} vs. PLC_{int}: left: MNI peak coordinates [x, y, z]: −24, −4, −16, $F_{(1.00,101)} = 4.31$, on peak level $p_{FWE} = 0.002$; right: MNI peak coordinates [x, y, z]: 20, −2, −14, $F_{(1.00,101)} = 4.28$, on peak level $p_{FWE} = 0.002$). We found no further significant main or interaction effects of sex and treatment types for the hippocampus, insula and amygdala responses for the social memory effect (all *ps* > 0.05).

3.4.3. ROI analysis: sex differences

We observed a significant main effect of sex in the left amygdala responses to positive remembered stimuli compared to positive forgotten stimuli (MNI peak coordinates [x, y, z]: −28, −2, −24, $F_{(1.00,204)} = 12.37$, on peak level $p_{FWE} = 0.03$; see Fig. 4) and in the right amygdala responses to negative remembered stimuli compared to negative forgotten stimuli (MNI peak coordinates [x, y, z]: 28, 4, −16, $F_{(1.00,205)} = 12.23$, on peak level $p_{FWE} = 0.03$), but there were no significant interactions between sex and either treatment type. We extracted the parameter estimates from the significant main effect and analyses revealed that across treatments men exhibited significantly increased right amygdala responses to negative remembered stimuli relative to negative forgotten stimuli compared to women ($t_{(200)} = 3.16$, $p < 0.01$, $d = 0.45$; $BF_{10} = 15.17$). In contrast, women showed a stronger activation than men in response to positive remembered stimuli relative to positive forgotten stimuli in the left amygdala ($t_{(200)} = -2.85$, $p < 0.01$, $d = -0.40$; $BF_{10} = 6.50$).

To further disentangle the observed three-way interaction in the left hippocampus, we examined sex differences in the parameter estimates for left hippocampal responses to remembered items compared to forgotten items separately for the treatment groups. Post-hoc two-sample t-tests of the extracted parameter estimates revealed that women exhibited nonsignificantly stronger hippocampal responses to remembered items than men under placebo (PLC_{tra} & PLC_{int}; $t_{(42)} = -2.10$, $p = 0.04$ [$p_{cor} = 0.17$], $d = -0.64$; $BF_{10} = 1.68$). This sex difference was reversed (i.e., men > women) in both the EST_{tra} & PLC_{int} group ($t_{(52)} = 1.77$, $p = 0.08$, [$p_{cor} = 0.33$], $d = 0.48$; $BF_{10} = 0.99$) and the PLC_{tra} & OXT_{int} group ($t_{(54)} = 2.25$, $p = 0.03$ [$p_{cor} = 0.11$], $d = 0.60$; $BF_{10} = 2.13$). Interestingly, the same pattern observed in the placebo group was again evident in the combined treatment group (women > men; $t_{(46)} = -2.42$, $p = 0.02$ [$p_{cor} = 0.08$], $d = -0.7$; $BF_{10} = 2.93$). Notably, a similar sex * EST_{tra} treatment * OXT_{int} treatment interaction emerged for the emotional memory effect in the left hippocampus ([Emotional $_{Remembered>Forgotten}$ > Neutral $_{Remembered >Forgotten}$]; MNI peak coordinates [x, y, z]: −14, −40, 10, $F_{(1.00,194)} = 16.66$, on peak level $p_{FWE} = 0.02$; see **SI**).

As an additional control analysis, we used a median-dichotomization and excluded EST_{tra}-treated women with large EST increase. In this subsample, the treatment-induced increases in EST levels were comparable between women and men within the treatment groups (all *ps* > 0.05; $BF_{10} < 0.6$) and the main behavioral and neural analyses yielded a similar pattern of results. In line with our reported main findings, we also found a significant three-way interaction of sex, OXT_{int}, and EST_{tra} treatment in the left hippocampus responses to remembered stimuli compared to forgotten stimuli (MNI peak coordinates [x, y, z]: −12, −38, 8, $F_{(1.00,165)} = 15.01$, $p < 0.01$ [$p_{cor} < 0.01$], $\eta_p^2 = 0.08$; $BF_{incl} = 208.56$), as well as a significant three-way interaction for the recognition mem-

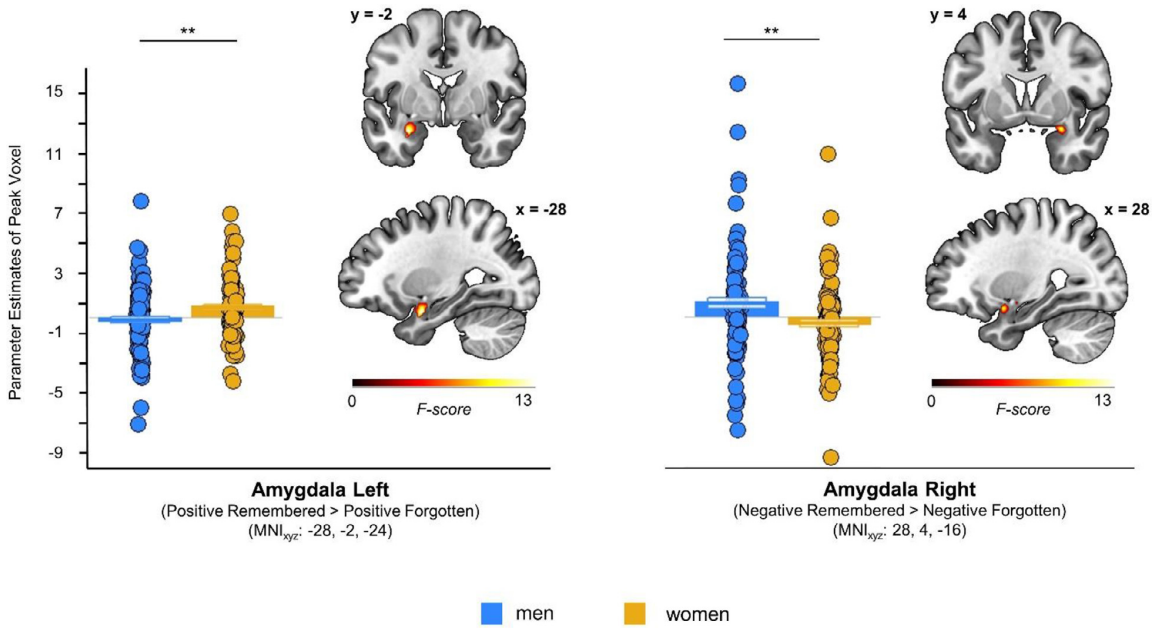

Fig. 4. *Sex-specific lateralization of amygdala activation.* Significant main effects of sex emerged in left amygdala responses to positive remembered stimuli compared to positive forgotten stimuli (MNI peak coordinates [x, y, z]: −28, −2, −24) and for right amygdala responses to negative remembered stimuli compared to negative forgotten stimuli (MNI peak coordinates [x, y, z: 28], 4, −16). Across treatments, analyses of the extracted parameter estimates showed that men exhibited significantly greater right amygdala responses to negative remembered stimuli relative to negative forgotten stimuli than women. However, the pattern in the left amygdala was reversed. Women showed a stronger activation than men in response to positive remembered stimuli compared to positive forgotten stimuli. There were no significant interactions between sex and either treatment type. $^{**}p < 0.01$.

ory ($F_{(1.00,165)} = 6.24$, $p = 0.01$, $\eta_p{}^2 = 0.04$; $BF_{incl} = 5.05$). Further analyses of the extracted parameter estimates revealed results that were comparable to our reported main findings (see **SI** and **Supplementary Figure S2**).

4. Discussion

The goal of the current study was to elucidate the effects of EST_{tra} and OXT_{int} and their interaction on the encoding of emotional and non-emotional scenes in healthy women and men. In contrast to our hypotheses, the treatments did not selectively affect the recognition memory of emotional material and we did not find significant treatment effects on amygdala or insula responses. Instead, we detected effects of EST_{tra} and OXT_{int} on the overall *d'* representing the general memory performance accompanied by neural changes in the hippocampus, but not in the amygdala or insula. Our results showed that under placebo women exhibited a better recognition memory than men and increased hippocampal responses to subsequently remembered items irrespective of emotional valence. Separate treatments with either EST_{tra} or OXT_{int} significantly diminished this mnemonic sex difference and reversed the hippocampal activation pattern. However, the combined treatments produced no significant effect, indicating an antagonistic effect of the two hormones at the administered doses. This pattern was also evident in a subsample with comparable treatment-induced EST increases in women and men. Given significant memory differences between the sexes in the placebo group, our data are consistent with the reported advantage of women in episodic memory (Andreano and Cahill, 2009; Fuentes and Desrocher, 2012; Heisz et al., 2013; Herlitz et al., 1997; Herlitz and Yonker, 2002; Loprinzi and Frith, 2018). The preponderance of studies with exclusively female samples to examine the effects of the "female" sex hormone EST_{tra} and exclusively male samples in the case of OXT_{int} might have obfuscated the contribution of these hormones to sex differences in episodic memory (Hammes and Levin, 2019; Taxier et al., 2020). Our study included both sexes and indicated an essential role of EST_{tra} and OXT_{int} as modulators of episodic memory in women and men.

In men, separate treatment with either EST_{tra} or OXT_{int} prior to encoding increased hippocampal activation and improved subsequent recognition memory, although the EST_{tra} effects on hippocampal activation and the behavioral OXT_{int} memory effect did not survive correction for multiple comparisons. Previous studies show that EST and OXT receptor signaling in the hippocampus regulate neuronal excitability, synaptic plasticity, and memory formation in male mice and rats (Frick et al., 2018; Lin and Hsu, 2018). For instance, EST induced spinogenesis in the hippocampus (Jacome et al., 2016) and improved long-term memory following pretraining administration of EST_{tra} in male rats (Locklear and Kritzer, 2014; Vázquez-Pereyra et al., 1995). Likewise, OXT has been found to enhance cortical information transfer in the hippocampus irrespective of sex by exciting fast-spiking interneurons (Owen et al., 2013). Furthermore, conditional deletion of OXT receptors in the hippocampus of male mice impaired the persistence of long-term social recognition memory (Lin et al., 2018), and treatment with OXT_{int} after experiencing a stressful event rescued recognition memory and hippocampal long-term potentiation in male rats (Park et al., 2017). In humans, some studies found improved recognition memory after an OXT_{int} administration before encoding (Guastella et al., 2008; Rimmele et al., 2009), while other studies observed the opposite effect (Heinrichs et al., 2004; Herzmann et al., 2012). In our study, the behavioral effect of the OXT_{int} administration did not survive correction for multiple comparisons, indicating that the mnemonic effects of 24IU OXT_{int} administration in men are not very robust. By contrast, EST_{tra} treatment significantly improved recognition memory in men independent of the emotional valence. We are not aware of any study probing the effects of exogenous EST_{tra} on memory performance in men, but our study provides first evidence that mnemonic EST effects in men are not emotion-specific.

Intriguingly, a single EST_{tra} treatment produced similar effects as OXT_{int}, but the OXT_{int}-induced increase in hippocampal activation was absent after EST_{tra} pretreatment. One possible explanation for this pattern of results is that EST_{tra} may have increased OXT receptor binding (Johnson et al., 1991), thereby mirroring the opposing effects previously observed for higher OXT_{int} doses in men (Spengler et al., 2017). Interestingly, EST_{tra}*OXT_{int} interactions may have contributed to the modulatory effects of hormonal contraception (Scheele et al., 2016) and to the sex-specific effects of OXT_{int} that have been found in various domains, including fear-related amygdala reactivity (Lieberz et al., 2020), the perception of competition (Fischer-Shofty et al., 2013), moral decision-making (Scheele et al., 2014), and emotional responses to couple conflict (Ditzen et al., 2013). In both sexes, we did not find the expected increase of endogenous OXT_{int} following the EST_{tra} treatment 4.5 h after gel administration, but there is preliminary evidence that EST_{tra}-induced OXT secretion would be most pronounced after 18–36 h (Chiodera et al., 1991). Thus, the chosen timepoint of the second blood sample was possibly too early to detect a potential increase in endogenous OXT levels.

The absence of a significant EST_{tra} effect in women may reflect sex-specific dose-dependent mechanisms. The EST_{tra} treatment produced a significantly larger increase in peripheral EST concentrations in women than in men, yielding concentrations comparable to the EST levels in pregnancy (Gressner and Arndt, 2013). Thus, the nonsignificant decrease in hippocampal activation is consistent with the notion of an inverted U-shaped dose-response function of EST_{tra} in women (Bayer et al., 2018). EST_{tra} levels within physiological ranges have been found to stimulate hippocampal activity, while levels within supraphysiological ranges can have the opposite effect. While low levels of EST were shown to activate the high-affinity receptor ERα, thus inducing synaptogenesis and enhancing blood oxygen level-dependent (BOLD) responses, high levels of EST also activated the low-affinity receptor ERβ, which in turn reduced synaptogenesis (Foster, 2012; Szymczak et al., 2006). As such, the EST_{tra}-enhanced anxiolytic action of OXT_{int} previously observed in female mice may also be dose-dependent (Young et al., 1998). In contrast, sex-specific effects of OXT_{int} on the amygdala response to fearful faces in women have been reported across a range of doses (Lieberz et al., 2020). Significant OXT_{int} effects in men, but no-significant effects in women, have been previously observed for hippocampal responses to cooperative interactions (Rilling et al., 2014) and chemosensory stress signals (Maier et al., 2019). Intranasal OXT administered after acquisition improved recognition memory of faces in a combined sample of 36 women and men (Savaskan et al., 2008), suggesting that the peptide's mnemonic effect may be modulated by the stimulus material or may differ between encoding and consolidation.

Similar time-sensitive effects have been found for other domains, with OXT_{int} increasing both fear conditioning (Eckstein et al., 2016) and fear extinction (Eckstein et al., 2015) in men depending on whether the peptide is administered before or after the fear learning. Nevertheless, a strong impact of OXT_{int} on consolidation processes in our study seems unlikely considering the kinetics of OXT_{int}. Limbic effects of OXT_{int} were already attenuated 75 min after nasal spray administration (Spengler et al., 2017) and blood OXT concentrations return to baseline 3–4 h after the treatment (Gossen et al., 2012). To account for the longer half-life time of EST_{tra} (~37 h, (Naunton et al., 2006)), we implemented a delay of 3 days between the encoding and the surprise recognition test. Furthermore, the recognition test was unannounced and we can therefore exclude that the treatments interacted with intentional memory strategies during the consolidation phase. Nevertheless, residual OXT or EST effects on consolidation processes may have contributed to the observed results and future human studies should apply OXT and EST immediately following encoding to further disentangle their roles in memory encoding and consolidation.

In addition, we replicated the sex-specific lateralization of amygdala recruitment (Cahill, 2006; Cahill et al., 2001; Canli et al., 2002, 2000), but there was no significant interaction of the treatments for the emotional memory effect in the amygdala. The effects of EST_{tra} and OXT_{int} on amygdala activation have been well established in animal studies (Acevedo-Rodriguez et al., 2015; Knobloch et al., 2012; Viviani et al., 2011), but translation to humans may depend on methodological details such as task design and stimulus material. Furthermore, while the availability of aromatase, the enzyme that catalyzes testosterone to estradiol, is comparable in the amygdala between women and men, higher aromatase availability was associated with lower memory performance in men, but not women (Alia-Klein et al., 2020). Thus, the effects of and interactions with other gonadal steroids should be taken into consideration. For instance, testosterone treatment shifted amygdala lateralization towards the right hemisphere in transgender boys (Beking et al., 2020) and exogenous progesterone increased amygdala responses to emotional faces in women (Van Wingen et al., 2008).

Our data replicated the known memory effect of emotional valence of the stimulus material, evident in better recognition memory of emotional vs. neutral images. In contrast to our hypotheses, however, the treatments did not selectively affect the recognition memory of emotional material and we did not find an emotion-specific female memory advantage. Null effects of EST on emotional memory performance have been previously observed after exogenous administration (Bayer et al., 2018) and while comparing different menstrual cycle phases (Gamsakhurdashvili et al., 2021). Moreover, we pre-selected the emotional scenes such that they were rated to be equally arousing by women and men (cf. **SI**) and better emotion-specific recognition memory in women was evident for emotional pictures that were also rated to be more arousing by women (Canli et al., 2002). Furthermore, OXT_{int} administered before the encoding has been found to improve the recognition memory for happy faces (Guastella et al., 2008) and the free recall of negative scenes (Striepens et al., 2012). We did not observe significant interactions between valence and treatments or between sociality and treatments on the behavioral level. This could be related to the use of scenes with a human person as social items instead of faces and may reflect differences between recognition and recall memory. Similarly, OXT_{int} has been shown to enhance emotion recognition of faces overall, while emotion-specific effects (i.e. happy or fearful faces) varied as a function of exposure time (Shahrestani et al., 2013). Our finding of an OXT_{int} effect on both social and non-social stimuli is in accordance with recent theoretical accounts describing OXT as an allostatic hormone that modulates both social and non-social behavior by maintaining stability through changing environments (Quintana and Guastella, 2020). On the neural level, OXT_{in} had no social-specific effects on hippocampus activity, but we observed a significant interaction of sex and OXT_{int} for amygdala responses to social memory (i.e. Social $_{Remembered>Forgotten}$ > Non-social $_{Remembered>Forgotten}$), suggesting that the moderation of OXT effects by sociality differs between brain regions.

The findings of the present study need to be considered in the context of the following limitations. Treatment-induced EST levels were higher in women than in men and although we included the treatment-induced hormone concentrations as control variables, this difference may have contributed to the observed sex-specific treatment effects. In addition, in both sexes, supraphysiological estradiol levels were induced due to the exogenous administration. It is conceivable that interactions between OXT_{int} and EST_{tra} in women would be evident at physiological EST levels occurring during the menstrual cycle. Along these lines, we tested women during the early follicular phase to control for changes in endogenous hormone levels, but this also means that we cannot extrapolate our findings to other cycle phases which are associated altered hippocampal gene expressions (Iqbal et al., 2020). Future studies should employ different doses in women and men and postlearning administration protocols to further delineate the sex-specific memory effects of EST_{tra} and OXT_{int}. Furthermore, we observed strong evidence for an interaction between sex, EST and OXT, but the hippocampal sex differences within the treatment groups did not survive correction for multiple comparisons and thus should be interpreted cautiously. Of note, millions of women around the world use steroid-based hormonal con-

traception as an effective way of birth control (Alkema et al., 2013). Some studies have shown an increased emotional memory recall in hormonal contraceptive users (Spalek et al., 2019), whereas other studies reported that neither pill phase (on and off) nor oral contraceptive use in general affected emotional memory (Kuhlmann and Wolf, 2005; Mordecai et al., 2017). Nevertheless, one has to keep in mind that different estrogen types and dosages are used for the preparation of oral contraceptives (Mawet et al., 2021), which may explain these conflicting findings. Thus, additional clinical trials using long-term applications are needed to further disentangle the hormones' impact on (emotional) episodic memory.

Collectively, our results provide evidence that EST_{tra} and OXT_{int} modulate episodic memory and hippocampal functioning in men. In contrast to our hypotheses, the treatments did not selectively affect the recognition memory of emotional material but rather the overall memory performance in men. Hence, future studies should consider sex as an important moderator variable and further explore the effects of EST-OXT interactions on (emotional) memory. Antagonistic effects of EST_{tra} and OXT_{int} may contribute to the previously observed sex-specific hormonal effects in hippocampus reactivity. Our findings support the increasingly recognized notion that it is vital to consider sex differences and hormonal interactions in pharmacological clinical trials.

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Data availability statement

A preprint of this article is published at the BioRxiv preprint server (https://doi.org/10.1101/2021.11.22.469500). The data that support the findings of the present study are openly available in the repository of the Open Science Foundation at https://osf.io/hvknp/ (doi:10.17605/OSF.IO/HVKNP). The unthresholded statistical maps of the fMRI results can be accessed at https://neurovault.org/collections/FBHLSKJX/. The code that supports the findings of the present study is openly available in the repository of the Open Science Foundation at https://osf.io/hvknp/ (doi:10.17605/OSF.IO/HVKNP).

Declaration of Competing Interest

The authors declare no competing interests.

Credit authorship contribution statement

Marie Coenjaerts: Conceptualization, Formal analysis, Methodology, Investigation, Visualization, Project administration, Writing – original draft, Writing – review & editing. **Isabelle Trimborn:** Formal analysis, Methodology, Investigation, Writing – review & editing. **Berina Adrovic:** Formal analysis, Methodology, Investigation, Writing – review & editing. **Birgit Stoffel-Wagner:** Resources, Writing – review & editing. **Larry Cahill:** Conceptualization, Writing – review & editing. **Alexandra Philipsen:** Writing – review & editing. **René Hurlemann:** Supervision, Writing – review & editing, Funding acquisition. **Dirk Scheele:** Conceptualization, Formal analysis, Methodology, Supervision, Funding acquisition, Writing – original draft, Writing – review & editing.

Data availability

The data is freely available on an online repository. The link has been included in the manuscript..

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Study approval statement

This study protocol was reviewed and approved by the institutional review board of the medical faculty of the University of Bonn [Approval number: 213/16]. Written informed consent was obtained from all participants included in this study.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2022.119689.

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3.3 Publication 3: Effects of exogenous oxytocin and estradiol on resting-state functional connectivity in women and men

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Effects of exogenous oxytocin and estradiol on resting-state functional connectivity in women and men

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Possible interactions of the neuropeptide oxytocin and the sex hormone estradiol may contribute to previously observed sex-specific effects of oxytocin on resting-state functional connectivity (rsFC) of the amygdala and hippocampus. Therefore, we used a placebo-controlled, randomized, parallel-group functional magnetic resonance imaging study design and measured amygdala and hippocampus rsFC in healthy men (n = 116) and free-cycling women (n = 111), who received estradiol gel (2 mg) or placebo before the intranasal administration of oxytocin (24 IU) or placebo. Our results reveal significant interaction effects of sex and treatments on rsFC of the amygdala and hippocampus in a seed-to-voxel analysis. In men, both oxytocin and estradiol significantly decreased rsFC between the left amygdala and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus compared to placebo, while the combined treatment produced a significant increase in rsFC. In women, the single treatments significantly increased the rsFC between the right hippocampus and the left anterior cingulate gyrus, whereas the combined treatment had the opposite effect. Collectively, our study indicates that exogenous oxytocin and estradiol have different region-specific effects on rsFC in women and men and that the combined treatment may produce antagonistic effects.

The hypothalamic peptide oxytocin (OXT) has a broad profile of effects ranging from labor induction and lactation[1] to social approach and avoidance behavior[2–4], romantic attachment[5–9], as well as fear[10–13] and trauma processing[14,15]. Neural effects of OXT in the hippocampus and amygdala are in line with higher expression of OXT receptors in these subcortical areas[1,16]. Furthermore, intranasal OXT has been found to modulate the functional communication between and within large-scale brain networks during resting state measured with electroencephalography[17] and functional magnetic resonance imaging (fMRI)[18–21]. Importantly, there is accumulating evidence that OXT exhibits sex-specific effects on neural responses during the perception or evaluation of socio-emotional stimuli[20,22–27] and on resting-state fMRI[28–30]. Preliminary evidence indicates that OXT affects resting-state functional connectivity (rsFC) between and within emotion and reward-related networks including the amygdala in a sex-dependent manner[28,31]. Additionally, as the amygdala is a set of functionally heterogeneous nuclei, a subregional-specific modulatory role of OXT on amygdala-centered emotion processing networks has been suggested[32,33], but sex-specific effects on rsFC of amygdala subregions have not been examined yet. Potential mechanisms contributing to the sex differences in rsFC include menstrual cycle effects and the interaction of OXT with sex hormones such as estradiol (EST)[28–30,34].

Fluctuations of sex hormones along the menstrual cycle impact rsFC[35] and effects of EST appear to be pronounced for the amygdala and the hippocampus. Both regions express a high density of EST receptors[36] and are sensitive to changes in estrogens[37,38]. For instance, elevated EST levels are related to an increase in hippocampal and amygdala gray matter volume[39–42]. In addition, higher EST levels positively correlate with hippocampal[39,43,44] and amygdala rsFC[45]. As yet, no study has probed the effects of exogenous EST on rsFC in men, but a recent study found that a single dose of exogenous testosterone modified the rsFC of the amygdala[46]. Given that testosterone

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is catalyzed to estrogen via the enzyme aromatase[47], an effect of exogenous EST on rsFC in men is therefore conceivable.

Evidence for EST-OXT interactions derive from animal models showing that EST and OXT modulate the synaptic plasticity in the medial nucleus of the amygdala in male rats[48] and that EST receptors induce an OXT production by binding in a dimerized form to the composite hormone response element of the OXT promotor gene[49–51]. Importantly, also in humans possible EST-OXT interactions have been discussed for various domains including social anxiety and migraine attacks[2,52]. There is preliminary evidence deriving from task-based fMRI studies that EST and OXT may antagonistically interact[23,53]. However, no study has simultaneously probed the modulatory effects of exogenous EST and OXT on rsFC and possible interactions in women and men. Over recent decades, large resting state fMRI (rsfMRI) datasets have been collected in neuroimaging consortia (e.g. the UK Biobank[54]) to decipher the functional integration of brain regions into interconnected networks. Machine learning approaches have been applied to rsfMRI data to predict demographic characteristics such as sex[55] and age[56]. Importantly, rsfMRI can help to identify neurophysiological subtypes of neuropsychiatric disorders like depression and these biomarkers may be useful to predict treatment responses[57]. For possible future clinical applications, a better understanding of sex differences and hormonal interaction effects is crucial. Previous studies observed that changes of rsFC significantly correlate with hormonal changes across the menstrual cycle[36], but the simultaneous alterations of multiple hormones hamper causal attributions. Furthermore, these findings cannot be extrapolated to men. By exploring the effects of exogenous OXT and EST on rsfMRI in women and men, we can overcome these limitations and control for possible context-dependent effects of OXT which have become evident in task-based studies. For instance, it has been found that OXT effects on amygdala activation are dependent on the emotional valence of faces[58–60] (but see also[61]), while other neural effects were moderated by previous experiences with the shown social stimuli[6,8]. Therefore, we conducted a pre-registered, randomized, placebo-controlled, parallel-group fMRI study involving healthy men and free-cycling healthy women in their early follicular phase to test the effects of exogenous EST, OXT, and their interaction on rsFC. Participants were scanned under four experimental conditions: 1. Transdermal placebo gel and intranasal placebo (PLC_{tra} & PLC_{int}), 2. Transdermal placebo and intranasal OXT (PLC_{tra} & OXT_{int}), 3. Transdermal EST and intranasal placebo (EST_{tra} & PLC_{int}), and 4. Transdermal EST and intranasal OXT (EST_{tra} & OXT_{int}).

Our first hypothesis was that under placebo men and women would differ in their amygdala and hippocampus rsFC[62–65]. We further expected that OXT_{int} would have opposing effects on hippocampal and amygdala rsFC in women and men[23,29,31]. However, due to the mixed results of OXT_{int} effects (e.g., increased and decreased rsFC), we refrained from formulating a directed hypothesis. Additionally, the observed correlation between higher EST levels and increased amygdalar and hippocampal rsFC[40,44–46], led to the hypothesis that the EST_{tra} treatment would increase the amygdalar and hippocampal rsFC in women and alter it in men. Due to limited research on EST effects in men, we abstained from hypothesizing a direction for the EST_{tra} effects in men. Furthermore, due to the possible antagonistic relation between OXT and EST, we hypothesized that the single treatment effects of either EST_{tra} or OXT_{int} would be reduced or inverted in the combined treatment group in both sexes[23]. In additional explorative analyses, we examined possible treatment and sex effects on the rsFC of amygdala subregions[33,34]. Furthermore, given that both OXT[66,67] and EST[68] have been found to modulate the default mode network (DMN), we also explored effects on rsFC of the DMN.

Results

Sex differences under placebo. To probe sex differences under placebo, we examined rsFC of the hippocampus, the amygdala, and the amygdala subregions as separate seed regions in a seed-to-voxel analysis (cluster defining threshold $p < 0.001$; significance threshold $p < 0.05$ false discovery rate-corrected, p_{FDR}) in placebo-treated (PLC_{tra} & PLC_{int}) women and men. Analyses revealed that women showed a decreased rsFC between the right hippocampus and the left anterior cingulate gyrus in contrast to men (MNI_{xyz}: - 4, 40, 26, $k = 133$, $p_{FDR} = 0.002$). The rsFC of the left hippocampus and the bilateral amygdala were not significantly different between the sexes under PLC (all $ps > 0.05$). However, an analysis of the amygdala subregions showed that for the right superficial amygdala as a seed region, the rsFC with the left cerebrum was increased in men compared to the women (MNI_{xyz}: - 32, 10, 18, $k = 80$, $p_{FDR} = 0.03$).

Significant sex * EST_{tra} treatment * OXT_{int} treatment interactions. We observed significant sex * EST_{tra} treatment * OXT_{int} treatment interactions in rsFC of both the hippocampus and the amygdala as separate seed regions in a seed-to-voxel analysis. Significant interactions were identified in rsFC of the right hippocampus with the left anterior cingulate gyrus (MNI_{xyz}: - 4, 38, 26, $k = 98$, $p_{FDR} = 0.02$) and in rsFC of the left amygdala with the right lingual gyrus (MNI_{xyz}: 16, - 52, 00, $k = 147$, $p_{FDR} = 0.002$) and the left cuneus (MNI_{xyz}: - 16, - 78, 16, $k = 88$, $p_{FDR} = 0.02$). There was no significant three-way interaction for the right amygdala or the left hippocampus. Additional analyses of the amygdala subregions revealed significant three-way interactions in rsFC of the left centromedial amygdala with the left cuneus (MNI_{xyz}: - 16, - 78, 16, $k = 122$, $p_{FDR} = 0.003$), the left lingual gyrus (MNI_{xyz}: - 20, - 50, - 4, $k = 112$, $p_{FDR} = 0.003$), and the right calcarine gyrus (MNI_{xyz}: 24, - 50, 4, $k = 173$, $p_{FDR} < 0.001$). By contrast, for the right superficial amygdala as a seed region, we observed a significant three-way interaction on rsFC with the right frontal lobe (MNI_{xyz}: 20, 36, 00, $k = 89$, $p_{FDR} = 0.047$). Importantly, we examined whether these group effects might be driven by motion and we observed no significant main or interaction effects with respect to the mean framewise-displacement (all $ps > 0.05$). The reported significant interaction effects are decomposed by examining treatment effects within the sexes.

Treatment effects within the sexes. To disentangle the observed three-way interactions, we further examined treatment effects separately for each sex. In women, a significant EST_{tra} treatment * OXT_{int} treatment

interaction was identified in rsFC of the right hippocampus with the left anterior cingulate gyrus (MNI_{xyz}: - 4, 38, 26, $k = 92$, $p_{FDR} = 0.04$; see Fig. 1). By contrast, the interaction effects on amygdala rsFC were evident in men, but not significant in women (see Fig. 2). In men, we found significant interactions between the two treatments in rsFC of the left amygdala with the right and left lingual gyrus (right: MNI_{xyz}: 16, - 50, - 2, $k = 202$, $p_{FDR} < 0.001$; left: MNI_{xyz}: - 20, - 50, - 4, $k = 102$, $p_{FDR} = 0.005$), the right calcarine fissure (MNI_{xyz}: 12, - 76, 16, $k = 107$, $p_{FDR} = 0.005$), and the right superior parietal gyrus (MNI_{xyz}: 12, - 60, 72, $k = 77$, $p_{FDR} = 0.02$). Further analyses of the amygdala subregions again showed a significant two-way interaction specifically in rsFC of the left centromedial amygdala with the left rolandic operculum (MNI_{xyz}: - 54, 2, 14, $k = 91$, $p_{FDR} = 0.03$) in the male subsample, but not in the female subsample.

Interestingly, we also observed significant two-way interaction effects on rsFC of the DMN in men. Specifically, interaction effects were identified in rsFc of the DMN with the left supramarginal gyrus (MNI_{xyz}: 54, - 24, 26, $k = 130$, $p_{FDR} = 0.003$), the right and left superior dorsolateral frontal gyrus (right: MNI_{xyz}: 16, 38, - 18, $k = 123$, $p_{FDR} = 0.003$; left: MNI_{xyz}: - 16, 34, - 22, $k = 120$, $p_{FDR} = 0.004$), and the left cerebellum Crus 1 (MNI_{xyz}: - 32, - 74, - 26, $k = 130$, $p_{FDR} = 0.003$).

To further analyze the significant two-way interactions, we extracted the parameter estimates of the significant peak voxels and employed t-tests to compare the activation between the treatment groups.

Treatment effects in women. Analyses of the extracted parameter estimates revealed that EST_{tra} significantly increased rsFC between the right hippocampus and the left anterior cingulate gyrus after PLC_{int} treatment (EST_{tra} & PLC_{int} > PLC_{tra} & PLC_{int}: $t_{(51)} = 4.40$, $p_{cor} < 0.001$, $d = 1.21$), but significantly reduced the rsFC in women who received OXT_{int} (EST_{tra} & OXT_{int} < PLC_{tra} & OXT_{int}: $t_{(56)} = -2.13$, $p_{cor} = 0.04$, $d = -0.56$). Likewise, OXT_{int} significantly enhanced rsFC between the right hippocampus and the left anterior cingulate gyrus after PLC_{tra} treatment (PLC_{tra} & OXT_{int} > PLC_{tra} & PLC_{int}: $t_{(57)} = 3.66$, $p_{cor} < 0.01$, $d = 0.96$), but decreased rsFC in women who received EST_{tra} (EST_{tra} & OXT_{int} < EST_{tra} & PLC_{int}: $t_{(50)} = -3.08$, $p_{cor} < 0.01$, $d = -0.85$).

Treatment effects in men. After the PLC_{int} treatment, EST_{tra} decreased rsFC between the left amygdala and the right and left lingual gyrus (EST_{tra} & PLC_{int} < PLC_{tra} & PLC_{int}: right: $t_{(57)} = -2.03$, $p_{cor} = 0.047$, $d = -0.53$; left: $t_{(57)} = -2.38$, $p_{cor} = 0.02$, $d = -0.62$) and the right superior parietal gyrus (EST_{tra} & PLC_{int} < PLC_{tra} & PLC_{int}: $t_{(57)} = -4.42$, $p_{cor} < 0.001$, $d = -1.15$). The opposite effect was evident in the OXT_{int} treatment groups, with EST_{tra} increasing rsFC between the left amygdala and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus (EST_{tra} & OXT_{int} > PLC_{tra} & OXT_{int}: right lingual gyrus: $t_{(55)} = 4.91$, $p_{cor} < 0.001$, $d = 1.31$; left lingual gyrus: $t_{(55)} = 4.14$, $p_{cor} < 0.001$, $d = 1.10$; right calcarine fissure: $t_{(55)} = 4.14$, $p_{cor} < 0.001$, $d = 1.10$; right superior parietal gyrus: $t_{(55)} = 2.46$, $p_{cor} = 0.03$, $d = 0.65$). The single treatment with OXT_{int} produced similar effects as the single treatment with EST_{tra}. OXT_{int} decreased rsFC between the left amygdala and the right and

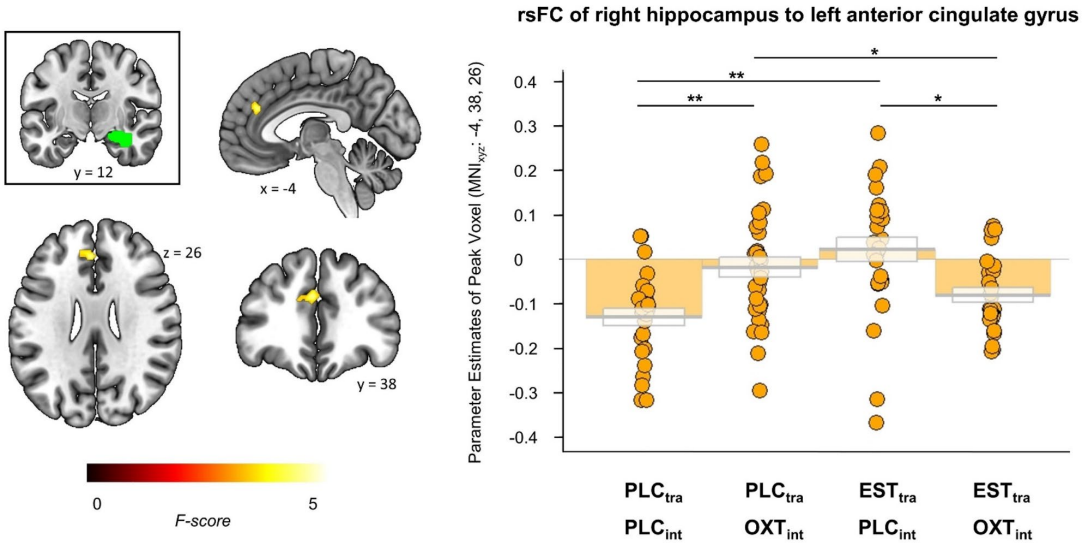

Figure 1. Treatment effects on the resting-state functional connectivity (rsFC) between the right hippocampus (green cluster) and the left anterior cingulate gyrus in women. The single treatment with either estradiol or oxytocin significantly increased rsFC between the right hippocampus and the left anterior cingulate gyrus. However, the combined treatment led to an rsFC between the right hippocampus and the left anterior cingulate gyrus comparable to that of the placebo group. Error bars indicate standard errors of the mean. PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol. *$p < 0.05$, **$p < 0.01$.

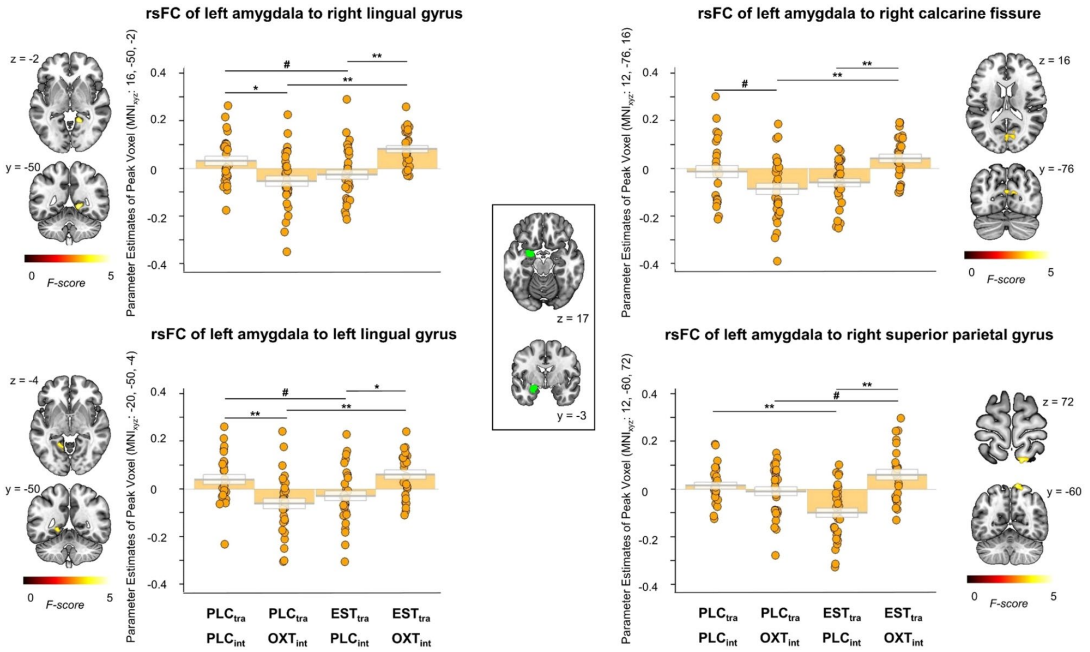

Figure 2. Treatment effects on the resting-state functional connectivity (rsFC) between the left amygdala as seed region (green cluster) and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus in men. The single treatment with either estradiol or oxytocin significantly decreased rsFC, while the combined treatment led to an rsFC comparable to that of the placebo group. Error bars indicate standard errors of the mean. PLC_{tra} = transdermal placebo gel; PLC_{int} = intranasal placebo; OXT_{int} = intranasal oxytocin; EST_{tra} = transdermal estradiol. #$p < 0.1$, *$p < 0.05$, **$p < 0.01$.

left lingual gyrus (PLC_{tra} & OXT_{int} < PLC_{tra} & PLC_{int}: right: $t_{(56)} = -2.89$, $p_{cor} = 0.01$, $d = -0.76$; left: $t_{(56)} = -3.35$, $p_{cor} < 0.01$, $d = -0.88$). However, after the EST_{tra} treatment, OXT_{int} increased rsFC (EST_{tra} & OXT_{int} > EST_{tra} & PLC_{int}: right lingual gyrus: $t_{(56)} = 4.08$, $p_{cor} < 0.001$, $d = 1.08$; right calcarine fissure: $t_{(55)} = 4.12$, $p_{cor} < 0.001$, $d = 1.10$; left lingual gyrus: $t_{(56)} = 3.18$, $p_{cor} < 0.01$, $d = 0.84$; right superior parietal gyrus: $t_{(56)} = 5.38$, $p_{cor} < 0.001$, $d = 1.42$). The same pattern of results was also evident for parameter estimates of rsFC of amygdala subregions, and DMN (cf. Supplementary Information). Taken together, both single treatments with EST_{tra} and OXT_{int} produced sex-specific effects on rsFC which were reversed in the combined treatment group.

Neuroendocrine parameters. We collected blood samples before the treatments and after the fMRI (approx. 4.5 h after gel administration) to measure the concentrations of the hormones EST, OXT, testosterone, and progesterone. At baseline, women had significantly higher EST concentrations ($t_{(157.61)} = 7.29$, $p < 0.001$, $d = 1.00$), but lower testosterone ($t_{(114.65)} = -28.50$, $p < 0.001$, $d = -3.71$) and OXT levels ($t_{(221)} = 2.90$, $p < 0.01$, $d = -0.39$) than men. The progesterone baseline concentrations were comparable between the two sexes ($t_{(107.21)} = -1.60$, $p = 0.11$, $d = -0.22$). Importantly, all baseline levels were comparable between treatment groups (all $ps > 0.05$).

The EST_{tra} administration significantly increased blood EST levels in both sexes (see Supplementary Table S1; time * EST_{tra} treatment: $F_{(1, 206)} = 303.10$, $p < 0.001$, $\eta_p^2 = 0.60$), with women exhibiting a significantly larger increase than men (time * sex * EST_{tra} treatment: $F_{(1, 206)} = 13.87$, $p < 0.001$, $\eta_p^2 = 0.06$). There were no significant main or interaction effects of the OXT_{int} treatment on EST levels (all $ps > 0.05$), indicating that the OXT_{int} treatment did not modulate the EST increase.

As an additional control analysis, we used a median-dichotomization and excluded EST_{tra}-treated women with large EST increase. In this subsample, the treatment-induced increases in EST levels were comparable between women and men within the treatment groups (all $ps > 0.05$) and the rsFC analyses yielded a similar pattern of results (see SI).

OXT_{int} administration significantly increased blood oxytocin levels in both sexes (see Supplementary Table S2; time * OXT_{int} treatment: $F_{(1,213)} = 347.92$, $p < 0.001$, $\eta_p^2 = 0.52$). There were no significant interaction effects of OXT_{int} and sex on the OXT levels, as well as no significant main or interaction effects of the EST_{tra} treatment on the OXT levels (all $ps > 0.05$), indicating that the EST_{tra} treatment did not modulate the OXT increase.

To examine whether the OXT and EST baseline values, as well as changes in OXT and EST levels affected neural treatment effects, we included the baseline values and the hormonal changes (after treatment minus baseline) as separate covariates in the analyses and all observed sex * treatment interactions remained significant.

Discussion

The goal of the current study was to elucidate the effects of exogenous EST_{tra} and OXT_{int} treatments and their interaction on hippocampus and amygdala rsFC in healthy women and men. Our results show significant interactions of sex and the treatments on hippocampus and amygdala rsFC. In women, the single treatment with either EST_{tra} or OXT_{int} significantly increased rsFC between the right hippocampus and the left anterior cingulate gyrus, while the combined treatment had the opposite effect. In men, both hormones significantly decreased the rsFC between the left amygdala and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus. Again, the combined treatment produced the opposite effect. Collectively, our study indicates an essential role of EST_{tra} and OXT_{int} as modulators of rsFC. We observed sex-specific differences in the localization of the rsFC effects, but the combined treatment reversed the single treatment effects in both sexes and produced effects comparable to the placebo groups, indicating an antagonistic effect of the two hormones at the administered doses.

While some studies found no significant sex differences in rsFC[69], recent machine learning approaches were able to reliably classify sex based on sex specifics in the functional brain organization both within sample and across independent samples[55]. Specifically, there is accumulating evidence that women and men differ in the rsFC of the amygdala[62–64] and hippocampus[64,65]. However, the direction of these changes (e.g. increased or decreased functional connectivity) varies between studies. In contrast to our hypothesis that women and men would differ in their amygdala and hippocampus rsFC under placebo[62–65], our results only showed a decreased rsFC between the right hippocampus and the left anterior cingulate cortex (ACC) in women compared to men, but no sex difference in rsFC of the amygdala. However, previously observed sex differences appear to depend on methodological details such as analysis method or sample characteristics. In the current study, we exclusively tested free-cycling women in their early follicular phase, which is associated with low levels of fluctuating steroid hormones, and excluded women using oral contraceptives. In contrast, previous sex-specific rsFC effects were reported in studies involving women in different cycle phases[63], and in studies not focusing on cycle phase or the use of oral contraceptives[62,64,65]. Thus, previously observed sex differences in rsFC of the amygdala might reflect the impact of other sex steroids fluctuating across the menstrual cycle, such as progesterone, or the influence of oral contraceptives on rsFC[70,71].

In our study, the single treatment with EST_{tra} did not significantly affect amygdala rsFC in women. Our hypothesis that EST_{tra} would affect amygdala rsFC in women was based on a previous study[46], which showed an association between increased amygdala rsFC and higher estradiol levels. We used an exogenous administration of estradiol to selectively modulate EST levels in healthy women in the early follicular phase. Thus, natural fluctuations of other hormones may have contributed to the previously observed association with estradiol. However, in line with our hypothesis, we observed that EST_{tra} increased hippocampal rsFC to the left ACC in women. The effect of estrogens on the hippocampus has been often investigated in female rodents[72] and most of the studies demonstrated a positive effect of estrogens on the hippocampal neurogenesis and dendritic morphology. Previous rsFC research on effects of EST on hippocampal connectivity revealed prefrontal regions[45], but also in line with our results the ACC as key target region[73–75]. Following a treatment with a gonadotropin releasing hormone agonist, which caused reduced EST levels, the functional connectivity between the hippocampus and the ACC was decreased[73]. In another study, postpartum women, who experience a sudden decline in EST levels after birth, demonstrated a decrease in rsFC of the hippocampus to the ACC[74,75]. Estrogen-dependent modulations of the hippocampus morphology and activation have been mostly examined in women and studies on EST effects in men are scarce. In our study, a single EST_{tra} treatment produced no significant effect in the male hippocampal rsFC. As yet, no study probed the effects of exogenous EST on rsFC in men. There is some evidence that changes in rsFC of the hippocampus depend on hippocampal neurogenesis in female mice[76]. However, previous studies[77,78] on hippocampal neurogenesis did not detect significant EST effects in male rats, either, and suggest an androgen-dependent mechanism, but it is unclear whether and how altered neurogenesis translates to altered rsFC in humans.

The single OXT_{int} treatment significantly increased hippocampal rsFC to the left ACC in women. Most published work on OXT_{int} effects focuses exclusively on males, but previous studies on OXT_{int} effects in women did not report significant modulation of hippocampus rsFC following an OXT_{int} treatment[18,32,66]. These differences might be rooted in experimental differences, as two of the studies examined women in different cycle phases[32,66] or additionally used a higher OXT dosage[66]. While Bethlehem and colleagues[18] only included women in their early follicular phase of their menstrual cycle and used the same dosage as in this study, they applied an independent component analysis (ICA) to examine how connectivity between-circuits differ across placebo and OXT_{int}. Based on our hypotheses about rsFC of the hippocampus and amygdala, we employed a seed-to-voxel approach which may produce conceptually different results than ICA[79]. In men, the OXT_{int} treatment significantly decreased left amygdala rsFC to the left and right lingual gyrus compared to the placebo group. While some previous OXT_{int} rsFC studies reported an enhancement of the amygdala rsFC to frontal regions after an OXT_{int} treatment[80], other studies found a decreased rsFC to the precuneus, prefrontal regions, or the lingual gyrus[33,81,82]. Interestingly, we also observed a significant effect of OXT_{int} on rsFC of the centromedial amygdala, which has been identified as a key target region of possible anxiolytic mechanisms of OXT[34].

Overall, our results show that a single OXT_{int} treatment yields effects similar to the EST_{tra} treatment, whereas the effects in the combined treatment groups were comparable to the placebo groups. Interestingly, the same pattern of results was evident in our previous study about hippocampus-dependent episodic memory effects of both hormones[53]. This pattern might be the result of an increased OXT receptor binding induced by the EST_{tra} pretreatment[83], which matches previously observed opposing effects for higher OXT_{int} doses in men[84]. Therefore, the antagonistic interaction of EST_{tra} and OXT_{int} may have contributed to previously observed sex-specific effects of OXT[23,27,85] and to the modulatory effects of hormonal contraception[86]. However, in contrast to our hypothesis,

a pre-treatment with EST_{tra} did not modulate OXT effects on rsFC in the same seed region in women and men. As such, the OXT-EST interactions cannot completely explain the observed sex-specific effects of OXT and future studies are warranted to probe the interaction with other sex hormones like progesterone.

The present study has some limitations that need to be addressed in future research. We tested women during the early follicular phase of their menstrual cycle to control for changes in endogenous hormone levels. Nevertheless, in both sexes, supraphysiological EST levels were induced and it is conceivable that treatment or interaction effects would be altered at physiological EST levels occurring during the menstrual cycle. In addition, the treatment-induced EST levels were higher in women than in men, which may have contributed to the observed sex-specific treatment effects. However, the inclusion of treatment-induced changes in hormonal levels as covariates did not alter our results and importantly, the baseline levels of EST, OXT, testosterone, and progesterone were comparable between treatment groups. Yet, we cannot extrapolate our findings to other cycle phases, which involve the fluctuation of other steroid hormones, or hormonal contraceptives, as different estrogen types and dosages are used for their preparation[87]. Thus, future rsFC studies are warranted to examine possible interactions between endogenous hormones by comparing the effects of experimentally induced release of endogenous OXT (e.g. via synchronous social interactions[84]) between different phases of the menstrual cycle. Future studies on exogenous effects should employ different doses and long-term applications in women and men to further disentangle the impact of exogenous hormones on rsFC.

Collectively, our results provide support for the notion that hippocampus and amygdala rsFC are modulated by sex and by the single and interactive effects of EST_{tra} and OXT_{int}. Previous findings associating OXT or EST and rsFC may have been affected by other fluctuating hormones and their potentially interactive effects. Thus, integrating sex and hormonal effects into research designs is vital to further decipher the interaction of neurobiological factors modulating hippocampus and amygdala rsFC.

Methods

Ethics and enrolment. The study was part of a larger project (for further results see[53]). It was approved by the institutional review board of the Medical Faculty of the University of Bonn and was carried out in accordance with the latest revision of the Declaration of Helsinki. The study was registered in the ClinicalTrials.gov database (Identifier: NCT04330677) and the data analyses were pre-registered (https://osf.io/gkd6s/). The participants were enrolled in the study after giving informed consent. The participants received monetary reimbursement.

Participants. In total, 295 participants (160 women) were invited to a screening session prior to the testing session. The 246 participants (122 women) who met the inclusion criteria (see below) were tested. The participants were randomly assigned to one of four experimental conditions: (1. PLC_{tra} & PLC_{int}; 2. PLC_{tra} & OXT_{int}; 3. EST_{tra} & PLC_{int}; 4. EST_{tra} & OXT_{int}). The data of 7 participants were excluded due to technical malfunctions. Furthermore, two participants were excluded due to excessive head movements (> 20% volumes were identified as outliers by ART). Additional 9 participants were excluded due to anatomical (n = 2) or hormonal (n = 7) abnormalities and one participant did not finish the study. Thus, after the exclusion of 19 participants from all analyses, our final sample included 227 participants (PLC_{tra} & $PLC_{int:}$ 27 men, 26 women; PLC_{tra} & OXT_{int}: 31 men, 33 women; EST_{tra} & PLC_{int}: 32 men, 27 women; EST_{tra} & OXT_{int}: 26 men, 25 women). For demographic and psychometric baseline characteristics see Supplementary Table S3.

Screening session and exclusion criteria. The participants were screened in a separate session prior to the test session. The participants were right-handed, non-smoking, and between 18 and 40 years old. Exclusion criteria were MRI contraindications, current pregnancy and the use of hormonal contraceptives. Additionally, participants reported to be free of current or past physical or psychiatric illnesses assessed by the Mini-International Neuropsychiatric Interview[88] and were naïve to prescription-strength psychoactive medication. Furthermore, participants had not taken any over-the-counter psychoactive medications in the four weeks prior to the study and were asked to abstain from alcohol intake on the day of the experiment. After completing the screening session, the participants were invited to the fMRI testing session. To ensure that the women were tested in their early follicular phase of their menstrual cycle, they were scanned simultaneously with the onset of their menstruation (days 1–6), which was determined via self-report. To further validate the cycle phase, blood assays were obtained on the testing day. Female participants showing estradiol pre-treatment values larger than 145 pg/ml were excluded, because it can be assumed that they were not in the follicular phase of their menstrual cycle[89].

Treatments. *Estradiol/placebo gel treatment.* EST_{tra} gel (Estramon, 2 mg EST, Hexal AG, Holzkirchen, Germany) or placebo gel (2 mg ultrasonic gel) was transdermally applied to the participants' backs. The 2 mg dose was chosen in line with a pharmacokinetic study[90] to reduce the possibility of side effects. The same dose has also been found to increase emotional vicarious reactivity in men when watching a distressed other[16].

Intranasal oxytocin/placebo treatment. The OXT dosage of 24 International Units (IU) was chosen on the basis of one of our previous studies targeting amygdala functioning[60], which determined the most effective dose (24 IU, in contrast to 12 IU or 48 IU) and dose-test interval (30–60 min). The participants self-administered 24 IU of synthetic OXT_{int} (Sigma-Tau Industrie Farmaceutiche Riunite S.p.A., Rome, Italy) or placebo via nasal spray prior to the fMRI scanning under supervision of a trained research assistant and in accordance with the latest standardization guidelines[91]. The placebo solution contained identical ingredients as the OXT_{int} solution except for the peptide itself. An interpuff interval of approx. 45 s was chosen and the amount of administered substance was weighed and supplemented until the 24 IU were reached. There is compelling evidence that OXT_{int} bypasses the blood–brain barrier and elevates OXT concentrations in the cerebrospinal fluid[92,93] and brain[94].

Resting state paradigm. Each participant was positioned in the MRI scanner with their heads comfortably placed and stabilized with cushions to reduce head motion. Participants were instructed to relax and to look at a white fixation cross on a black screen for ten minutes.

Experimental design. We used a randomized, placebo-controlled, double-blind, parallel-group study design. The fMRI day commenced with the gel administration. The OXT_{int} or placebo spray was administered three hours after gel administration in line with our pharmacokinetic pre-study (see **SI**). The imaging data collection included a high-resolution structural MRI scan and a resting-state scan, followed by two tasks (for further results see[53]). The resting-state data collection commenced 35 min after OXT_{int} administration, because the strongest limbic effects can be expected for dose-test interval of 30–60 min[60]. Blood samples were collected at baseline and immediately after the fMRI testing session (approx. 4.5 h after gel administration).

Data analysis. *fMRI data acquisition.* All fMRI data were acquired using a 3 T Siemens TRIO MRI system (Siemens AG, Erlangen, Germany) with a Siemens 32-channel head coil. Following a fieldmap acquisition, resting state data were acquired using T2*-weighted echoplanar (EPI) sequence [repetition time (TR) = 2690 ms, echo time (TE) = 30 ms, ascending slicing, matrix size: 96 × 96, voxel size: 2 × 2 × 3 mm^3, slice thickness = 3.0 mm, distance factor = 10%, FoV = 192 mm, flip angle 90°, 41 axial slices] for ten minutes. High-resolution T1-weighted structural images were collected on the same scanner (TR = 1660 ms, TE = 2.54 ms, matrix size: 256 × 256, voxel size: 0.8 × 0.8 × 0.8 mm^3, slice thickness = 0.8 mm, FoV = 256 mm, flip angle = 9°, 208 sagittal slices).

fMRI data preprocessing. The resting state data was analyzed employing the Functional Connectivity Toolbox for SPM (CONN; http://www.nitrc.org/projects/conn/)[95]. The CONN preprocessing pipeline included realignment and unwarping using the fieldmap, slice time correction, segmentation, spatial normalization, and smoothing with a 6 mm Gaussian kernel. Furthermore, to limit the impact of head movements, the artifact detection tool (ART) implemented in CONN was used to identify high motion volumes using a volume-to-volume shift of > 1.5 mm and a volume-to-volume change in mean signal intensity of > 3 standard deviations. Artifacts were treated as regressors of no interest in the following analysis. Participants with > 20% volumes identified as outliers by ART were excluded.

fMRI data analysis. Amygdalar and hippocampal functional resting-state connectivity was probed in a whole brain seed-to-voxel analysis. To assess the connectivity of the amygdala and hippocampus, seed-to-voxel connectivity maps were estimated for each participant using CONN. The seeds (left and right hippocampus and left and right amygdala) were anatomically defined using the aal atlas in the Wake Forest University PickAtlas, version 3.0. Statistical analyses were conducted using CONN. Connectivity maps for each of the seeds were compared using analyses of variance (ANOVAs) with the between-subjects variables "nasal spray treatment" (PLC_{int} or OXT_{int}), "gel treatment" (EST_{tra} or PLC_{tra}), and "sex" (female or male). We probed significant three-way interactions and two-way interactions separately for men and women. Parameter estimates of significant peak voxels of the significant two-way interactions (cluster defining threshold $p < 0.001$; significance threshold $p < 0.05$, false discovery rate-corrected, p_{FDR}) were extracted and further analyzed with SPSS 27 (IBM Corp., Armonk, NY). Post-hoc analyses employed two-sample t-tests comparing activation between subgroups, corrected for multiple comparisons with the Bonferroni-Holm method (p_{cor}).

Further exploratory analyses. We explored the rsFC of amygdala subregions, and the DMN with whole brain seed-to-voxel analyses. The basolateral, centromedial, and superficial amygdala were defined as seeds based on cytoarchitectonic probabilistic maps[96] implemented in the Anatomy toolbox[97]. The four DMN main nodes (medial prefrontal cortex (MPFC), left and right lateral parietal regions, and the posterior cingulate cortex (PCC)) were based on the pre-defined seeds implemented in the CONN toolbox. We employed the four seeds separately to detect possible differences in the resulting connectivity maps. The preprocessing and the data analysis of the amygdala subregions, and the DMN were the same as described for the amygdala and hippocampus whole brain seed-to-voxel analysis.

Statistical analyses. Neuroendocrine and demographic data were analyzed in SPSS 27 using standard procedures including analyses of variances (ANOVAs) and post-hoc *t*-tests. Post hoc *t*-tests were Bonferroni-Holm-corrected (p_{cor}). If the assumption of sphericity was significantly violated, a Greenhouse–Geisser correction was applied. As measures of effect sizes, partial eta-squared and Cohen's d were calculated. Changes in hormone concentrations were examined with mixed-design ANOVAs with the between-subject factors "OXT_{int} treatment", "EST_{tra} treatment", and "sex" and the within-subject variable "time" (baseline, after fMRI). Furthermore, to explore the potential moderating effects of treatment-induced hormonal changes, the magnitude of the increases in hormone concentrations (levels of EST, OXT, testosterone, and progesterone after the fMRI session minus baseline) as well as autistic-like traits and social anxiety scores were considered covariates in the main analyses with significant neural outcomes (i.e., parameter estimates of significant contrasts of interests).

Ethical approval. This study protocol was reviewed and approved by the institutional review board of the medical faculty of the University of Bonn [Approval number: 213/16]. Written informed consent was obtained from all participants included in this study.

Data availability

The data that support the findings of the present study are openly available in the repository of the Open Science Foundation at https://osf.io/xubts/ (https://doi.org/10.17605/OSF.IO/XUBTS). The code that supports the findings of the present study is openly available in the repository of the Open Science Foundation at https://osf.io/xubts/ (https://doi.org/10.17605/OSF.IO/XUBTS).

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Author contributions
M.C. and D.S. designed the experiment; M.C., I.T., and B.A. conducted the experiments; M.C. and D.S. analyzed the data. M.C. and D.S. wrote the manuscript. All authors read and approved the manuscript in its current version.

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4. Discussion with references

The goal of the current thesis was to elucidate estradiol's sex-specific impact on economic decision-making (study 1), as well as sex differences in estradiol's and oxytocin's single and interactive effects on emotional memory (study 2) and rsFC (study 3). In particular, the first research question addressed the effects of estradiol on the sensitivity to the perceived fairness of economic offers in women and men and the influence of stereotypical beliefs associated with an estradiol treatment. The results indicate that under placebo women are more sensitive to fairness frames than men, which supports previous findings reporting that women show a stronger propensity to adjust their behavior with changing frames (Fehr and Gachter, 2002; Miller and Ubeda, 2012). Yet, the estradiol treatment sex-specifically altered the fairness sensitivity by reducing the acceptance rate of fair-framed offers in women and elevating it in men, whereas it did not significantly change responses to unframed offers. In contrast to unframed offers, the intentionality of the proposer is evident in framed offers (Radke et al., 2012). Thus, in women, it is conceivable that low estradiol levels might increase their ability to incorporate the proposer's intentionality into their decision-making, apposite to previous findings on low estradiol levels elevating their attentional vigilance for emotional information (Albert and Newhouse, 2019). However, in men, the estradiol treatment may have increased their sensitivity for the intentionality of bargaining offers by possibly facilitating perspective taking (Guroglu et al., 2011). Yet, estradiol's influence expanded beyond its direct hormonal effects. Irrespective of sex, participants in the placebo group, who believed in being treated with estradiol showed an increased acceptance rate of unfair-framed offers in comparison to those believing in receiving a placebo treatment. Therefore, the first study underlines the importance to consider the impact of hormones and sex on economic decision-making but also highlights the necessity to investigate potential belief effects about specific hormonal treatments.

The second study probed estradiol's and oxytocin's behavioral and neural effects on emotional memory, in addition to their possible interactions. Contrary to our hypotheses, both treatments did not emotion-specifically affect memory performance at the administered doses. The treatments rather induced mnemonic effects independent of emotional valence, which were accompanied by neural changes in the hippocampus, but not in the amygdala. Null effects of estradiol on emotional memory and associated

amygdala reactivity have previously been reported (Bayer et al., 2018), and oxytocin's emotion-specific effects are discussed to potentially be time-sensitive (Shahrestani et al., 2013) and task-dependent (Striepens et al., 2012). The results showed that under placebo women exhibited a better overall memory performance and increased hippocampal activation to subsequently remembered items compared to men. The single treatments attenuated this mnemonic sex difference and reversed the sex-specific hippocampus responses. Contrasting, the co-administration of both hormones yielded no significant effects, suggesting an antagonistic relation between estradiol and oxytocin at the administered doses. Interestingly, a similar pattern of results emerged, while exploring the third research question on the sex-specific impact of estradiol and oxytocin on rsFC (study 3). In men, rsFC between the left amygdala and the right and left lingual gyrus, the right calcarine fissure, and the right superior parietal gyrus were decreased following the separate treatment with either estradiol or oxytocin, whereas in women, the single treatments enhanced rsFC between the right hippocampus and the left anterior cingulate gyrus compared to placebo. Intriguingly, the combined treatment reversed the sole treatment effects in both sexes. Therefore, the second and third study displayed a resembling pattern of results, since the effects induced by the estradiol treatment were comparable to those of the oxytocin treatment, while their co-administration produced similar effects as the placebo group. Possible mechanisms explaining this pattern might be rooted in the estradiol treatment eliciting an enhancement of the oxytocin receptor binding (Johnson et al., 1991), which corresponds to preceding findings on oppositional effects for higher oxytocin doses in men (Spengler et al., 2017). Thus, the results of the current thesis propose the notion of a potentially antagonistic relation between both hormones at the administered doses. These antagonistic effects might have contributed to priorly reported sex differences in oxytocin effects (Ditzen et al., 2013; Lieberz et al., 2020; Rilling et al., 2014). Nevertheless, since oxytocin's rsFC effects were not modified by the estradiol treatment in the same seed region in men and women, an interaction of both hormones cannot exhaustively elucidate the priorly reported sex differences in oxytocin effects. Hence, further research is needed to examine the ancillary impact of other fluctuating sex hormones on the single and interactive effects of estradiol and oxytocin. Furthermore, both hormones' neural effects might additionally be modulated by methodological characteristics. Despite numerous animal studies reporting estradiol and

oxytocin altering amygdala responses (Acevedo-Rodriguez et al., 2015), a translation to humans might depend on task design and stimulus material, as both hormones affected amygdala rsFC in men, but did not modify mnemonic-related amygdala responses. In conclusion, the current thesis extended the knowledge of estradiol's impact on economic decision-making in both sexes, as well as of estradiol's and oxytocin's sex-specific influence on episodic memory and rsFC.

4.1 Limitations and outlook

The current thesis needs to be considered in the context of the following limitations. Based on the erroneous perception that estradiol is a "female" sex hormone due to its close link to female reproduction, previous estradiol studies primarily concentrated on women, while the majority of oxytocin studies focused on men (Hammes and Levin, 2019). Yet, the current thesis demonstrates that exclusively studying women or men, or aggregating the analysis of both sexes, would conceal sex-specific effects. Consequently, future studies should include women and men in the same experimental protocol and particularly pharmacological trials should incorporate the influence of sex in their research designs (Bale, 2019; Tannenbaum et al., 2019).

In addition to sex, future research should try to decipher the impact of naturally fluctuating hormones and their interactive effects. The second and third study detected significant interaction effects of estradiol and oxytocin on memory and rsFC. Since the first study solely addressed estradiol's role in economic decision-making, future studies could expand their focus on additional interactive effects with oxytocin, as oxytocin's previously observed sex-specific effects on decision-making (Feng et al., 2015) might derive from interactions with fluctuating sex hormones including estradiol. Nonetheless, extrapolating the results of the second and third study to economic decision-making or other non-investigated task domains or contexts would be invalid, and hence further work is needed.

In the current thesis, solely free-cycling women in their early follicular phase were tested. This provides the advantage of low levels of circulating sex hormones, including estradiol, and therefore comparable baseline conditions between women and men. Yet, the estradiol treatments triggered a higher increase in estradiol levels in women compared to men, which might have affected the observed sex-specific treatment effects. Moreover, the treatments induced supraphysiological estradiol and oxytocin levels in both sexes,

which underlines the importance to examine how naturally occurring fluctuations of both hormones alter the reported treatment and interaction effects. As endogenous oxytocin levels can be elevated inter alia via synchronous social interactions (Spengler et al., 2017), future studies could explore the impact of experimentally increased oxytocin levels on different menstrual cycle phases to further decipher the interactive effects of both hormones. Focusing on other cycle phases could delineate not just estradiol's, but also other sex hormones' effects. For instance, variations in progesterone levels along the menstrual cycle have been shown to affect economic decision-making, memory formation, and rsFC as well (Ambrase et al., 2021; Arélin et al., 2015; Barros et al., 2015).

In addition to the investigation of endogenous estradiol and oxytocin fluctuations, future research could pursue examining effects elicited by exogenous hormonal treatments. The current thesis proposes a potentially antagonistic relation between estradiol and oxytocin at the administered doses. However, previous studies suggested that exogenous estradiol and oxytocin effects are dose-dependent and that estradiol might even exhibit an inverted U-shaped dose-response function (Bayer et al., 2018, Spengler et al., 2017). Therefore, future studies should elucidate the influence of different dosages and also long-term applications, such as oral contraceptives, on the observed treatment effects.

Conclusively, antecedent studies suggest that estradiol might play a role in the onset and development of anxiety disorders, which occur more frequently in women relative to men (Cover et al., 2014). As oxytocin has evolved as a potential candidate compound for treating various psychiatric disorders due to its prosocial and anxiolytic effects (Heinrichs et al., 2009), additional clinical trials using long-term applications are needed to further disentangle the hormones' interactive effects and to deduce possible direct implications for the development and treatment of anxiety disorders (Bale, 2019; Lieberz et al., 2020).

4.2 Conclusion

Summarizing, the current thesis indicates region- and sex-specific effects of estradiol and oxytocin. Additionally, the results provide unprecedented evidence for a potentially antagonistic relation between both hormones. Therefore, future studies are warranted to continue deciphering the single and interactive effects of exogenously applied or endogenously fluctuating estradiol and oxytocin, besides possible interactions with other sex hormones. In conclusion, the current thesis highlights the necessity to consider sex,

simple hormonal effects, and associated stereotypical beliefs, but also hormonal interactions as critical moderating factors in pharmacological and clinical trials.

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